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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE ANTIMICROBIAL EFFECT OF *N*-ACETYLCYSTEINE AND ITS INTERACTION WITH ANTIBIOTICS AGAINST *ACINETOBACTER BAUMANNII* ISOLATES

N-ASETİL SİSTEİNİN ACINETOBACTER BAUMANNII İZOLATLARINA KARŞI ANTİMİKROBİYAL ETKİSİ VE ANTİBİYOTİKLERLE ETKİLEŞİMİ

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ABSTRACT

Objective: *The objective of this study was to delve into the effect of N-acetylcysteine (NAC) molecule on the minimum inhibitory concentration (MIC) of meropenem, ciprofloxacin, and gentamicin in clinical isolates of Acinetobacter baumannii, aiming to find potential alternatives for the treatment of bacterial infections that are resistant to conventional antibiotics.*
Material and Method: *The study included 25 A. baumannii isolates that were confirmed to be resistant to meropenem, ciprofloxacin, and gentamicin. The susceptibility of antibiotics was re-evaluated in the presence of NAC using the microdilution method. FIC indexes were calculated based on the checkerboard test results to determine the effect of the combination, defined as synergistic or additive.*
Result and Discussion: *The study demonstrated that NAC molecule, when used alongside meropenem, ciprofloxacin, and gentamicin, effectively reduced the MIC values of these antibiotics against Acinetobacteria. Furthermore, NAC molecule exhibited a synergistic effect when combined with meropenem. Additive effects were observed in all isolates for the GEN-NAC and CIP-NAC combinations. In conclusion, the findings suggest that NAC molecule could serve as a new alternative for combined drug therapy, offering a promising approach to treatment.*
Keywords: *Acinetobacter baumannii, ciprofloxacin, gentamicin, meropenem, N-acetylcysteine (NAC)*

ÖZ

Amaç: *Dirençli bakteriyel enfeksiyonların tedavisi için yeni antimikrobiyal bileşiklerin sentezlenmesi çalışmalarının yanısıra, inhibitör moleküllerin antibiyotiklerle kombine kullanılmasına yönelik araştırmalar da yapılmaktadır. Çalışmamızda, NAC molekülünün Acinetobacter baumannii klinik izolatlarında, meropenemin, siprofloksasinin ve gentamisinin minimum inhibitör konsantrasyonu (MİK) üzerine etkisinin saptanması araştırılarak, tedavi için yeni potansiyel alternatifler bulmak amaçlanmıştır.*
Gereç ve Yöntem: *Çalışmamızda kullanılmak üzere meropeneme, siprofloksasine ve gentamisine dirençli olduğu doğrulanan 50 A. baumannii izolatı çalışmaya alınmıştır. Antibiyotiklerin duyarlılığı NAC varlığında yeniden araştırılmıştır. Antimikrobiyal duyarlılık testleri mikrodilüsyon yöntemi ile yapılmıştır. Dama tahtası testi sonuçlarına göre FİK indeksleri hesaplanmış ve kombinasyonun etkisi sinerjik ya da aditif olarak tanımlanmıştır.*
Sonuç ve Tartışma: *Bu çalışmada, NAC molekülünün meropenem, siprofloksasin ve gentamisin ile*

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birlikte kullanıldığında bu antibiyotiklerin Acinetobakterlere karşı MİK değerlerini etkili bir şekilde azalttığı gösterilmiştir. Ayrıca NAC molekülü meropenem ile birlikte kullanıldığında sinerjik etki göstermiştir. GEN-NAC ve CIP-NAC kombinasyonları için tüm izolatlarda aditif etki gözlenmiştir. Sonuç olarak elde edilen bulgular, NAC molekülünün kombine ilaç tedavisine yeni bir alternatif olarak kullanılabileceği ve tedaviye umut verici bir yaklaşım sunabileceği göstermiştir. NAC molekülünün farklı mikroorganizmalar ve antimikrobiyal maddelerle birlikte kullanılmasının da mümkün olabileceği ve ileride yapılacak çalışmalar açısından çalışmadaki bulguların yararlı olduğu düşünülmüştür.

Anahtar Kelimeler: *Acinetobacter baumannii, gentamisin, meropenem, N-asetilsistein (NAC), siprofloksasin*

INTRODUCTION

Infections acquired during the process of receiving healthcare that were not present or in the incubation period during admission to the hospital or developed 48-72 hours after hospitalization or within 10 days after leaving the hospital are defined as nosocomial infections [1-2]. *Acinetobacter* spp., non-fermentative, Gram-negative coccobacilli, have emerged as causes of hospital infections in recent years. While the overall mortality rate of patients due to *A. baumannii* infections is 5% in general hospital wards, it can reach 54% in intensive care units [3]. In a multicenter study conducted to investigate nosocomial Gram-negative bacterial infections in intensive care units in Turkey, Acinetobacter spp. were reported as the third most common bacteria among Gram-negative bacilli, and antibiotic resistance rates were also found to be quite high [4]. Acinetobacter spp. have also been identified as causative agents of ventilator-associated pneumonia in intensive care units and have been reported to be the leading cause of hospital-acquired pneumonia [5,6]. In Trakya University Education Application and Research Center, Acinetobacter species are frequently isolated and cause serious problems, especially in intensive care units and surgical services.

Among the Acinetobacter genus, *Acinetobacter baumannii* is the most important species in terms of the infections it causes. *A. baumannii* can be found in nature and in human skin flora or may be isolated from clinical specimens [7-8]. Resistance to antibiotics used in infections caused by these strains has become a significant global health problem, as increasing multi-antibiotic resistance reduces the possibility of treatment [9,10]. Many Acinetobacter species have shown resistance to antibiotics such as quinolones, carbapenems, and cephalosporins. Colistin is currently used as the only treatment option for such incurable cases; however, colistin-resistant strains have also been reported in recent years. Therefore, new treatment strategies need to be investigated for this and other multi-drug resistant bacterial infections [9,11]. The use of combined antibiotics for treatment or the addition of non-antibiotic compounds to the combination are the most preferred strategies in recent years [6]. These combinations have been reported to decrease the MIC values of the antibiotics or inhibit antimicrobial resistance [12].

One of these molecules, N-Acetylcysteine (NAC), has been extensively studied [13,14]. N-acetylcysteine (NAC) is an N-acetylated derivative of the amino acid L-cysteine and is a precursor molecule of glutathione [13-17]. NAC has provided an alternative pharmacological approach in antimicrobial resistance studies [14]. It is stated that the sulfur groups in the structure of NAC are effective in reducing the adhesion of bacteria to the surface and separating bacteria adhered to a surface [15-17].

The antimicrobial activity of NAC has been proposed to be explained by several mechanisms. These include competitive inhibition of cysteine utilization, reaction of NAC sulfhydryl group with bacterial proteins, and disruption of intracellular redox balance through indirect effects on cell metabolism and intracellular signal transduction pathways [18].

To detect the inhibitory, synergistic, or antagonistic effect of compounds in a combination, synergy tests are used, and the checkerboard assay is one of the most frequently used tests [19]. In the checkerboard test, the combination efficacy is tested by comparing the concentrations of drugs on a 96-well plate. The MIC values of the drugs are compared with the MIC values obtained from the combination, and the fractional inhibitory concentration (FIC) is found. Then, the FIC values of the drugs in the combination are summed, and the FIC index is calculated. The FIC value of each antimicrobial agent is obtained by dividing the lowest antimicrobial agent concentration in the well

without growth by the MIC of that agent against the same strain alone [19,20]. In this study, the aim was to determine the effect of the NAC molecule on the MIC value of antibiotics against meropenem, ciprofloxacin, and gentamicin-resistant clinical isolates of *A. baumannii*. For this purpose, FIC indices of meropenem-NAC, ciprofloxacin-NAC, and gentamicin-NAC combinations were calculated using the checkerboard test, and the effect of the combination was defined as synergistic or additive.

MATERIAL AND METHOD

In our study, as quality control strains recommended by CLSI M100-S28 [21] and European Committee on Antimicrobial Susceptibility Testing- EUCAST [22]; *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 27853, *A. baumannii* NCTC 1342 and 25 *A. baumannii* isolates isolated from various clinical samples sent to Trakya University Health Research and Application Center were used.

Method

Microdilution Method

Antimicrobial susceptibility tests were conducted in accordance with CLSI-M100-S28 recommendations. In microplates, in cation-adjusted Mueller Hinton broth (CA-MHB), NAC concentrations were between 4096-64 µg/ml. Concentrations of meropenem, ciprofloxacin and gentamicin were prepared in the range of 256-0.25 µg/ml. Bacterial suspensions were prepared at a density of 5 x 10^5 cfu/ml and were added to the wells. Microplates were incubated at 37ºC for 18-24 hours and the lowest drug concentration that inhibited growth was determined as MIC.

Checkerboard Method

In the checkerboard method, serial dilutions of the antibiotics (256-0.25 µg/ml) were dispensed into the first ten wells of the microplate from left to right, and serial dilutions of NAC (4096-64 µg/ml) were dispensed into the first eight-well from top to bottom of another microplate. The contents of the two plates were combined in another microplate. The concentration range of the antibiotics used was determined according to the MIC values. Bacterial inoculum prepared at a density of 5 x 10^5 cfu/ml was added to the wells. Plates were incubated at 37ºC for 18-24 hours. Evaluation of the combination test was performed according to the fractional inhibitory concentration (FIC) index (Figure 1) [5-7].

$FIC\ A = MICA_{combination}/MICA$

$FIC\ B = MICB_{combination}/MICB$

$FIC = FIC\ A + FIC\ B$

$FIC \leq 0{,}5$, synergistic effect

$0{,}5 < FIC \leq 4$, additive effect

$FIC > 4$, antagonistic effect

Figure 1. Calculation of Fractional Inhibitor Concentration (FIC) Values

RESULT AND DISCUSSION

MIC values determined as sensitive if the MIC value was ≤2 µg/ml, and resistant if ≥8µg/ml for meropenem [22]. All isolates were found to be resistant to meropenem. The detected MIC values were over 16 µg/ml.

MIC values obtained as a result of the susceptibility test performed with the microdilution method were determined as sensitive if the MIC value was ≤1 µg/ml, and resistant if ≥4 µg/ml for ciprofloxacin [22]. All isolates were found to be resistant to ciprofloxacin. The detected MIC values were over 32 µg/mL.

MIC values obtained as a result of the susceptibility test performed with the microdilution method were determined as sensitive if the MIC value was ≤4 µg/ml, and resistant if ≥16 µg/ml for gentamicin [22]. All isolates were found to be resistant to gentamicin. The detected MIC values were over 16 µg/ml.

According to the EUCAST guidelines all isolates were determined to be resistant to meropenem and gentamicin and ciprofloxacin. The MIC values were given in Table 1.

Table 1. MIC values of the agents against *A. baumannii* isolates

	MER*(µg/ml)	**GEN**(µg/ml)**	**CIP***(µg/ml)**
2	16	64	64
3	16	256	128
7	16	256	128
8	128	256	128
11	64	256	256
13	8	256	256
14	16	256	256
15	16	64	128
17	32	64	64
19	16	64	64
20	32	256	256
21	256	64	64
24	16	256	128
25	64	256	256
26	32	64	64
28	32	32	32
29	128	64	64
30	32	64	64
31	256	256	256
32	16	64	64
33	16	32	256
35	16	16	128
36	64	64	64
41	64	64	64

*MER: Meropenem,**GEN: Gentamicin,***CIP: Ciprofloxacin

In the presence of NAC, a four-fold or more decrease was found in the MER MIC values to 19 (76%) of 25 *A. baumannii* isolates included in the study (Table 2). Despite the decrease in 4 of 19 isolates, the MIC value were still above the resistance limit value. In the presence of NAC, 8 (32%) of 25 resistant isolates was determined to be susceptible.

In the presence of 2038 mg/l NAC, a four-fold or more decrease was found in the CIP MIC values of 11 (44%) of 25 *A. baumannii* isolates included in the study (Table 3). Although there was a decrease in 8 of 11 isolates, the MIC value is still above the resistance limit value. In the presence of 2038 mg/L NAC 3 (12%) of 25 isolates resistant to GEN became susceptible to GEN.

In the presence of 2038 mg/l NAC, a four-fold or more decrease was found in the CIP MIC values of 4 (16%) of 25 *A. baumannii* isolates included in the study (Table 4). However, the MIC value was still above the resistance limit value in all isolates.

Table 2. Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	MER* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+MER MIC (µg/ml)**	**MER-NAC Combinations (µg/ml)**	**FIC*** Index**	**Interaction type**
2	16	2038	8	8/509	1	Additive
3	16	2038	0.5	16-2038	1.06	Additive
7	16	2038	8	16-509	1.5	Additive
8	128	2038	8	8-509 16-127 32-64	0.31 0.187 0.28	Synergy Synergy Synergy
11	64	2038	16	2-1018 16-509	0.53 0.49	Additive Synergy
13	8	2038	4	4-509	1	Additive
14	16	1018	4	4-509	1.5	Additive
15	16	2038	8	4-1018	0.62	Additive
17	32	2038	4	8-509	0.5	Synergy
19	16	2038	1	1-509	0.31	Synergy
20	32	2038	16	16-64	0.56	Additive
21	256	2038	4	8-254 8-509	0.15 0.28	Synergy Synergy
24	16	1018	4	16-509	1.5	Additive
25	64	2038	4	4-1018	0.56	Additive
26	32	2038	0.5	16-509	0.75	Additive
28	32	2038	4	4-1018	0.62	Additive
29	128	2038	4	4-1018 8-509 16-509 16-127 16-64	0.53 0.31 0.37 0.187 0.031	Additive Synergy Synergy Synergy Synergy
30	32	2038	0.5	0.5-1018 1-1018 8-509	0.51 0.53 0.5	Additive Additive Synergy
31	256	4075	8	16-256	0.31	Synergy
32	16	2038	8	16-509	1.5	Additive
33	16	2038	0.5	8-509	0.75	Additive
35	16	2038	8	8-509	1	Additive
36	64	2038	16	16-1018	0.75	Additive
41	64	2038	1	32-64	0.53	Additive
43	16	2038	1	1-509	0.31	Synergy
A. baumannii NCTC 1342	16	4075	1	1-509	0.31	Synergy

*MER: Meropenem, **NAC: N-acetylcysteine, ***FIC: Fractional inhibitory concentration

Table 3. Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	GEN* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+GEN MIC (µg/ml)**	**GEN-NAC Combinations (µg/ml)**	**FIC** Index**	**Interaction type**
2	64	2038	4	4-1018	0.56	Additive
3	256	2038	32	256-1018	1.5	Additive
7	256	2038	16	16-1018	0.56	Additive

Table 3 (*continue*). Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	GEN* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+GEN MIC (µg/ml)**	**GEN-NAC Combinations (µg/ml)**	**FIC** Index**	**Interaction type**
8	256	2038	64	64-1018	0.75	Additive
11	256	2038	256	256-1018	1.5	Additive
13	256	2038	64	64-1018	0.75	Additive
14	256	2038	256	256-1018	1.5	Additive
15	64	2038	4	4-1018	0.56	Additive
17	64	2038	64	256-1018	1.5	Additive
19	64	2038	64	256-1018	1.5	Additive
20	256	2038	64	64-1018	0.75	Additive
21	64	2038	2	2-1018	0.53	Additive
24	256	2038	128	128-1018	1	Additive
25	256	2038	256	256-1018	1.5	Additive
26	64	2038	16	32-1018	1	Additive
28	32	2038	32	32-1018	1.5	Additive
29	64	2038	64	32-1018	1	Additive
30	64	2038	64	32-1018	1	Additive
31	256	2038	64	64-1018	0.75	Additive
32	64	2038	32	32-1018	1	Additive
33	32	2038	32	32-1018	1.5	Additive
35	16	2038	8	256-1018	1.5	Additive
36	64	2038	16	256-1018	1.5	Additive
41	64	2038	64	32-1018	1	Additive
43	32	2038	16	16-1018	1	Additive
A. baumannii NCTC 1342	64	2038	64	4-1018	0.56	Additive

*GEN: Gentamicin, **NAC: N-acetylcysteine, ***FIC: Fractional inhibitory concentration

Table 4. Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	CIP* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+CIP MIC (µg/ml)**	**CIP-NAC Combinations (µg/ml)**	**FIC** Index**	**Interaction type**
2	64	2038	32	32-1018	1	Additive
3	128	2038	64	64-1018	1	Additive
7	128	2038	32	32-1018	0.75	Additive
8	128	2038	32	32-1018	0.75	Additive
11	256	2038	128	128-1018	1	Additive
13	256	2038	256	128-1018	1	Additive
14	256	2038	256	256-1018	1.5	Additive
15	128	2038	64	64-1018	1	Additive
17	64	2038	64	32-1018	1	Additive
19	64	2038	64	32-1018	1	Additive
20	256	2038	128	128-1018	1	Additive
21	64	2038	64	32-1018	1	Additive
24	128	2038	128	128-1018	1.5	Additive
25	256	2038	256	256-1018	1.5	Additive
26	64	2038	32	32-1018	1	Additive
28	32	2038	32	32-1018	1.5	Additive

Table 4 (*continue*). Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	CIP* MIC (µg/ml)	NAC** MIC (µg/ml)	NAC+CIP MIC (µg/ml)	CIP-NAC Combinations (µg/ml)	FIC** Index	Interaction type
29	64	2038	64	32-1018	1	Additive
30	64	2038	64	32-1018	1	Additive
31	256	2038	128	128-1018	1	Additive
32	64	2038	4	4-1018	0.56	Additive
33	256	2038	256	256-1018	1.5	Additive
35	128	2038	64	64-1018	1	Additive
36	64	2038	64	64-1018	1.5	Additive
41	64	2038	32	32-1018	1	Additive
43	128	2038	16	16-1018	0.62	Additive
A. baumannii NCTC 1342	64	2038	32	32-1018	1	Additive

*CIP: Ciprofloxacin, **NAC: N-acetylcysteine, ***FIC: Fractional inhibitory concentration

While a synergistic effect was detected between NAC and MER in 9 isolates, an additive effect was observed in other isolates. When GEN-NAC and CIP-NAC combinations were examined, additive effects were observed in all isolates.

Studies conducted in recent years focus on synthesizing new antimicrobial compounds while addressing the problem of resistance. However, in addition to synthesizing new antimicrobial compounds, research on inhibiting resistance is also gaining importance. Combined use of inhibitory compounds and antibiotics can be considered as an alternative treatment method. Based on this idea, in our study, which aimed to determine the effect of NAC on the MIC value of antibiotics and to calculate the concentration of CIP + NAC, MER + NAC and GEN + NAC, which eliminates antibiotic resistance, it was shown that the combination of MER + NAC increased the sensitivity of MER.

De angelis et al. [23] studied the combination of NAC with various antibiotics against a total of 30 isolates, 15 of which are carbapenem-resistant *K. pneumoniae* and *15 A. baumannii* isolates. It was shown that the MIC values of the antibiotics alone decreased with the addition of NAC. The combination of meropenem+NAC was noted to show synergism in all strains tested. However, the combination of Rifampin+ NAC was shown to be synergistic in 3/15 (20%) strains. On the other hand, it was stated that no synergism was found in the combinations of Tigecycline+NAC and Colistin+NAC. In our study, synergy was observed between MER-NAC combinations in 9 of 25 isolates.

Pollini et al. [24], synergistic activity of Colistin+NAC combinations was investigated against 16 *A. baumannii isolates*, 9 of which were colistin-sensitive (MIC range 0.5-1 mg/l) and 7 colistin-resistant (MIC range 16-256 mg/l) isolates. In the study combinations of 8000 mg/l NAC and 2 mg/l colistin showed synergy in colistin-resistant strains, while no synergism was observed in colistin-susceptible strains. In addition, synergistic activity of 8 mg/l colistin and 8000 mg/l NAC combination has been demonstrated in all strains (colistin-resistant and colistin-sensitive).

Goswami et al. determinated [25]; The combination of NAC with ampicillin against various bacteria has been investigated. In most isolates, the combination of ampicillin + NAC has been shown to significantly decrease the MIC of ampicillin alone. In contrast, Moon et al. [26] found that the antibiotic susceptibility of *Prevotella intermedia* was not affected by NAC, and ampicillin activity was reduced in the presence of NAC.

Likewise, Rodriguez-Bertram et al. [27] investigated combinations of various antibiotics with NAC against *E. coli*, *P. aeruginosa*, and *A. baumannii*. While an additive effect was observed in the combination of NAC + antibiotics against *P. aeruginosa* isolates, less effect was noted with the combination of NAC + meropenem. Nevertheless, some isolates susceptible to imipenem have been shown to become resistant after combined use with NAC.

Landini et al. [28], investigated the activity of NAC alone and with various antibiotics against

Gram positive and Gram negative bacteria. They determined no synergistic effect with the combination of NAC and antibiotics against isolates. Besides, the effect of carbapenems on isolates decreased after being combined with NAC. This situation was observed more in imipenem than in meropenem.

Çetinkaya et al. [29] detected a four-fold or more reduction in CIP MIC values in the presence of 25 mg/l PAβN in 15.5% of 58 *A. baumannii* isolates. Kaynak Onurdağ et al. [30] also reported a four-fold or more reduction in CIP MIC values in the presence of 25 mg/l PAβN in 43.28% of CIP-resistant *A. baumannii* isolates.

Kuyucuklu et al. [31], reported that 41 ampicillin-resistant *staphylococcus* isolates became susceptible to ampicillin in the presence of appropriate NAC concentrations for 60.98% of the isolates. Likewise, they found a 2-32-fold decrease in vancomycin MIC values in the combination of vancomycin and NAC. According to these results, they observed that the NAC molecule, which has no antimicrobial effect and therefore has a very high MIC value on its own, is an effective chemical agent against staphylococcal isolates, together with ampicillin and vancomycin and reduces the MIC values of antibiotics.

In our study, in the presence of 2038 mg/l NAC, 4 (16%) of the 25 *A. baumannii* isolates included in the study had a four-fold or greater decrease in CIP MIC values. When using NAC as an antimucolytic in adults, the serum concentration dose is 400-600 mg per day. However, 140 mg/kg is used as a loading dose in paracetamol poisoning and the toxic dose is quite high. In our study, the NAC concentration in all antibiotic-NAC combinations was between 2038-64 mg/l, well below the toxic dose [32].

Similar to this study, it was reported in different studies that the combined effect of NAC with different antibiotics (rifampicin, tigecycline, ciprofloxacin) against bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *S. aureus*) caused a decrease in the MIC values of antibiotics [33].

Soudeiha *et al*. [34] in 2017 showed that there was only additive effect and there was no synergism when evaluating combination of colistin and meropenem in *Acinetobacter baumanni.*

It was determined that NAC molecule showed the predicted effect on MIC values of antibiotics when used together with antibiotics. It is thought that the use of NAC molecule together with antibiotics will facilitate the treatment of infections caused by microorganisms that are especially difficult to treat and are resistant to antibiotics. For this reason, based on the observed effect of the combination of MER + NAC in Acinetobacter infections, it can be concluded that NAC may serve as a new alternative for combined drug therapy.

In summary, it was determined that i) NAC molecule, when used together with meropenem, ciprofloxacin, gentamicin, decreased the MIC values of these antibiotics affecting Acinetobacteria, and ii) NAC molecule had a synergistic effect when used together with meropenem. Based on the results obtained, the potential of using NAC in combination with other microorganisms and antimicrobial agents for novel treatment approaches is considered promising.

Some mechanisms have been proposed to understand the antimicrobial activity of NAC. These are: competitive inhibition of cysteine utilization, reaction of the NAC sulfhydryl group with bacterial proteins, indirect effects of disruption of intracellular redox balance on cell metabolism and intracellular signal transduction pathways [18]. In our previous study, it was stated quinolone resistance determined in 73 *A. baumannii* isolates are related to that mutations in the *gyrA, gyrB, parC* genes [35]. Also in one of our studies which studied with the same clinical isolates; it has been found that overexpression of adeA, adeB and adeC genes which are related to efflux pump resistance and it was shown that inhibiting the resistance by an efflux pump inhibitor reduces the MIC values of ciprofloxacin [36]. There are also other studies conducted with different antibiotics and inhibitors in which antimicrobial resistance is associated with the expression levels in *adeABC* genes [37].

It is thought that the mechanism that effects resistance inhibition determined in this study may be due to these previous mechanisms. For this reason we think that this study should be improved with the previous molecular studies including NAC.

AUTHOR CONTRIBUTIONS

Concept: A.S.S.; Design: A.S.S.; Control: A.S.S., S.Ö.; Sources: A.S.S.; Materials: A.S.S., S.Ö.;

Data Collection and/or Processing: A.S.S.; Analysis and/or Interpretation: A.S.S.; Literature Review: A.S.S.; Manuscript Writing: A.S.S.; Critical Review: A.S.S., S.Ö.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

Approval was received from T.R. Trakya University Faculty of Medicine Scientific Research Ethics Committee on 29.04.2015 with the approval number TÜTF-BAEK-2015/55-08/10.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DETERMINATION OF BIOLOGICAL ACTIVITIES OF *MICROMERIA MYRTIFOLIA* BOISS. & HOHEN

MICROMERIA MYRTIFOLIA BOISS. & HOHEN'İN BİYOLOJİK AKTİVİTELERİNİN BELİRLENMESİ

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ABSTRACT

Objective: *Lamiaceae family has a wide variety of well-known and lesser-known plants with strong medicinal qualities. The genus Micromeria Benth. is a member of this family consisting of herbaceous plants with a variety of significant biological, phytochemical, and ethnobotanical uses. In this study, the biological activities of methanol and ethanol extracts of Micromeria myrtifolia were evaluated.*
Material and Method: *To demonstrate the antioxidant activity DPPH radical scavenging activity and total phenolic content assays were done. The effects of the extracts on acetylcholinesterase (AChE) and monoamine oxidase-A were then assessed.*
Result and Discussion: *Methanol extract showed the highest DPPH scavenging activity, at the dose of 10 mg/ml with a value of 96.55%. For the highest concentration that can be applicable, AChE inhibitions for the methanol and ethanol extracts were 25% and 27%, respectively. On the other hand, the inhibitory effects of the ethanol and methanol extracts of the plant on MAO-A were determined; for the ethanol extract IC_{50} value was found as 32.5876 ± 0.89 µg/ml, and for the methanol extract it was found as 34.6544 ± 0.76 µg/ml. It can be told that M. myrtifolia can act as a potential antioxidant. With further research and investigation, it is thought that Micromeria myrtifolia could be used as a natural source for the treatment of various neurological diseases.*
Keywords: *Acetylcholinesterase, antioxidant activity, Micromeria myrtifolia, monoamine oxidase-A, neurodegenerative diseases*

ÖZ

Amaç: *Lamiaceae familyası, güçlü tıbbi niteliklere sahip bilinen veya az bilinen değişik bitkileri içeren bir bitki ailesidir. Micromeria Benth cinsi bu familyaya ait, çok çeşitli biyolojik, fitokimyasal ve etnobotanik kullanımı olan, çoğunlukla otsu bitkilerden oluşan cinslerden biridir. Bu çalışmada, Micromeria myrtifolia'nın metanol ve etanol ekstreleri, bitkinin biyolojik aktiviteleri gösterilmek üzere seçilmiştir*
Gereç ve Yöntem: *Antioksidan aktiviteyi göstermek için DPPH radikal süpürücü aktivitesi ve total fenolik içerik deneyleri yapılmıştır. Daha sonra ekstrelerin asetilkolinesteraz (AChE) ve monoamin oksidaz-A (MAO-A) üzerindeki etkileri değerlendirilmiştir.*
Sonuç ve Tartışma: *Çalışma sonunda metanol ekstraktı, 10 mg/ml dozunda % 96.55 değeriyle en yüksek DPPH radikal süpürücü aktivitesi göstermiştir. Uygulanabilecek en yüksek*

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konsantrasyonlarda metanol ve etanol ekstreleri için AChE inhibisyonları sırasıyla %25 ve %27 olarak saptanmıştır. Ayrıca bitkinin MAO-A üzerine etkisi de tespit edilmiştir. Buna göre etanol ekstresi için IC_{50} değeri 32.5876 ± 0.89 µg/ml ve metanol ekstresi için ise 34.6544 ± 0.76 µg/ml olarak bulunmuştur. Elde edilen sonuçlara göre M. myrtifolia'nın potansiyel bir antioksidan etkisi gösterebileceği söylenebilir. Daha ileri çalışmalar ile Micromeria myrtifolia'nın çeşitli nörolojik hastalıkların tedavisi için doğal bir kaynak olarak kullanılabileceği düşünülmektedir.
Anahtar Kelimeler: *Antioksidan aktivite, asetilkolinesteraz, Micromeria myrtifolia, monoamin oksidaz-A, nörodejeneratif hastalıklar*

INTRODUCTION

The pathogenetic pathways of many illnesses, including neurological disorders are linked to oxidative stress [1,2]. Besides, acetylcholinesterase (AChE) and monoamine oxidases (MAOs) are very important enzymes for their significance in the treatment of these diseases. So, their activities and inhibitions play crucial roles in treating some neurologic conditions [3,4].

Since ancient times plants have been used for the therapy of different illnesses. Today, practically all pharmacopoeias in the world recommend plant-derived medicines with actual therapeutic benefits. There are also some regions with unique herbal pharmacopoeias [5].

Lamiaceae family has strong biological effects with secondary metabolites whose structure has been elucidated and secondary metabolites that have not yet been elucidated [6]. *Micromeria* Benth., a genus of this family, mostly consists of herbaceous plants with a variety of significant biological, phytochemical, and ethnobotanical uses. *Micromeria* genus is represented by eighty-nine species in the world and nine species in Türkiye. There are seventeen taxa in Türkiye and five of them are endemic [7,8]. *Micromeria myrtifolia* Boiss. & Hohen. (Ayaklı kekik, Boğumluçay) used for flu, gallstones, gastrointestinal disorders, pleasure, relaxing, stomachache, and throat disease [9-12].

Studies on the phytochemistry of *Micromeria* species have revealed the presence of several flavonoid chemicals, saponins, tannins, anthraquinones, and essential oils [13-15]. Numerous investigations have established that certain *Micromeria* species have biological properties that include antifungal, antimicrobial, antioxidant, anticholinesterase, anti-inflammatory, gastroprotective, hepatoprotective, and cytotoxic effects [6,16-19]. According to Formisano et al., the methanol extracts of *M. myrtifolia* were found to be substantially more active antioxidants than chloroform and hexane extracts, when tested using DPPH radical scavenging and ferric ion reduction (FRAP) assays [20].

Here in this study, some of the biological functions of *Micromeria myrtifolia* were examined. Our goal was to demonstrate the DPPH activity of the extracts of the plant and the relationship between that activity and the phenolic content of the plant. AChE and MAO-A are two crucial enzymes that have drawn a lot of interest from researchers due to their significance in the therapy of neurodegenerative illnesses. We also tried to find out how the extracts of *Micromeria myrtifolia* influenced these two enzymes.

MATERIAL AND METHOD

Plant Materials

In June 2021, *M. myrtifolia* was collected from Gemlik, Bursa, Türkiye. Assistant Professor Dr. Ebru Ozdemir Nath observed and identified the plant sample. Herbarium of Altınbaş University Faculty of Pharmacy (HERA) has received a specimen of the plant with the herbarium number HERA255.

Preparation of the Extract

The plant parts were ground into a fine powder using a mechanic's grinder, air dried at room temperature in a dark, shaded environment, and weighed with a digital balance. *M. myrtifolia* powder was macerated with a ratio of 1 part of plant (25 g) soaked in 10 parts of solvent (250 ml), by using 96% ethanol and methanol solvents, in a tightly closed container for 24 hours, and protected from light, while stirring frequently. Under a rotary evaporator (Heidolph Hei-VAP Advantage rotary evaporator), the solvent evaporated to dryness. *M. myrtifolia* plant extract was kept at +4ºC until biological activity

research.

DPPH Radical Scavenging Activity

Brand-Williams method was used to determine the DPPH activity of the extracts of *M. myrtifolia* [21]. 10 µl of ethanol/methanol extracts of the plant sample in different concentrations (0.5-10 mg/ml) were combined with 240 ml DPPH radical and incubated in the dark at room temperature for 10 minutes. By measuring the absorbance at 517 nm, the decrease of the DPPH radical was quantified. Quercetin was used as the standard in this activity test and the result is given in Table 1.

Table 1. DPPH radical scavenging activity of quercetin

Concentration (mg/ml)	DPPH %
0.250	86.853
0.125	75.148
0.062	49.864
0.031	30.740
0.015	21.124
0.008	9.850
0.004	4.736

The formula below was used to determine the radical scavenging activity (Inh %) as a percentage of DPPH discoloration.

$$\text{Inh \%} = [1- (Abs_{extract} / Abs_{DPPH})] \times 100$$

Total Phenolic Content

The total phenolic contents of the extracts of *M. myrtifolia* were found using a modified Folin Ciocalteu technique [22]. The reduction that resulted in a blue color was observed at 760 nm. Various concentrations (0.5-10 mg/ml) of the ethanol/methanol extracts of the plant samples were combined with distilled water and Folin Ciocalteu reagent, respectively. The combination underwent a room-temperature incubation. 15 µl of 2% Na_2CO_3 were added after 3 minutes. After 2 hours of incubation at room temperature in the dark, the absorbance at 760 nm was measured using a multimode microplate reader, the BioTek Synergy H1 (Agilent). Gallic acid was used as the reference solution.

Acetylcholinesterase Activity

The acetylcholinesterase activities of the extracts of *M. myrtifolia* were measured with the AChE activity of *Electrophorus electricus* (electric eels) spectrophotometrically using the Ellman Method [23]. The substrate used in the study was acetylthiocholine iodide (ATC). The reactions were carried out at 25°C, in 100 mM Tris HCl (pH 8.0) buffer. To start each enzymatic reaction approximately 0.05 U/ml AChE was added to the reaction mixture. The breakdown of acetylthiocholine was detected with a UV spectrophotometer (Carry 60 Single Beam spectrophotometer, Agilent Technologies, USA) over an elevation in absorbance at 412 nm.

Monoamine Oxidase-A (MAO-A) Activity

Spectrofluorometric analysis and the previously described protocols were used to determine the effects of the *M. myrtifolia* extracts on MAO-A activity [24,25]. Each reaction was carried out in a 100 mM potassium phosphate buffer at a pH of 7.4. Kynuramine was used as the substrate of MAO-A. The substrate, buffer, and extracts had a 10-minute preincubation at 37°C. MAO-A was added to the assay mixtures to start the reactions, and the processes were then maintained at 37°C for an additional 20 minutes. 1000 µl of distilled water was added after the reactions were stopped by NaOH (2N). A multimode microplate reader was used to measure the quantity of fluorescence at 310 nm for excitation and 400 nm for emission. The related IC_{50} values were used to express the extracts' inhibitory potencies.

To compare the IC_{50} values of the plant clorgyline was used.

RESULT AND DISCUSSION

The DPPH radical scavenging activity and the total phenolic content of different concentrations of these extracts were determined. The results are summarized in Table 2. The antioxidant activity of methanol extracts was higher than that of ethanol extract. Methanol extract showed the highest DPPH scavenging activity, at the dose of 10 mg/ml with a percentage value of 96.55%. In this concentration, quercetin equivalent was obtained as 0.4392 mg. As the amount of tested dose increases, the DPPH activity increases for both extracts. DPPH percentage of the 0.5 mg/ml of the methanol extract was found to be close to the IC_{50} value (46.0967%). Similar to antioxidant activity assays, the highest TPC was found in the methanol extracts at the dose of 10 mg/ml with gallic acid equivalent 0.8629 mg/ml, and the gallic acid the equivalent phenolic content of methanol extract was higher at all doses. Furthermore, it was found that, as the TPC was getting higher, the DPPH activity test was found to be higher. All these findings suggest that *Micromeria myrtifolia* methanol extracts have high antioxidant content, even in low doses, and have a high potential in terms of antioxidant product development.

Table 2. DPPH radical scavenging activity and TFC of the methanol and ethanol extracts of *Micromeria myrtifolia*

Extract	Concentration (mg/ml)	DPPH Quercetin Equivalent (mg/ml)	DPPH, %	TFC, Gallic acid Equivalent (mg/ml)
Ethanol Extracts	10	0.2603±0.024	83.1417±1.795	0.5836±0.040
	5	0.1059±0.001	64.1762±0.010	0.3214±0.018
	1	0.0333±0.001	39.4157±0.707	0.0789±0.002
	0.5	0.0162±0.001	23.8984±1.764	0.0368±0.003
Methanol Extracts	10	0.4392±0.003	96.5517±2.441	0.8629±0.045
	5	0.2540±0.012	83.0459±1.005	0.6106±0.012
	1	0.0586±0.038	59.6024±1.221	0.1860±0.009
	0.5	0.0456±0.004	46.0967±2.083	0.0901±0.002

For the inhibitory effects of *Micromeria myrtifolia*'s ethanol and methanol extracts on AChE, a wide range of concentrations were screened. The highest (final) concentration that could be tested was 1.5 mg/ml for each extract. The inhibition percentages for the methanol and ethanol extracts were 25% and 27%, respectively. At higher concentrations, transparency and measurability in the cuvette were greatly reduced, preventing tests from being performed with higher concentrations.

On the other hand, the inhibitory effects of the ethanol and methanol extracts of the plant on MAO-A were determined and the IC_{50} values were calculated. For the ethanol extract the IC_{50} value was found as 32.5876 ± 0.89 μg/ml and for the methanol extract it was found as 34.6544 ± 0.76 μg/ml which is a very close result to the former. We have identified the IC_{50} value for the well-known inhibitor "clorgyline" as 3.128 nM $\pm$ 0.023. On the other hand, the IC_{50} values of another selective inhibitor of MAO-A "moclobemide" is reported as 6.1 μM [26]. According to these results, it can be assumed that the plant has a moderate inhibitory effect on MAO-A activity.

There are various studies on the antioxidants, anti-tyrosinase, anti-amylase, and antidepressant activities of *Micromeria* species, but very limited of them have been done with *M. myrtifolia* species [27,28]. In the study that have been conducted by *M. myrtifolia* collected from Lebanon the DPPH test revealed that the methanol extracts exhibited higher antioxidant activity compared to the extracts than that of the chloroform and hexane extracts. Finally, *Micromeria myrtifolia* collected from Antalya was studied for its antidepressant activity. It was determined that the methanol extract of the plant was shown to have antidepressant activity both in vivo and in vitro [29].

In this work, we have identified some of the biological activities of *M. myrtifolia*'s ethanol and

methanol extracts. In the study, we found that methanol extract showed high activity in all doses administered by DPPH. If we compare the findings we obtain with the ones in the literature, the results we found for the antioxidant tests were consistent with the results obtained from different species of the same genus. They were also parallel to that of the same species collected from different locations. So, it can be accepted that our plant *M. myrtifolia* can act as an antioxidant.

On the other hand, our research is the first to examine the inhibitory effects of the methanol and ethanol extracts of *M. myrtifolia* on AChE and the first to address the inhibitory effects of the ethanol extracts of *M. myrtifolia* on MAO-A. The results are beneficial and encouraging. With further research and investigation, it is thought that *M. myrtifolia* could be used as a natural source for the treatment of various neurological diseases.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PHYTOCHEMICAL PROFILING, ANTIOXIDANT, ANTIDIABETIC, AND ADMET STUDY OF *DIOSPYROS MESPILIFORMIS* HOCHST. EX A. DC. (EBENACEAE) LEAF

DIOSPYROS MESPILIFORMIS HOCHST. EX A. DC. (EBENACEAE) YAPRAKLARININ FİTOKİMYASAL PROFİLLENDİRMESİ, ANTİOKSİDAN, ANTİDİYABETİK VE ADMET ÇALIŞMASI

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ABSTRACT

Objective: *This study aimed to carry out phytochemical profiling, antioxidant, antidiabetic, and ADMET study on the crude ethanol extract (CR) of Diospyros mespiliformis (DM) and its ethyl acetate (EEF) and aqueous fractions (AQF).*
Material and Method: *The phytochemicals were identified by GC-MS. The antioxidant activity was determined in vitro and silico while the antidiabetic and ADMET were in silico.*
Result and Discussion: *Exactly 54 and 44 compounds were respectively identified in the EEF and AQF. At 300 µg/ml, the CR demonstrated a significantly ($p < 0.05$) higher ascorbic acid equivalent (AAE) total antioxidant capacity (TAC) (73.59 ± 0.011 µg/ml) than the EEF (41.28 ± 0.003 µg/ml AAE) and AQF (31.28 ± 0.005 µg/ml AAE). The total reducing power (TRP) of the AQF (106.84 ± 3.46 µg/ml) was significantly ($p <0.05$) higher than the CR (93.23 ± 5.63 µg/ml AAE) and EEF (92.35 ± 6.96 µg/ml AAE) at 100 µg/ml. A significantly ($p < 0.05$) higher percentage inhibition (48.38% ± 4.61) was demonstrated by the EEF at 1 mg/ml in the ferric thiocyanate and a lower malonaldehyde concentration (0.75 ± 0.01 nmol/ml) in the thiobarbituric acid methods. The AQF demonstrated a significantly ($p < 0.05$) higher (82.72% ± 1.88) peroxide scavenging activity at 100 µg/ml than the CR (33.33% ± 2.16) and EEF (63.64% ± 2.66). Compound VII exhibited the lowest binding affinity (BA) and inhibition constant (Ki) of -8.8 kcal/mol and 0.35 µM, respectively with xanthine oxidase and -8.0 kcal/mol and 1.35 µM, respectively with NADH oxidase. X exhibited the lowest BA (-8.5 kcal/mol) and Ki (0.58 µM) interacting with CytP450 21A2. Compound III exhibited the lowest BA (-7.5 kcal/mol) and Ki (3.14 µM) with PTP1B while compound X had BA and Ki values of -8.5 kcal/mol and 0.58 µM, respectively with PPARγ. The result of ADMET showed some of the compounds might be strong candidates for antioxidant and antidiabetic drugs. All the extracts possess significant antioxidant activity and some of the identified compounds might be candidates for novel antioxidants and antidiabetic drugs.*
Keywords: *ADMET, antidiabetic, antioxidant, molecular docking, molecular dynamics*

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ÖZ

Amaç: *Bu çalışmada Diospyros mespiliformis (DM) ham etanol ekstraktı (CR), etil asetat (EEF) ve sulu fraksiyonları (AQF) üzerinde fitokimyasal profilleme, antioksidan, antidiyabetik ve ADMET çalışmasının yapılması amaçlandı.*

Gereç ve Yöntem: *Fitokimyasallar GC-MS ile tanımlandı. Antioksidan aktivite in vitro ve silico olarak belirlenirken, antidiyabetik ve ADMET in silico olarak belirlendi.*

Sonuç ve Tartışma: *EEF ve AQF'de sırasıyla tam olarak 54 ve 44 bileşik tanımlandı. 300 µg/ml'de CR (73.59 ± 0.011 µg/ml), EEF (41.28 ± 0.003 µg/ml AAE) ve AQF (31.28 ± 0.005 µg/ml AAE)'den önemli ölçüde ($p<0.05$) daha yüksek askorbik asit eşdeğeri (AAE) toplam antioksidan kapasitesi (TAC) gösterdi. 100 µg/ml'de AQF'nin (106.84 ± 3.46 µg/ml) toplam indirgeme gücü (TRP), CR'den (93.23 ± 5.63 µg/ml AAE) ve EEF'den (92.35 ± 6.96 µg/ml AAE) önemli ölçüde ($p <0.05$) daha yüksekti. 1 mg/ml EEF, ferrik tiyosiyanat yönteminde önemli ölçüde ($p<0.05$) daha yüksek bir inhibisyon yüzdesi (%48.38 ±4.61) ve tiyobarbitürik asit yönteminde daha düşük bir malonaldehid konsantrasyonu (0.75 nmol/ml ±0.01) gösterdi. AQF, 100 µg/ml'de CR (%33.33 ± 2.16) ve EEF'den (%63.64 ± 2.66) önemli ölçüde ($p < 0.05$) daha yüksek (%82.72 ± 1.88) peroksit temizleme aktivitesi gösterdi. Bileşik VII, ksantin oksidaz ile sırasıyla -8.8 kcal/mol ve 0.35 µM ve NADH oksidaz ile sırasıyla -8.0 kcal/mol ve 1.35 µM ile en düşük bağlanma afinitesini (BA) ve inhibisyon sabitini (Ki) sergiledi. X, CytP450 21A2 ile etkileşime giren en düşük BA (-8.5 kcal/mol) ve Ki'yi (0.58 uM) sergiledi. Bileşik III, PTP1B ile en düşük BA (-7.5 kcal/mol) ve Ki'yi (3.14 µM) sergilerken; bileşik X, PPARy ile sırasıyla -8.5 kcal/mol ve 0.58 µM BA ve Ki değerlerine sahipti. ADMET sonucu, bazı bileşiklerin antioksidan ve antidiyabetik ilaçlar için güçlü adaylar olabileceğini gösterdi. Tüm ekstraktlar önemli antioksidan aktiviteye sahiptir ve tanımlanan bileşiklerin bazıları yeni antioksidanlar ve antidiyabetik ilaçlar için aday olabilir.*

Anahtar Kelimeler: *ADMET, antidiyabetik, antioksidan, moleküler yerleştirme, moleküler dinamik*

INTRODUCTION

Diabetes entails endocrine metabolic disorders characterized by a loss of glycemic control leading to the development of acute and subsequent chronic complications, including macro- and micro-vascular complications [1,2]. Various strategies are employed in the management of diabetes including synthetic drugs to achieve glycemic control. However, proper diet and exercise are the usual recommendations. Synthetic drugs employed in the management of diabetes are often reported to possess side effects, thus undesirable for prolonged use [3]. Furthermore, the specificity of these drugs towards their target and the nature of diabetes as a metabolic disorder makes a single therapy a challenge for some individuals. The need for combined therapy leads to more side effects [3]. Additionally, the cost of diabetic therapy is often expensive for some individuals, especially in low-income countries [4,5]. Therefore, the need for an affordable, safe, and efficient alternative.

Drugs from plant sources are often considered as alternate sources especially for low-income countries and rural communities due to their efficacy and low cost with minimized side effects. The use of plant-based drugs in diabetes is due to their phytoconstituents associated with various individual and synergistic modes of action [6]. In some cases, these phytoconstituents are isolated and studied for their antidiabetic activities while in others as crude extracts [6]. Identification of these compounds has gained attention in recent times as these compounds are sources of novel drugs for the management of ailments [7]. Different studies previously reported the application of different plants and compounds of plant origin with antidiabetic activities. These studies often involve animal models and in other cases *in vitro* studies to justify their use in folkloric medicine [8-13]. Furthermore, in traditional practice, plants of different types including *D. mespiliformis* are formulated in various preparations, including decoction and maceration to extract these compounds for medicinal applications.

D. mespiliformis is a plant native to West Africa and often called West African ebony or African ebony. It is an evergreen tree growing up to 25 meters in drylands, often used as a source of food because of its edible fruit and for medicinal purposes [14]. In folkloric medicine, the roots and bark of the plant are employed in the treatment of infections, toothaches, fever, wound healing, malaria, leprosy, and headache [15]. Furthermore, previous studies reported that the plant exerts pharmacological activities, including hypoglycemic, antioxidant, neuropharmacological, analgesic, antiproliferative, and

antimicrobial properties [16]. However, the determination of the activity and identification of the constituent compounds of the different fractions of the plant present gaps in knowledge. This includes the interactions of these constituents with the proteins involved in the pathology of oxidative stress and diabetes. Additionally, the ADMET properties of the compounds also present unexplored gaps. Thus, in our study, we investigated the phytochemical profile, antioxidant, antidiabetic, and ADMET properties of the crude ethanol extract (CR) of *Diospyros mespiliformis* and its ethyl acetate (EEF) and aqueous fractions (AQF). Additionally, we investigated the ADMET properties of the identified compounds and their interaction with different antioxidant and antidiabetic targets.

MATERIAL AND METHOD

Sample Collection and Preparation

Fresh leaves sample of *D. mespiliformis* (DM) was obtained from Mayo-belwa local government of Adamawa State. The plant was authenticated by a Forest technologist with a voucher specimen (ASP/FT/078) deposited in the Department of Forestry Technology. The plant was air-dried and ground to powder then 7 days of maceration of 1 kg of the powder in 70% (v/v) ethanol. This was followed by filtering and concentrating the filtrate to dryness at 40ºC with a rotary evaporator (Buchi Rotavapor R-200) yielding 150 g of the crude extract (green-colored).

Extract Fractionation

Exactly 100 g of the crude extract (CR) was completely dissolved in 250 ml of distilled water and transferred to a separating funnel for partitioning with ethyl acetate until a clear ethyl acetate layer was observed. The ethyl acetate layer was collected as the ethyl acetate fraction (EEF) while the aqueous layer as the aqueous fraction (AQF). Both fractions were subjected to the same drying procedure stated for the CR yielding 28.04 g of EEF (reddish brown color) and 67.12 g of AQF (dark green color). The extract and its fractions were stored at 4°C until needed for analysis.

Phytochemical Screening

The secondary metabolites (phytochemicals), including alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids were detected by the methods previously described by Evans [17] to ascertain their presence or absence.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

A combined GC-MS (Agilent 19091-433HP, USA) fused with a silica column was used to identify the compounds present in DM. The procedures and instrumentation settings were as we described previously [18].

Antioxidant Assay

Total Antioxidant Capacity (TAC)

The TAC of the samples was determined according to the protocol described by Prieto *et al.*, [19]. Briefly, 0.5 ml of the sample (300 µg/ml) was incubated with the phosphor-molybdate reagent while the same volume of varying concentrations (20-100 µg/ml) of ascorbic acid [AA (standard)] was used to obtain the standard calibration curve of concentration versus absorbance. The blank was made up of the phosphor-molybdate reagent and distilled water incubated in the same condition as the sample and AA. The TAC of the samples was expressed as AA equivalent (AAE) in µg/ml. All values were determined in triplicates.

Total Reducing Power (TRP)

The protocol described by Oyaizu [20] was employed to determine the TRP of samples. Briefly, 0.25 ml of the sample (100 µg/ml) dissolved in distilled water was used while the same volume of varying concentrations of AA (20-100 µg/ml) incubated similarly to the samples was used to obtain the AA calibration curve. The blank was a mixture of the reagents without samples. The TRP was expressed

as AAE in µg/ml. All values were determined in triplicates.

Ferric Thiocyanate Method (FTC)

The procedure described previously by Kikuzaki and Nakatani [21] was employed to determine the lipid peroxidation inhibitory potential of the samples. Briefly, 4 ml of the sample (1 mg/ml) dissolved in absolute ethanol was used whereas the same volume and concentration of AA dissolved in absolute ethanol was used as a standard. A mixture without the sample was used as blank. The percentage inhibition was determined according to Equation 1 [22]. All values were determined in triplicates.

$$\% \text{ Inhibition} = 100 - \frac{\text{At}}{\text{Ac}}\ x\ 100 \qquad \text{Equation 1}$$

Where At = Absorbance of sample while Ac = Absorbance of control

Thiobarbituric Acid Method (TBA)

The protocol described by Kikuzaki and Nakatani [21] was adopted to further determine the anti-lipid peroxidation effects of the samples. Briefly, 1 ml of 1 mg/ml of the sample and ascorbic acid (AA) solutions from the FTC method were used with AA regarded as a standard while the mixture without sample was blank. The malonaldehyde (MDA) concentration was determined by equation 2 using the extinction coefficient 156 mM^{-1} cm^{-1} as previously described [23]. All values were determined in triplicates.

$$\text{MDA concentration} = \frac{\text{OD}}{\text{EC}}\ x\ \text{Sample volume} \qquad \text{Equation 2}$$

Where OD = Absorbance of the sample while EC = Extinction coefficient

Hydrogen Peroxide (H_2O_2) Scavenging Assay

The procedure described by Zang [24] was followed to determine the H_2O_2 scavenging potential of the samples. Briefly, 2 ml of varying concentrations (20-100 µg/ml) of the sample and AA was used with AA regarded as a standard while the reaction mixture without the sample was used as a blank for the titration. All values were determined in triplicates.

***In silico* Study**

Drug-likeness

The identified compounds were further screened for drug-like properties and subjected to molecular docking and molecular dynamics studies. The drug-likeness screening was carried out using the DruLiTo software by applying Lipinski's rule, Veber rule, and Ghose Filter. Only compounds that passed Lipinski's rule and either Veber or Ghose filters were further subjected to the molecular docking and molecular dynamics study.

Molecular Docking (MD) and Molecular Dynamics Simulation (MDS)

The compounds were downloaded from the PubChem website in SDF formats and energy-minimized by PyRx software (PyRx- Python Prescription 0.8) for molecular docking. The docking pockets were identified using the PrankWeb: Ligand Binding Site Prediction online server [24]. The target proteins were downloaded from the RSCB protein data bank website and prepared using the AutoDockTools software version 1.5.7 [25] by removing identical chains, heteroatoms, and water molecules. The PyRx software was used for the molecular docking where only the top 10 compounds with the lowest binding energy (BA) were selected. The 2D and 3D interactions of the docked complexes were depicted using the LigPlot$^+$ (version 2.2.8.) [27] and PyMol (Version 2.0 Schrödinger, LLC) softwares, respectively while the salt bridges were identified using the Protein-Ligand Interaction Profiler online server [28]. The compounds with their PubChem ID are listed in Table 1 while the targets, including their PDB IDs and docking coordinates in Table 2. The molecular dynamics simulations were done with the Webnm online server [29] to determine the chain and residue displacements of the docked complexes with the lowest BA identified above. The inhibition constant was determined from the BA

by the equation; Ki=exp ΔG/RT, where T=298.15 K (temperature), R=1.985 x 10^{-3} $kcal^{-1}$ mol^{-1} k^{-1} (the universal gas constant), and ΔG=binding affinity [30].

Table 1. List of the compounds screened for drug-likeness

Names of the Compounds	Designation	PubChem ID
(2,2,6-trimethylbicyclo [4.1.0] hept-1-yl) methanol	I	535115
1-(4-Bromobutyl)-2-piperidinone	II	536377
4a-methyl-7-(propan-2-yl) octahydronaphthalen-2(1H)-one	III	41133
2,3-Dimethylfluorobenzene	IV	96489
2,7-Octanedione	V	74196
3-(Acetyloxy)-2-isopropyl-2-methyl butyl acetate	VI	538234
3-(azepan-1-yl)-1,2-benzothiazole 1,1-dioxide	VII	535203
4-Phenylpiperidine	VIII	69873
Butyraldehyde, semicarbazone	IX	9601714
Caryophyllene oxide	X	1742210
cis-(Z)-alpha.-Bisabolene epoxide	XI	91753574
Cubedol	XII	11276107
Ethyl 4-isopropenyl-6-methyl-2-oxo-6-heptenoate	XIII	543224
Farnesol	XIV	445070
Isometheptene	XV	22297
Lilac alcohol A	XVI	526973

Table 2. List of docking targets for the MD

Activity	Target	PDB ID	Docking coordinates		
			X	Y	Z
Antioxidant	Xanthine Oxidase (XO)	3NVZ	23.23	-16.02	35.78
	Cytochrome P450 21A2 (CytP450 21A2)	4Y8W	-14.71	12.04	28.68
	NADH Oxidase (NOX)	5ER0	-6.76	-53.62	50.91
Antidiabetic	Protein tyrosine phosphatase 1B (PTP1B)	2ZMM	46.08	15.49	3.12
	Peroxisome proliferator-activated receptor gamma (PPARγ)	4EMA	14.16	6.91	44.56

ADMET Study

The compounds with top interactions (lowest BA) identified from the MD were further screened for drug-like properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) using the pkCSM – pharmacokinetics online server [31].

Statistics

The results obtained were expressed as mean ± standard error of the mean (± SEM) and statistically evaluated by one-way analysis of variance followed by Tukey's multiple comparison tests at $p < 0.05$ level of significance. The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 22 software.

RESULT AND DISCUSSION

The phytochemical components of the CR, EEF, and AQF of the DM leaf are shown in Table 3. Alkaloids and steroids were detected in the CR and EEF only. Saponins were detected in the CR and its fractions while flavonoids were absent in the EEF.

Phytochemicals have different affinity for different solvents depending on the solvent polarity and the type of phytochemical extracted depends on the solvent employed during extraction [33-34]. Moreover, during the partitioning process, the phytochemicals with higher affinity towards a solvent will partition in that solvent [35-37]. Thus, saponins detected in all the solvents might be attributed to

their amphiphilic nature and affinity towards ethyl acetate and water. The detection of flavonoids in only the CR and AQF might be due to the presence of polar flavonoids. Moreover, the steroids detected might be hydrophobic, thus, they were not detected in the AQF. Similar results were previously reported [38,39].

Table 3. Phytochemical composition of CR, EEF, and AQF of DM leaf

Phytochemical	CR	EEF	AQF
Alkaloids	+	+	-
Saponins	+	+	+
Steroids	+	+	-
Glycosides	-	-	-
Terpenoids	-	-	-
Flavonoids	+	-	+

*(+: Present, -: Absent)

GC-MS Analysis

Table 4 shows the identified compounds in the EEF of DM. Exactly 54 compounds were identified in the EEF of DM made up of mostly long-chain aliphatic and aromatic compounds. Squalene was the most abundant (33.41%) next to Lilac alcohol A and Caryophyllene oxide with peak areas of 13.23 and 5.17 %, respectively. The least abundant compound was 8-Methylenepentadecane (0.01%). Furthermore, Figure 1 shows the structures of the compounds while the GC-MC chromatogram is shown in 3a.

Squalene identified as the most abundant compound in the EEF of DM might be attributed it its non-polar nature as ethyl acetate is a moderately polar solvent. Thus, extracting squalene in higher abundance than distilled water (AQF) [32-34]. Moreover, this compound has been associated with pharmacological actions against hypoglycemia, LDL-C, and cardiovascular diseases [40]. Therefore, it might contribute to the antidiabetic effects reported in our study. Lilac alcohol A (2-(5-Methyl-5-vinyltetrahydro-2-furanyl)-1-propanol) is another compound detected in the EEF next to squalene in abundance which might be due to its moderate solubility. Caryophyllene oxide is another moderately soluble compound identified in the EEF in high abundance. This compound has been previously reported to exert antidiabetic [41], antioxidant activities [41], and anti-obesity [42] activities. Therefore, it might also be a notable contributor to the antidiabetic effect reported in our study. Moreover, some of the compounds reported were previously associated with antidiabetic properties including, humulene [43], Caryophyllene [44], Nerolidol [45], Farnesol [46], and 2- Chloropropionic acid, octadecyl ester [47].

Table 5 presents the compounds identified in the AQF of DM. Exactly 44 compounds were identified with linoleic acid as the most abundant with a peak area of 29.86% next to palmitic acid (15.13%) and phytol (5.90%) while 2, 7-Octanedione was the least abundant (0.02%). Additionally, the structure of the identified compounds in the AQF is presented in Figure 2 while Figure 3b shows the GC-MS chromatogram.

Linoleic acid, palmitic acid, and phytol were detected in high abundance in the AQF. These compounds were not partitioned into the EEF and, thus, left in the AQF. Linoleic acid (an omega-6 fatty acid) has been previously reported to exert hypoglycemic effects in diabetic rats [48]. Additionally, some of the identified compounds in AQF were reported to exert antidiabetic effects including squalene [49].

Table 4. Compounds identified in the EEF of DM

S/N	Name	RT	Area (%)	MW
1	*N*-Nitrosopiperazine	5.12	0.02	115.13
2	8-Methylenepentadecane	5.19	0.01	224.42
3	5-Methyl-1-undecene	5.27	0.01	168.32

Table 4 (*continue*). Compounds identified in the EEF of DM

S/N	Name	RT	Area (%)	MW
4	1-Chloro-2-methylazetidine	6.82	0.03	105.56
5	4-Phenylpiperidine	6.94	0.08	161.24
6	Caryophyllene	7.26	0.83	204.35
7	Humulene	7.61	0.47	204.35
8	Santolina triene	7.72	0.08	136.23
9	2,3-Dimethylfluorobenzene	8.01	1.37	124.16
10	Cubedol	8.16	1.17	222.37
11	Nerolidol	8.48	0.46	222.37
12	Patchulane	8.67	4.29	206.37
13	4-Methylenecyclohexanemethanol	8.87	1.52	126.20
14	*(E, Z)*-alpha-Farnesene	9.12	1.70	204.35
15	Farnesol	9.27	1.10	222.37
16	Lilac alcohol A	9.61	13.23	170.25
17	Caryophyllene oxide	9.78	5.17	220.35
18	Cycloheptanone, 2-(2-methylpropylidene)-	10.09	1.13	166.26
19	Ethyl 4-isopropenyl-6-methyl-2-oxo-6-heptenoate	10.34	0.63	224.30
20	Nerolidyl propionate	10.43	0.70	278.40
21	Palmitic acid, methyl ester	10.61	1.36	270.45
22	*cis-(Z)*-alpha.-Bisabolene epoxide	10.87	0.52	220.35
23	Octadecoxy ethanol	10.95	0.68	314.55
24	Cembrane	11.15	0.20	280.53
25	1-Octacosanol	11.22	0.23	410.76
26	cis-10-Nonadecenoic acid	11.49	2.63	296.49
27	2- Chloropropionic acid, octadecyl ester	11.58	2.52	361.00
28	11-Cyclopentylheneicosane	11.73	0.95	364.69
29	Hexadecyl 2-chloropropanoate	11.93	2.51	332.95
30	17-Pentatriacontene	12.06	2.30	490.93
31	Stearyl vinyl ether	12.09	0.79	296.53
32	Eicosyl perfluorobutyrate	12.14	1.72	494.60
33	1-Bromo-11-iodoundecane	12.24	1.75	361.10
34	Docosane	12.39	2.73	310.60
35	Octadecanal	12.80	1.51	268.48
36	1,19-Eicosadiene	12.91	1.45	278.52
37	Docos-1-ene	13.16	0.96	308.58
38	*(Z, Z)* -3,13-Octadecadienyl acetate	13.19	0.31	308.50
39	Hexacos-1-ene	13.23	1.92	364.69
40	3-(azepan-1-yl)-1,2-benzothiazole 1,1-dioxide	13.53	0.64	264.35
41	Propane, 1,1,3-tricyclohexyl-	13.62	1.10	290.53
42	Octadeca-1-ene	13.87	1.20	252.48
43	Hentriacontane	14.27	0.17	436.84
44	Trichlorooctadecyl silane	15.22	0.18	387.93
45	Squalene	16.62	33.41	410.72
46	Diethyl malonic acid, monochloride, 2-acethylphenyl ester	17.24	0.41	296.74
47	Hexadecyl ether	17.91	1.22	466.87
48	Tetratriacontane	18.43	0.02	478.92
49	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	18.59	0.02	412.70
50	1,26-Dibromohexacosane	19.22	0.03	524.50
51	Octatriacontyl pentafluoropropionate	19.24	0.01	697.00
52	Octacosane	19.79	0.14	394.76
53	Hexatriacontane	19.83	0.13	506.97
54	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	19.94	0.06	414.50

RT= Retention time, MW= Molecular weight

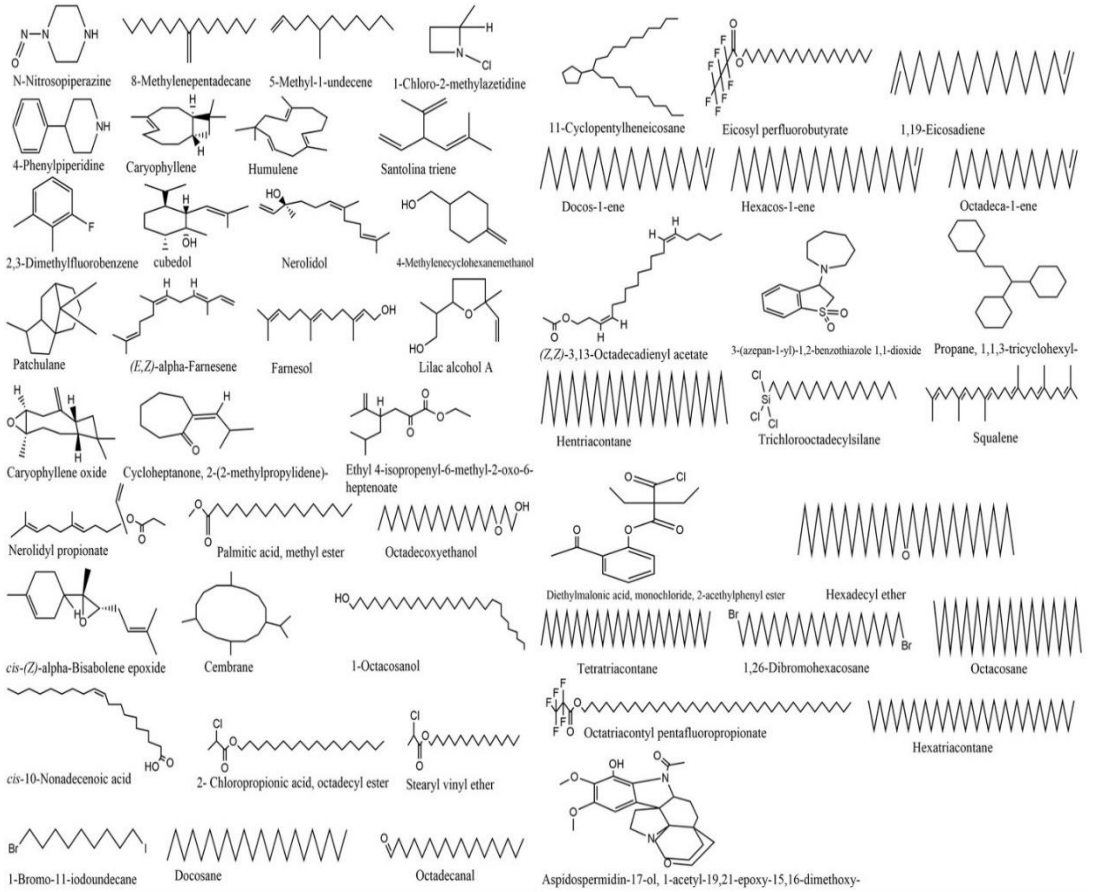

Figure 1. Structural formulas of compounds identified in the EEF of DM

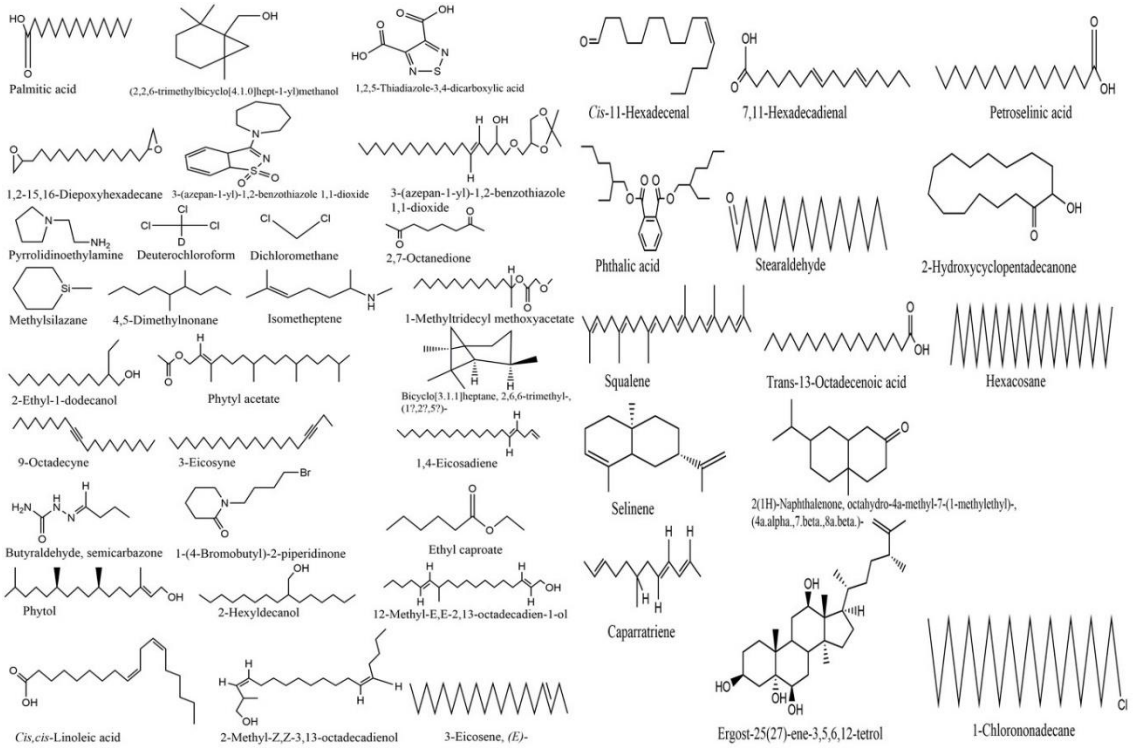

Figure 2. Structural formulars of compounds identified in the AQF of DM

Table 5. Compounds Identified in the AQF of DM

S/N	Name	RT	Area (%)	MW	Formula
1	Deuterochloroform	8.16	0.10	120.38	$CDCl_3$
2	Dichloromethane	8.79	0.13	84.93	CH_2Cl_2
3	2,7-Octanedione	9.22	0.02	142.20	$C_8H_{14}O_2$
4	Methyl silazane	11.59	0.07	113.25	$C_6H_{13}Si$
5	4,5-Dimethylnonane	13.76	0.16	156.31	$C_{11}H_{24}$
6	1,2,5-Thiadiazole-3,4-dicarboxylic acid	14.26	0.26	174.14	$C_4H_2N_2O_4S$
7	3-(Acetyloxy)-2-isopropyl-2-methylbutyl acetate	15.21	0.13	230.30	$C_{12}H_{22}O_4$
8	Isometheptene	15.44	0.07	141.25	$C_9H_{19}N$
9	1-Methyltridecyl methoxyacetate	16.46	0.55	286.40	$C_{17}H_{34}O_3$
10	Octadecan	16.58	0.74	254.49	$C_{18}H_{38}$
11	2-Ethyl-1-dodecanol	16.78	0.18	214.39	$C_{14}H_{30}O$
12	Phytyl acetate	16.89	3.13	338.6	$C_{22}H_{42}O_2$
13	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1α,2β,5α)-	16.97	3.90	138.25	$C_{10}H_{18}$
14	Octadec-9-yne	17.23	0.86	250.46	$C_{18}H_{34}$
15	3-Eicosyne	17.39	1.29	278.52	$C_{20}H_{38}$
16	1,4-Eicosadiene	17.43	2.20	278.5	$C_{20}H_{38}$
17	Methyl isohexadecanoate	18.00	2.60	270.45	$C_{17}H_{34}O_2$
18	Butyraldehyde, semicarbazone	18.26	0.78	129.16	$C_5H_{11}N_3O$
19	1-(4-Bromobutyl)-2-piperidinone	18.36	1.15	234.13	$C_9H_{16}BrNO$
20	Ethyl caproate	18.64	4.95	144.21	$C_8H_{16}O_2$
21	Palmitic acid	19.11	15.13	256.42	$C_{16}H_{32}O_2$
22	Phytol	19.90	5.90	296.53	$C_{20}H_{40}O$
23	2-Hexyldecanol	20.54	1.33	242.44	$C_{16}H_{34}O$
24	*cis*,*cis*-Linoleic acid	20.90	29.86	280.45	$C_{18}H_{32}O_2$
25	12-Methyl-*E*,*E*-2,13-octadecadien-1-ol	21.88	0.12	280.50	$C_{19}H_{36}O$
26	2-Methyl-*Z*,*Z*-3,13-octadecadienol	21.95	1.48	280.5	$C_{19}H_{36}O$
27	3-Eicosene, *(E)*-	22.28	0.91	280.53	$C_{20}H_{40}$
28	*cis*-11-Hexadecenal	22.96	0.68	238.41	$C_{16}H_{30}O$
29	1,2-15,16-Diepoxyhexadecane	23.10	0.35	254.41	$C_{16}H_{30}O_2$
30	7,11-Hexadecadienal	23.41	0.46	236.39	$C_{16}H_{28}O$
31	Phthalic acid	23.54	1.72	390.56	$C_{24}H_{38}O_4$
32	Petroselinic acid	23.98	0.67	282.46	$C_{18}H_{34}O_2$
33	Stearaldehyde	25.05	0.18	268.48	$C_{18}H_{36}O$
34	2-Hydroxycyclopentadecanone	25.33	0.90	240.38	$C_{15}H_{28}O_2$
35	3-(azepan-1-yl)-1,2-benzothiazole-1,1-dioxide	26.00	0.17	264.35	$C_{13}H_{16}N_2O_2S$
36	Squalene	26.47	4.57	410.72	$C_{30}H_{50}$
37	Trans-13-Octadecenoic acid	27.17	1.03	282.46	$C_{18}H_{34}O_2$
38	Hexacosane	27.94	0.90	366.71	$C_{26}H_{54}$
39	Selinene	28.45	1.05	204.35	$C_{15}H_{24}$
40	2(1*H*)-Naphthalenone, octahydro-4a-methyl-7-(1-methyl-ethyl)-, (4a. alpha.,7. beta.,8a. beta.)-	28.49	0.08	208.34	$C_{14}H_{24}O$
41	Caparratriene	28.52	0.22	206.37	$C_{15}H_{26}$
42	(2,2,6-trimethylbicyclo [4.1.0] hept-1-yl) methanol	28.59	0.09	168.28	$C_{11}H_{20}O$
43	Ergost-25(27)-ene-3,5,6,12-tetrol	28.63	0.25	448.7	$C_{28}H_{48}O_4$
44	1-Chlorononadecane	32.62	1.66	302.97	$C_{19}H_{39}Cl$

RT= Retention time, MW= Molecular weight

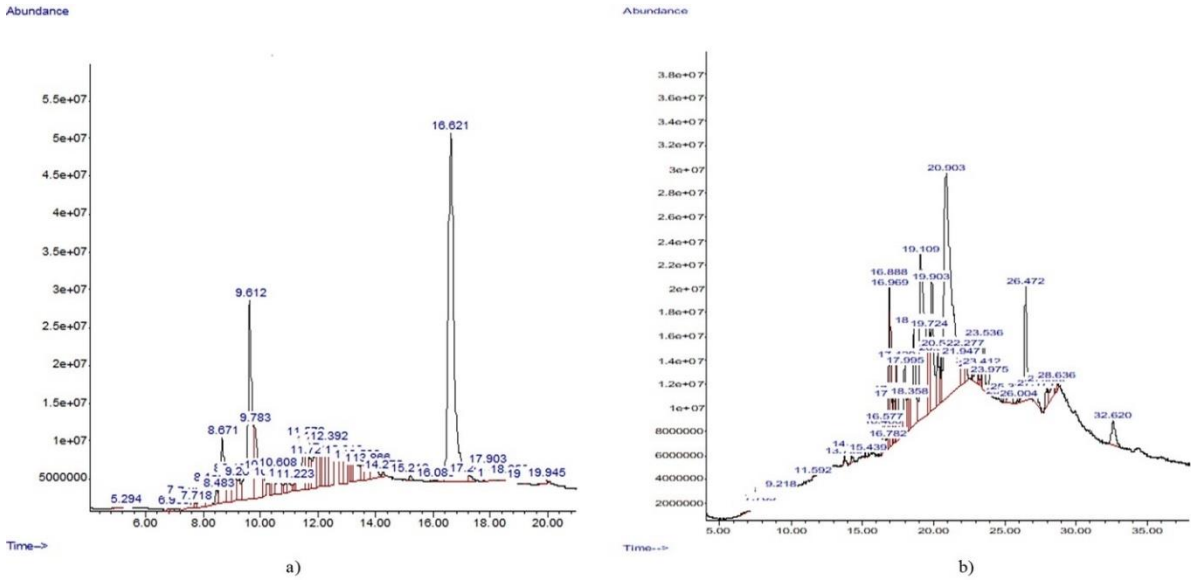

Figure 3. GC-MS chromatogram of compounds identified in the: a) EEF and b) AQF of DM

Antioxidant Potential

TAC

Figure 4 presents the AA calibration curve (a) and the AAE of the TAC of CR, EEF, and AQF of DM (b). At 300 µg/ml, the CR exhibited a significantly ($p < 0.05$) higher TAC (73.59 ± 0.011 µg/ml AAE) than EEF (41.28 ± 0.003 µg/ml AAE) and AQF (31.28 ± 0.005 µg/ml AAE). Furthermore, the EEF and AQF were not significantly different ($p > 0.05$) from each other.

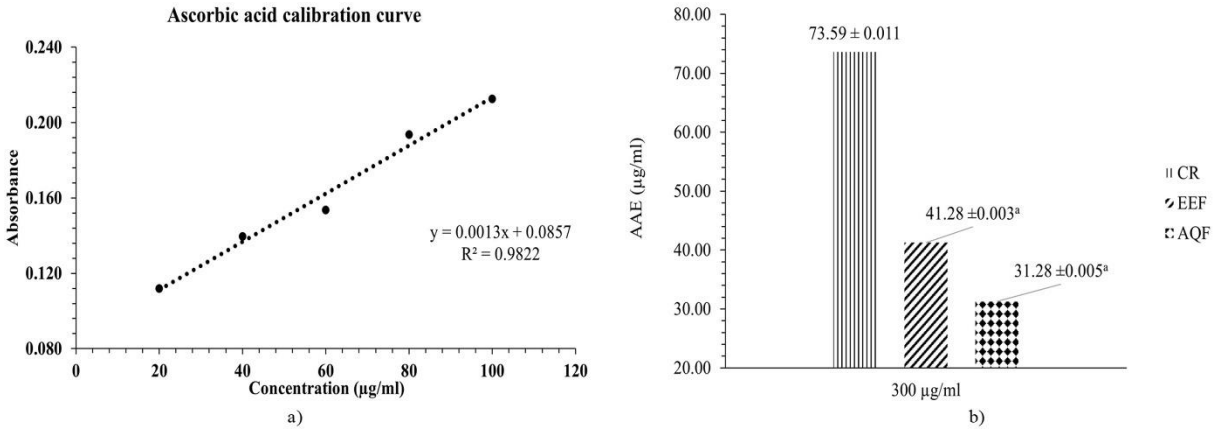

Figure 4. TAC of DM: a) AA calibration curve and b) AAE TAC of DM. Values with [a] superscript are significantly ($p < 0.05$) lower than CR

AA used as a standard in the determination of the antioxidant effect demonstrated a concentration-dependent absorbance yielding a standard calibration curve (Figures 4a and 5a). All the tested samples (CR, EEF, and AQF) exhibited lower total antioxidant capacity compared to AA. This is not surprising considering AA is a standard water-soluble antioxidant and the test medium was polar [50]. The CR extract demonstrating significantly higher total antioxidant capacity might be attributed to the synergistic and additivity properties of phytochemicals [51,52] as some compounds present in the EEF were absent in AQF. Therefore, their combined presence in the CR showed a higher total antioxidant capacity.

TRP

The AA calibration curve (a) and the AAE of the TRP of CR, EEF, and AQF (b) are presented in Figure 5. At 100 µ/ml, the AQF showed a significantly ($p < 0.05$) higher TRP (106.84 ± 3.46 µg/ml AAE) than the CR (93.23 ± 5.63 µg/ml AAE) and EEF (92.35 ± 6.96 µg/ml AAE). However, the CR and EEF were not significantly ($p > 0.05$) different from each other.

The AQF exhibited a slightly higher TRP than AA but significantly ($p < 0.05$) higher than CR and EEF. The TRP of a compound is due to its electron-donating ability [53]. The superior reducing power of the AQF might be attributed to the presence of compounds with high electron-donating potential in the AQF. Although these compounds might be present in the CR, the antagonist effects [52] of some compounds present in the CR but absent in AQF might negatively impact the antioxidant activity of the CR.

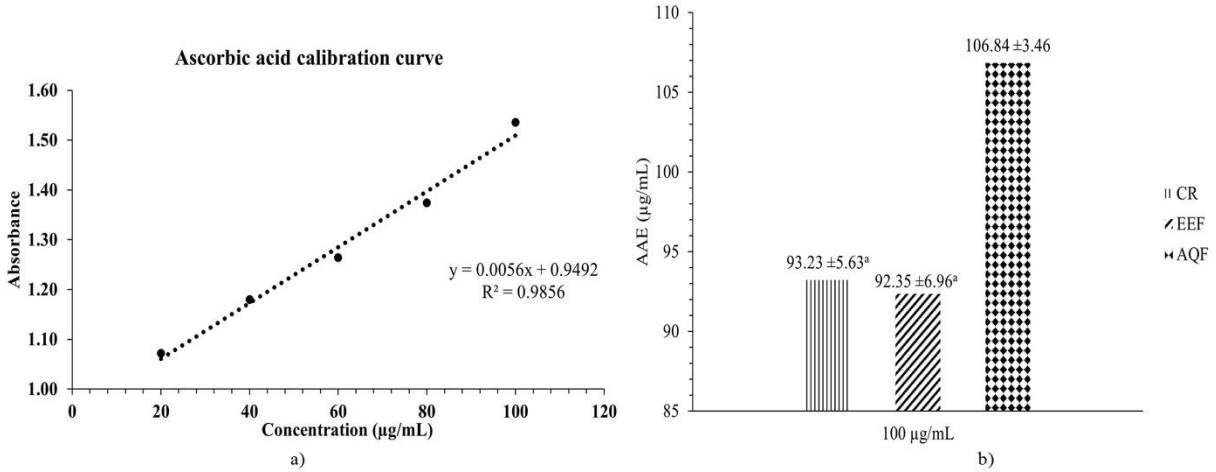

Figure 5. TRP of DM: a) AA calibration curve and b) AAE total reducing power of DM. Values with [a]superscript are significantly ($p < 0.05$) lower than AQF

FTC

The antioxidant capacity of CR, EEF, and AQF expressed by the FTC method is presented in Figure 6a. At 1 mg/ml, the EEF demonstrated a significantly ($p < 0.05$) higher inhibition (48.38% ±4.61) than ascorbic acid (32.64% ±4.40), CR (18.39% ±3.80), and AQF (11.23% ±3.66) in the final day of the experiment (day 6).

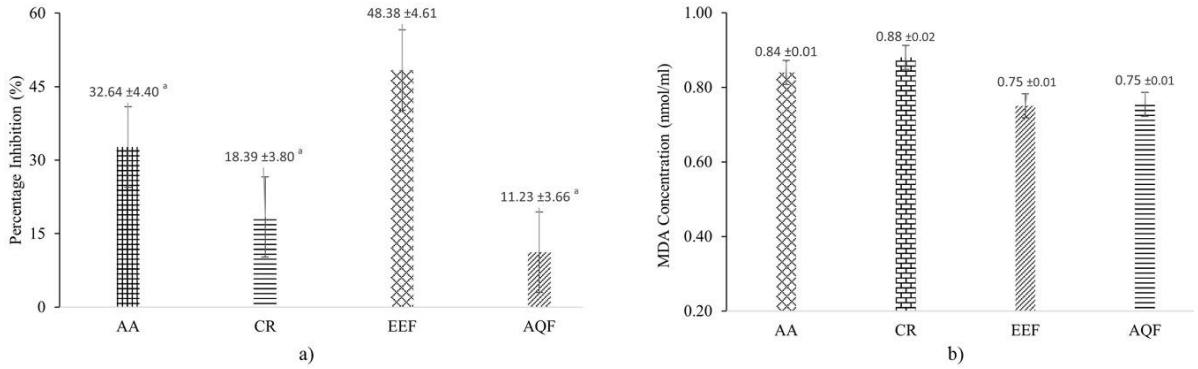

Figure 6. Antioxidant capacity of DM expressed by: a) FTC and b) TBA methods. Values with [a] superscript are significantly ($p < 0.05$) lower than EEF

TBA

The antioxidant potential of CR, EEF, and AQF expressed by the TBA method is shown in Figure 6b. At 1 mg/ml, the EEF and AQF demonstrated lower MDA concentration (0.75 ±0.01 nmol/ml) than the CR (0.88 ±0.02 nmol/ml) and AA (0.84 ±0.01 nmol/ml) on day 6, similar to the result observed for EEF in the FTC method.

H_2O_2 Scavenging Assay

Figure 7 presents the H_2O_2 scavenging activity of CR, EEF, and AQF. The AQF demonstrated significantly ($p < 0.05$) higher (82.72% ±1.88) scavenging at the 20 µg/ml concentration than AA (51.52% ±2.43), CR (33.33% ±2.16), and EEF (63.64% ±2.66). Furthermore, the AQF demonstrated significantly ($p < 0.05$) higher scavenging compared to the CR and EEF at 60, 80, and 100 µg/ml concentrations, with no significant ($p > 0.05$) difference with AA at 100 µg/ml concentration.

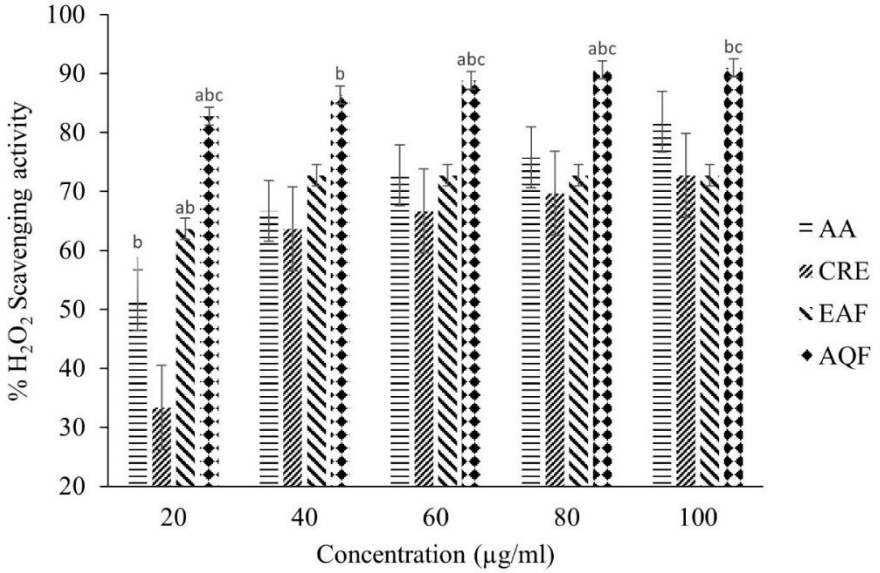

Figure 7. H_2O_2 scavenging activity of DM. Values with [a], [b], and [c] superscripts are significantly ($p < 0.05$) higher than AA, CR, and EEF, respectively

In the FTC method, the early stage of lipid peroxidation due to the production of peroxides is considered. Here, the peroxides combine with ferrous chloride yielding ferric ions which subsequently react with ammonium thiocyanate to produce the red-colored ferric thiocyanate [54]. The prevention of the formation of the red product by the samples is exploited here. Thus, a lower absorbance value translates to higher anti-peroxidation potential. In our study, the EEF exhibited the highest inhibition and lowest MDA concentration demonstrating a significantly higher anti-peroxidation capacity than all the extracts and ascorbic acid. The superiority of the EEF against ascorbic acid in the FTC method might be due to the weak antioxidant capacity of ascorbic acid in a non-polar medium [50] and the semi-polar nature of ethyl acetate. A similar observation was made in the TBA method with ethyl acetate demonstrating the lowest MDA concentration after day 6. The high antioxidant activity observed at the beginning of the experiment which diminished continuously for AA and CR demonstrated powerful anti-lipid peroxidation. However, the EEF and AQF demonstrated a longer antioxidant potential and, thus, a better anti-peroxidation activity. In the hydrogen peroxide scavenging method, the AQF exhibited the highest scavenging ability, significantly higher than the CR and EEF. This further justifies the high reducing power of the AQF reported earlier (Figure 5).

Persistent hyperglycemia associated with diabetes promotes oxidative stress over time *via* reactive oxygen species (ROS) generation and the depletion of the inherent antioxidant system [55]. Some of the problems encountered with antioxidants are storage instability and poor solubility [55] as

revealed by the poor performance of AA in the FTC and TBA method. Furthermore, lipid peroxidation due to persistent hyperglycemia is a major concern in diabetes [56]. Thus, the antioxidant activity demonstrated by CR, EEF, and AQF might contribute to the antidiabetic activity of DM by ameliorating oxidative stress.

In silico Study

MD and MDS

Antioxidant Activity

Table 6 exhibits the results of the docking interactions of XO with the top 10 compounds. Compound VII exhibited the least BA (-8.8 kcal/mol) and Ki (0.35μM) next to XI with respective BA and Ki of -7.4 kcal/mol and 3.71 μM. Moreover, VII exhibited a superior number of HBs (3) than XI (2) though the latter had more HBIs (11). The low BA, Ki, and higher HBs of VII might contribute to a stable and more favorable dock pose than the other compounds as lower BA translates to an energy-favorable docked complex. The BA defines an inverse of the strength and level of interaction between a macromolecule (target) and the ligand (small molecule or compound) [57] while the Ki defines the inhibition constant. XO is a crucial enzyme in the metabolism of purine and catalyzes the rate-limiting step [56]. However, it is linked to oxidative stress by utilizing oxygen as an electron acceptor rather than NAD^+ yielding superoxide anions free radical and other ROS [59]. Thus, the favorable binding of compound VII might affect the enzyme activity and minimize its effects. Moreover, Figure 8a shows the 2D and 3D docking interactions of XO with compounds VII and XI. The MDS result is shown in Figure 8b depicting the chain and residue displacement indicating hinge regions with higher displacement notably between residues 995 to 1028. Thus, further revealing the flexibility of the complex and possible XO inhibition. Additionally, high XO activity was reported to be associated with type 2 diabetes [60]. Therefore, a decrease in its activity might be associated with antidiabetic activity *via* alleviation of oxidative stress.

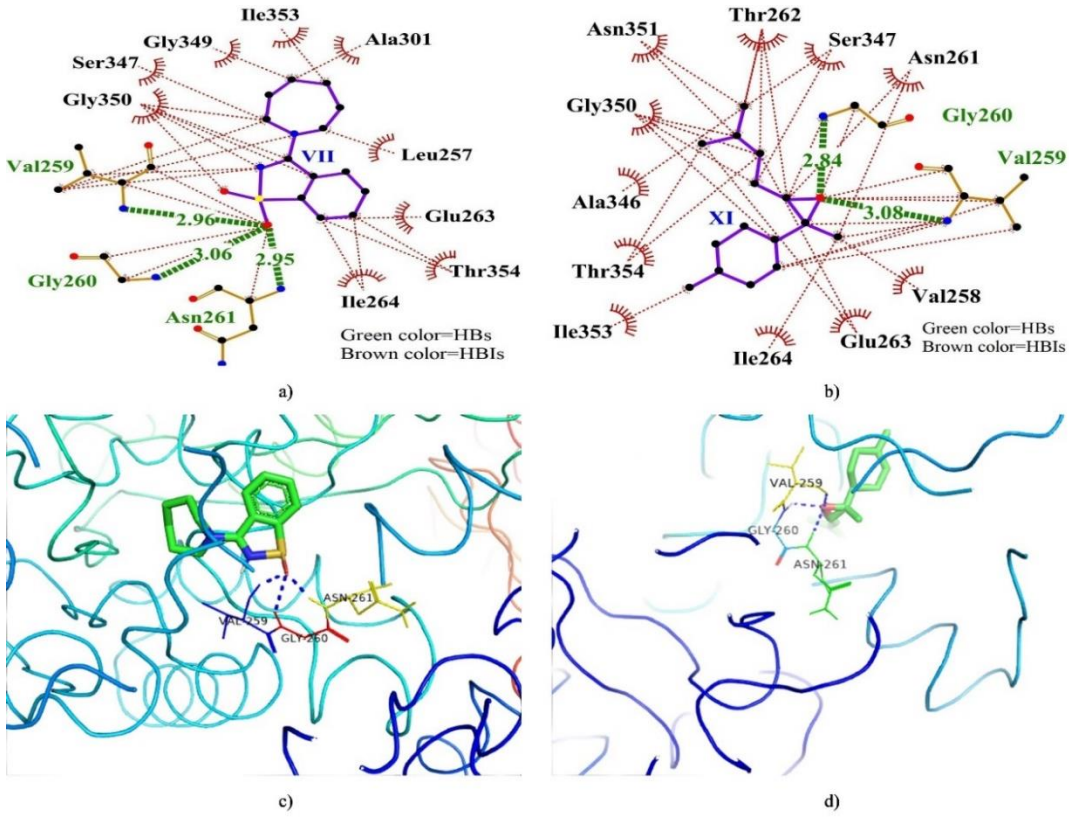

Figure 8a. Docked complex of XO with compounds VII (a and c) and XI (b and d)

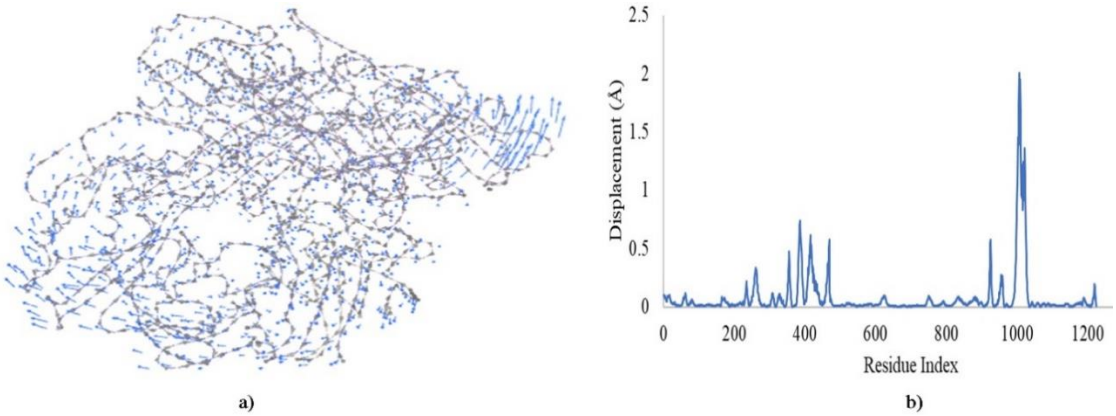

Figure 8b. MDS result of docked XO showing; a) cluster and b) residue displacements

Table 6. Docking interactions of XO with compounds VII and XI

Compounds	Binding Affinity (kcal/mol)	Ki (µM)	Hydrogen Bonds	Hydrophobic Interactions
VII	-8.8	0.35	3	9
XI	-7.4	3.71	2	11
X	-6.7	12.12	0	8
III	-6.5	16.99	1	6
XII	-6.5	16.99	0	8
XIV	-6.4	20.12	1	12
VIII	-6.2	28.20	0	8
VI	-5.9	46.82	3	10
XIII	-5.8	55.44	2	7
XVI	-5.7	65.65	3	8

Table 7 displays the docking interactions of CytP450 21A2 with the compounds. Compound X displayed the lowest BA (-8.5 kcal/mol) and Ki (0.58µM) next to XII with BA and Ki of -8.3 kcal/mol and 0.81 µM, respectively. However, XII exhibited a superior number of HBs (4) with slightly lower HBIs (8). Additionally, compounds VII and IV participated in SB (ASP288) and π-cationic (TRP202) interactions, respectively. Moreover, Figure 9a shows the interactions of CytP450 21A2 with compounds X and XI depicting the residues participating in both HBs and HBIs interactions. CytP450 21A2 belongs to the heme monooxygenases family with their activities associated with the generation of ROS by uncoupled catalytic action [61]. Thus, in ailments associated with depletion of the inherent antioxidant system such as diabetes, inhibition leading to its decreased activity might contribute to decreased oxidative stress.

Table 7. Docking interactions of CytP450 21A2 with compounds VII and XI

Compounds	Binding Affinity (kcal/mol)	Ki (µM)	Hydrogen Bonds	Hydrophobic Interactions	Salt Bridge	π-Stacking
X	-8.5	0.58	0	10		
XII	-8.3	0.81	4	8		
VII	-8.2	0.96	1	8	ASP288	
III	-7.9	1.60	1	9		
XII	-7.5	3.14	1	11		
VIII	-7.2	5.21	0	9		
I	-6.9	8.64	4	5		
XIV	-6.9	8.64	4	7		
XIII	-6.5	16.99	3	8		
IV	-6.4	20.12	0	4		TRP202

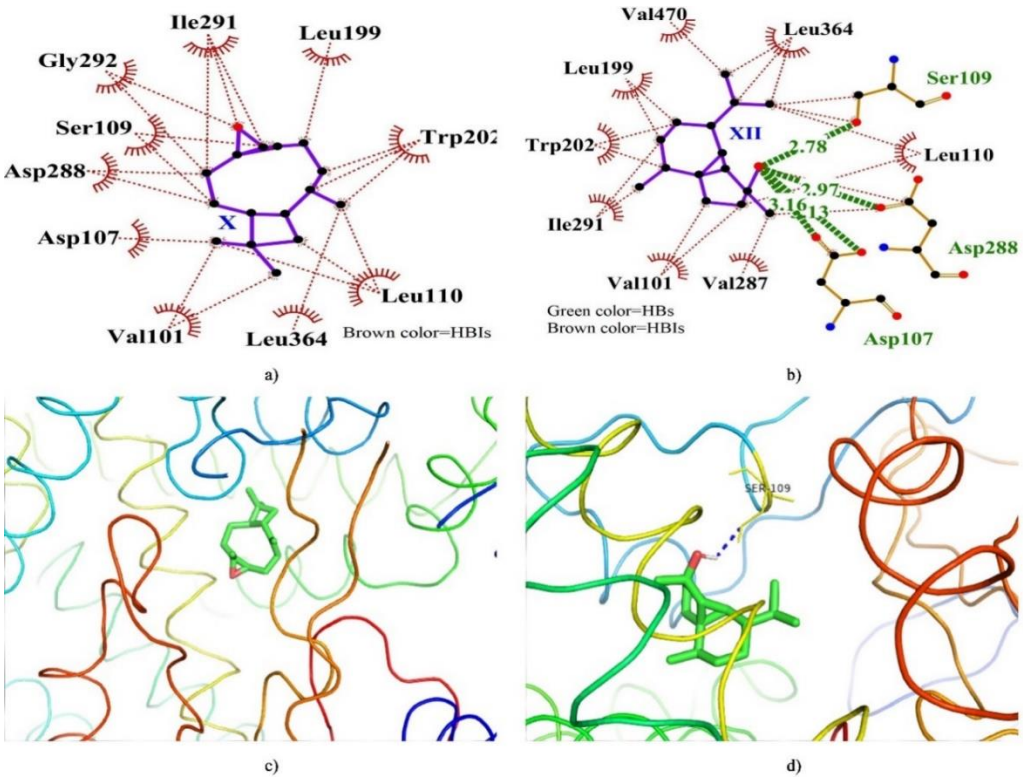

Figure 9a. Docked complex of CytP450 21A2 with compounds X (a and c) and XII (b and d)

Furthermore, the MDS result of the docked CytP450 21A2 complex is exhibited in Figure 9b showing high cluster and residue displacements (3.343 Å) close to the mid-chain residues which might disrupt its tertiary structure and its activity.

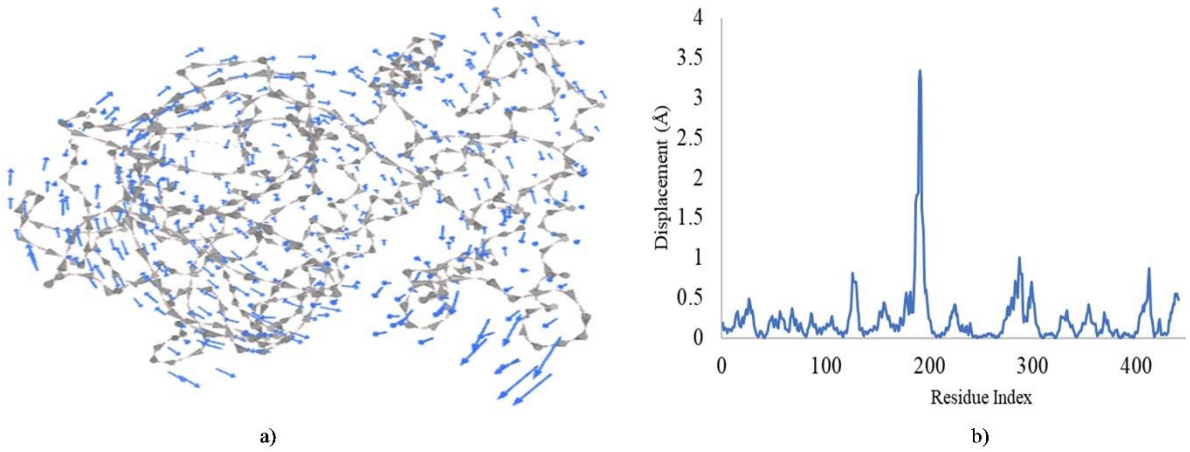

Figure 9b. MDS result of docked CytP450 21A2 showing; a) cluster and b) residue displacements

Table 8 reveals the docking interactions of NOX with the compounds. The lowest BA (-8 kcal/mol) and Ki (1.35µM) were demonstrated by compound VII followed by III with respective BA and Ki of -6.9 kcal/mol and 8.64 µM. Higher interaction was also observed for VII with a superior number of HBs and HBIs than the other compounds including an SB formed with GLU32. Moreover, compounds III, VIII, and XIII were involved in SB formation with GLU32, GLU32, and HIS10, respectively. NOX are groups of membrane-bound proteins transferring electrons across the membrane

to oxygen-generating superoxide anions and ROS such as the hydroxyl and H_2O_2 radicals [62]. Thus, in a state of impaired body antioxidant systems such as diabetes, ROS accumulate. Decreased activity of this enzyme in these ailments might lead to decreased oxidative stress and promote healing.

Table 8. Docking interactions of NOX with compounds VII and III

Compounds	Binding Affinity (kcal/mol)	Ki (μM)	Hydrogen Bonds	Hydrophobic Interactions	Salt Bridge
VII	-8	1.35	2	12	GLU32
III	-6.9	8.64	1	10	
XI	-6.7	12.12	0	12	
XII	-6.6	14.35	3	8	
XIV	-6.3	23.82	3	10	
VIII	-6.2	28.20	0	8	GLU32
X	-6.2	28.20	0	10	
XIII	-5.8	55.44	8	3	HIS10
III	-5.6	77.73	1	10	GLU32
IV	-5.6	77.73	0	10	

Figure 10a shows the HBs and HBIs formation by NOX with compounds VII and III depicting a stabilized docked complex. Figure 10b displays the MDS result depicting the cluster and residue displacements with notable displacements at both terminals. However, higher displacements up to 3.385 and 2.285 Å were observed at residues 365 and 426, respectively. This might disrupt the enzyme activity by disrupting its tertiary structure, further indicating a possible inhibition of the enzyme by the compounds.

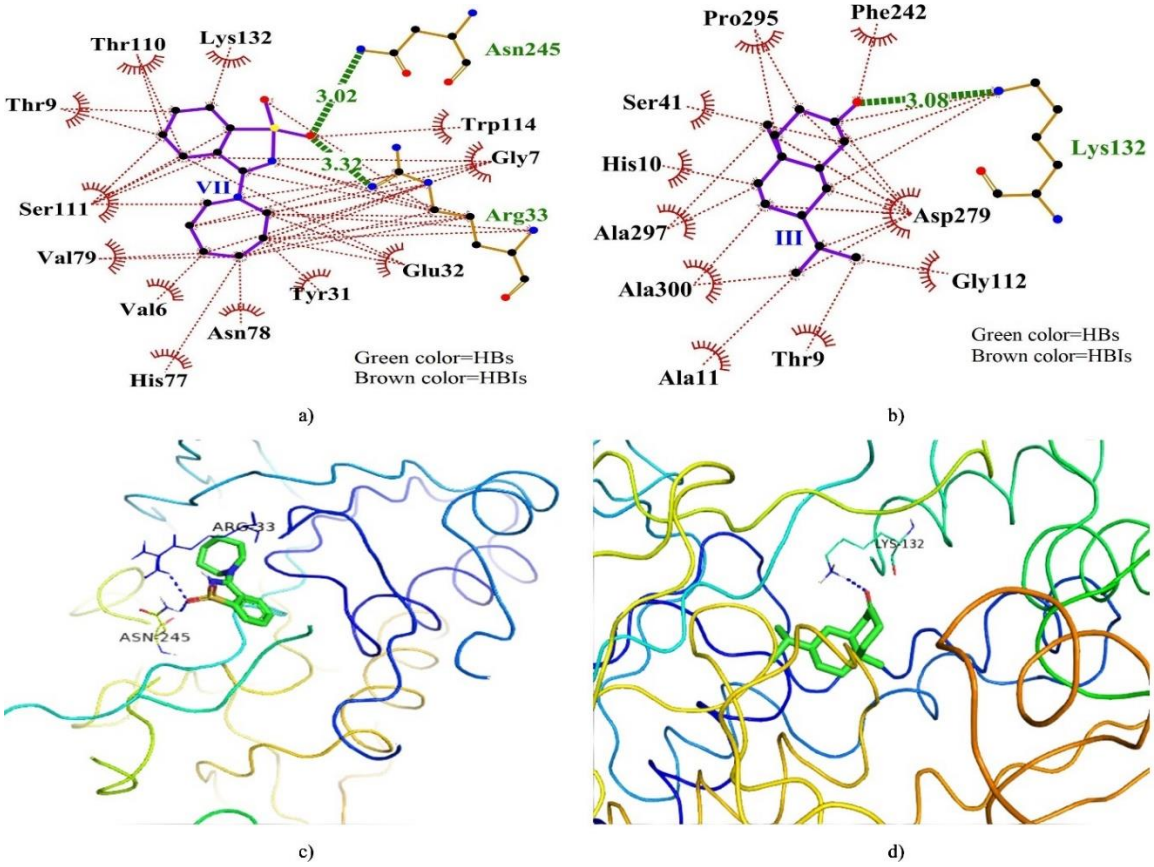

Figure 10a. Docked complex of NOX with compounds VII (a and c) and III (b and d)

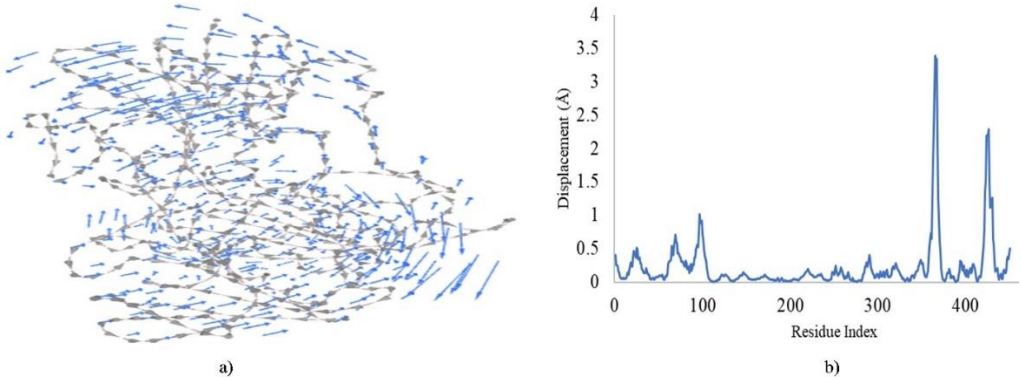

Figure 10b. MDS result of docked NOX showing; a) cluster and b) residue displacements

Antidiabetic Activity

Table 9 reveals the docking interactions of PTP1B with the compounds. Compound III exhibited the lowest BA (-7.5 kcal/mol) and Ki (3.14 µM) slightly lower than X with BA and Ki of -7.4 kcal/mol and 3.71 µM, respectively. Additionally, III exhibited 3 HBs and 7 HBIs, slightly higher and lower than X, respectively.

Figure 11a depicts the docking interactions of PTP1B with compounds III and X revealing the HBs and HBIs. Both compounds formed HBs with ARG221 and SER216 residues. Additionally, the MDS of the docked PTP1B complex is displayed in Figure 11b showing the chain cluster and residue displacements. Notable displacement was observed at 6 regions depicting hinge regions and cluster movements with the highest displacement (2.65 Å) at approximately mid-chain (residue 265).

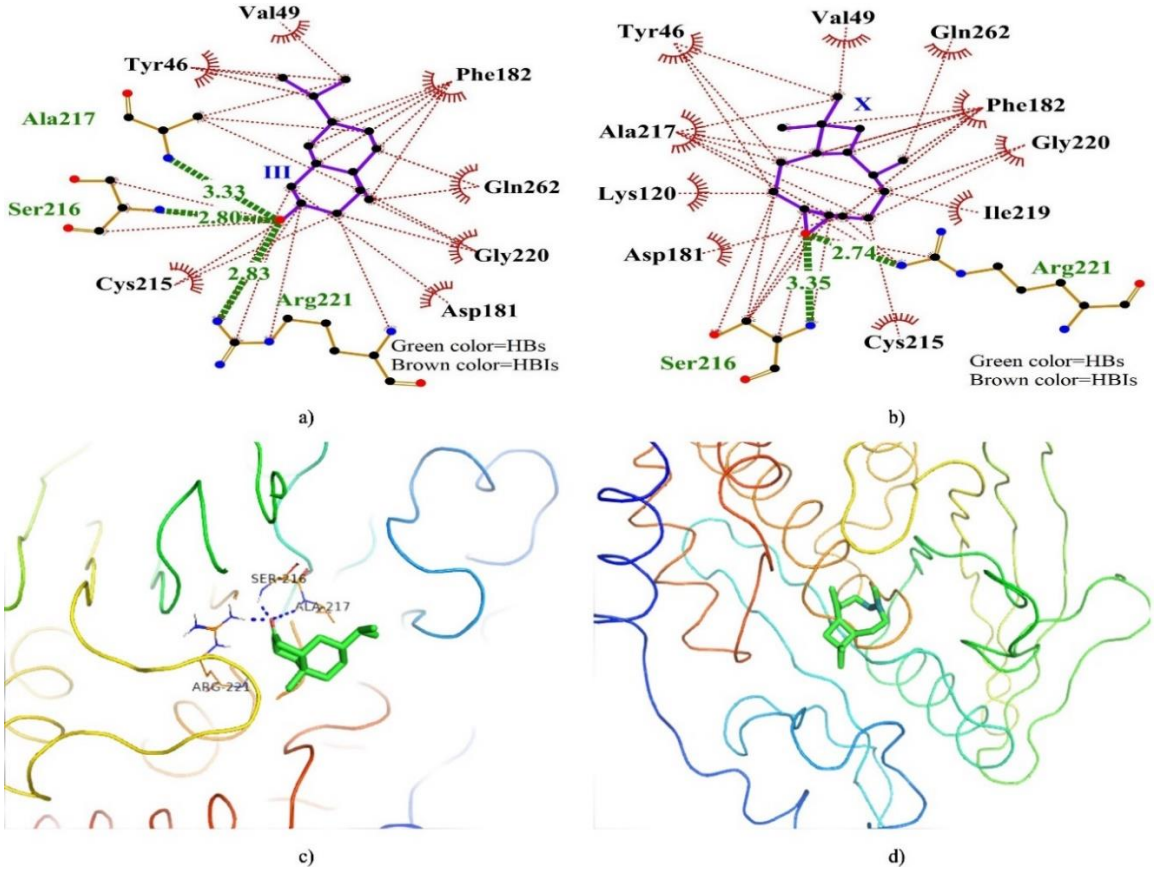

Figure 11a. Docked complex of PTP1B with compounds III (a and c) and X (b and d)

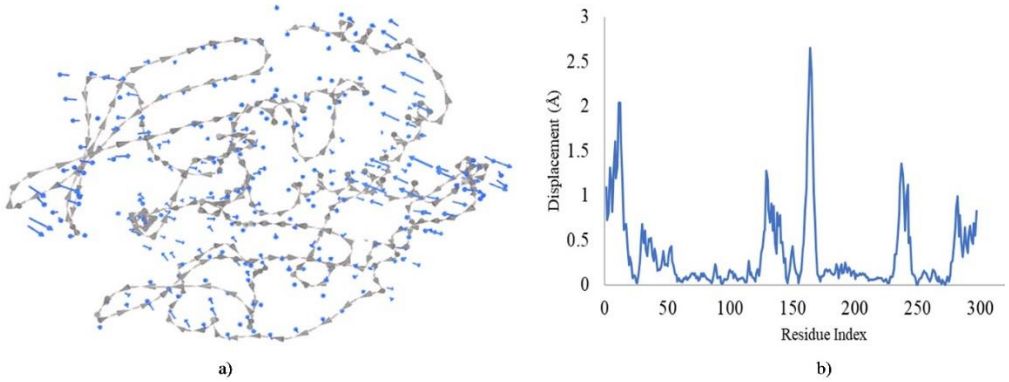

Figure 11b. MDS result of docked PTP1B showing; a) cluster and b) residue displacements

Table 9. Docking interactions of PTP1B with the compounds

Compounds	Binding Affinity (kcal/mol)	Ki (μM)	Hydrogen Bonds	Hydrophobic Interactions	π-Cation	Salt Bridge	Halogen Bond
III	-7.5	3.14	3	7			
X	-7.4	3.71	2	9			
VII	-7.2	5.21	2	7			
XI	-7.2	5.21	0	11			
XIV	-7	7.30	2	11			
VIII	-6.8	10.23	0	7			
XIII	-6.7	12.12	4	8		LYS120 ARG221	
XII	-6.3	23.82	1	10			
IV	-6.2	28.20	1	7	PHE182		LYS120
II	-5.8	55.44	1	8			

PTP1B is an insulin-desensitizing enzyme that downregulates the insulin receptor phosphorylation. Therefore, decreases insulin sensitivity in tissues *via* the insulin signaling pathway and promotes insulin resistance [63]. Inhibition of its activity promotes insulin signaling and absorption of glucose and decreases hyperglycemia. Thus, a promising target of diabetic therapy. In our study, the docking of the top two compounds in the PTP1B active site interacting with the amino acids might block the activity of the enzyme and decrease insulin resistance.

The docking interaction of PPARγ with the compounds is displayed in Table 10 depicting the various binding interactions. Compound X exhibited a slightly lower BA (-8.5 kcal/mol) and Ki (0.58 μM) than XII with a BA and Ki of -8.3 kcal/mol and 0.81 μM, respectively. However, the latter had a higher number of HBs (1) and HBIs (13). Furthermore, these docking interactions are displayed in Figure 12a showing the HBs and HBIs whereas the MDS of the docked PPARγ complex is shown in Figure 12b depicting the cluster and residue displacement.

Table 10. Docking interactions of PPARγ with the compounds

Compounds	Binding Affinity (kcal/mol)	Ki (μM)	Hydrogen Bonds	Hydrophobic Interactions
X	-8.5	0.58	0	6
XII	-8.3	0.81	1	13
VII	-8.2	0.96	1	10
III	-7.9	1.60	0	6
XII	-7.5	3.14	0	14
VIII	-7.2	5.21	0	12
I	-6.9	8.64	1	6
XIV	-6.9	8.64	3	10
XIII	-6.5	16.99	1	14
IV	-6.4	20.12	0	8

PPARγ is part of the nuclear hormone receptors family acting as ligand-modulated transcription factors that regulate lipid metabolism by simulating insulin sensitivity, proliferation, and cell differentiation [64]. These receptors have emerged as antidiabetic drug targets, notably the thiazolidinediones which lower insulin resistance and improve glycemic control [64]. In our study, compounds X and XII interacted with PPARγ with low BA and Ki in the thiazolidinediones binding pocket which might its activity and improve insulin resistance.

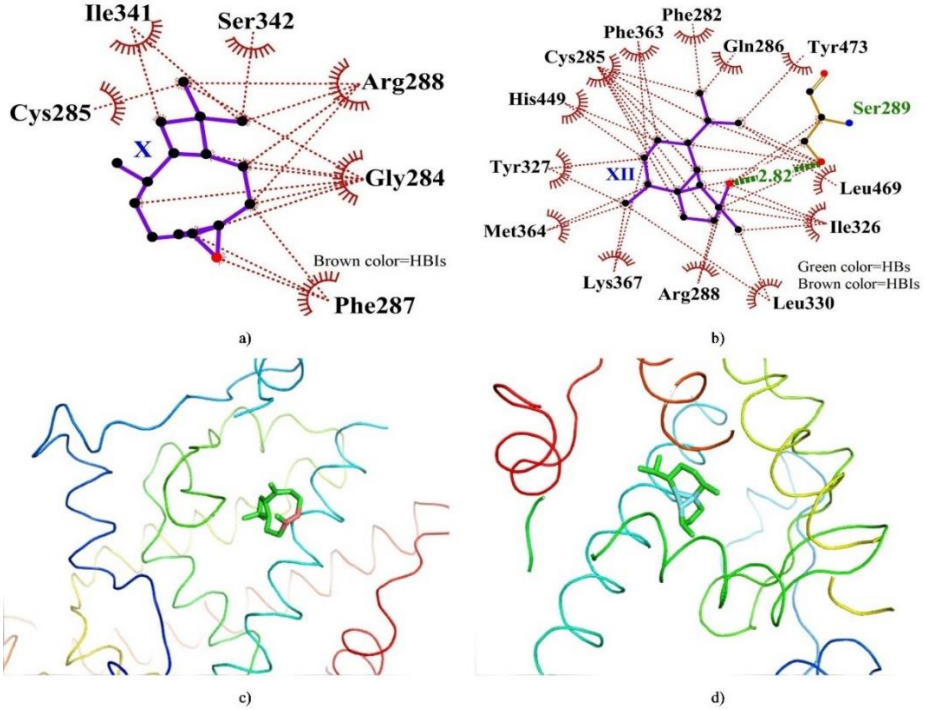

Figure 12a. Docked complex of PPARγ with compounds X (a and c) and XII (b and d)

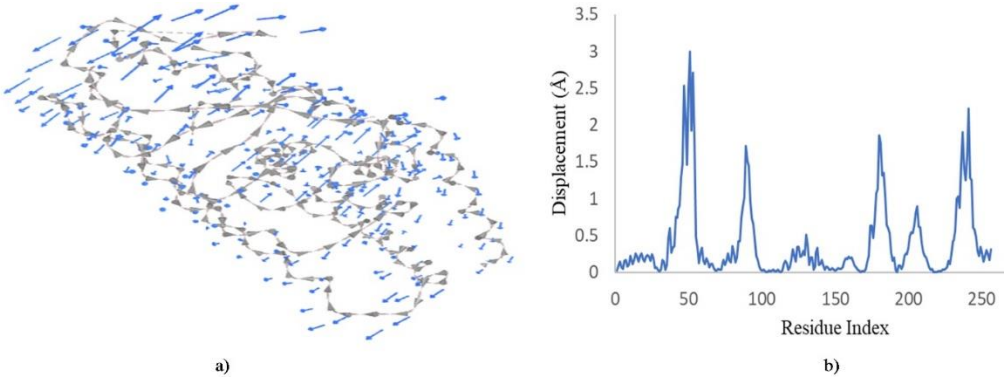

Figure 12b. MDS result of docked PPARγ showing; a) cluster and b) residue displacements

The ADMET predictions of the top two compounds with the least BA and Ki are displayed in Table 11. Compound VII was predicted to be slightly soluble (-2.87 log mol/L) while the others were insoluble with III (-4.98 log mol/L) being the least. However, the compounds were predicted to have high human intestinal absorption with compound X (95.88%) demonstrating the highest percentage of absorption while VII (94.24%) was the least. Furthermore, compounds VII (-3.60 log Kp), X (-3.07 log Kp), and XI (-3.27 log Kp) were predicted to be skin permeant while III and XII were relatively less permeant. Moreover, the compounds were predicted to be neither inhibitors of P-glycoproteins nor its

substrate. For a compound to exert pharmacological effects, it must be effectively absorbed and remain bioavailable for a while which also contributes to safe usage and predicts its onset and intensity [65]. In our study, some of the compounds were predicted to possess good absorption properties.

Table 11. ADMET properties of the compounds

ADMET Properties		**III**	**VII**	**X**	**XI**	**XII**
Absorption	Water solubility (log mol/L)	-4.98	-2.87	-4.42	-4.60	-4.55
	Human Intestinal absorption (%)	95.10	94.24	95.88	95.29	94.86
	Skin permeability (log Kp)	-1.76	-3.60	-3.07	-3.27	-2.23
	P-glycoprotein substrate	No	No	No	No	No
	P-glycoprotein I inhibitor	No	No	No	No	No
	P-glycoprotein II inhibitor	No	No	No	No	No
Distribution	The volume of distribution [VDss (log L/kg)]	0.45	0.01	0.59	0.53	0.55
	Human fraction unbound	0.21	0.26	0.33	0.33	0.19
	BBB permeability (log BB)	0.64	0.31	0.65	0.67	0.68
	CNS permeability (log PS)	-1.75	-2.85	-2.52	-2.57	-1.97
Metabolism	CYP2D6 substrate	No	No	No	No	No
	CYP3A4 substrate	Yes	No	No	No	Yes
	CYP1A2 inhibitor	No	No	Yes	No	Yes
	CYP2C19 inhibitor	No	No	Yes	Yes	Yes
	CYP2C9 inhibitor	No	No	Yes	No	No
	CYP2D6 inhibitor	No	No	No	No	No
	CYP3A4 inhibitor	No	No	No	No	No
Excretion	Total clearance (log ml/min/kg)	1.05	0.33	0.91	1.24	0.89
	Renal OCT2 substrate	No	No	No	No	No
Toxicity	Human max. tolerated dose (log mg/kg/day)	-0.21	0.12	0.19	0.22	-0.32
	hERG I inhibitor	No	No	No	No	No
	hERG II inhibitor	No	No	No	No	No
	LD_{50} [rats (mol/kg)]	1.56	2.68	1.55	1.48	1.69
	Hepatotoxicity	No	Yes	No	No	No
	Skin sensation	Yes	No	Yes	Yes	Yes

Compound X was predicted to have a higher (0.59 log L/kg) steady-state volume of distribution (VDss) than the others with the lowest VDss exhibited by VII (0.01 log L/kg). The VDss predicts the amount of the compound required to be equally distributed between tissues and plasma with higher values (log VDss > 0.45) indicating higher concentration in tissues while values < -0.15 and > 0.45 are considered low [31]. Thus, in our study, the compounds had higher VDss. Furthermore, X and XI had similar fraction unbound values (0.33) while XII had the lowest value (0.19). Compounds XII, XI, X, and III exhibited similar BBB (blood-brain barrier) permeability properties in order of decreasing values (0.69-0.64 log BB) while VII had the lowest (0.31 log BB). The log BB value accounts for the crossing of the BBB which determines brain toxicity and side effects as a log ratio of brain-plasma drug concentration [31]. In our study, the compounds were predicted to be BBB permeant. Moreover, VII showed the lowest (-2.85 log PS) CNS (central nervous system) permeability next to XI (-2.57 log PS) and X (-2.52 log PS) while III (-1.75 log PS) had the highest. A CNS permeability value (log PS) >-2 is considered CNS permeant while < -3 is not. Thus, the compounds were predicted to be CNS permeant in our study.

All the compounds were predicted to be non-CYP2D6 substrates but III and XII were CYP3A4 substrates. X and XII were CYP1A2 and CYP2C19 inhibitors while XI inhibited only the latter. Additionally, X was predicted to be a CYP2C9 inhibitor while none were CYP2D6 and 3A4 inhibitors. CytP450 is associated with the metabolism and subsequent excretion of many compounds and their inhibitors can alter their effects on drugs [31]. CYP2D6 and CYP3A4 metabolize many compounds. In our study, compounds III and XII were predicted to be metabolized by these enzymes while VII was neither a substrate nor inhibitor of the cytP450 enzymes. Compound XI had the highest (1.24 log ml/min/kg) total clearance rate next to III (1.05 log ml/min/kg) while VII (0.33 log ml/min/kg) had the

lowest. Additionally, all the compounds were not renal OCT2 (organic cation transporter 2) substrates. The renal OCT2 is crucial in the clearance and disposition of drugs in the kidney [31], thus, the compounds can easily undergo renal clearance and disposition.

Compounds X (0.19 log mg/kg/day) and XI (0.22 log mg/kg/day) were predicted to have slightly different maximum tolerable doses in humans while XIII had the lowest (-0.32 log mg/kg/day). A maximum tolerated dose < 0.477 log (mg/kg/day) is considered low while above the value is high [31]. Thus, the compounds were within the low range. Moreover, the compounds were predicted not to be hERG I and II inhibitors. Compound VII has the highest (2.68 mol/kg) LD50 value while III (1.56 mol/kg), X (1.55 mol/kg), and XI (1.48 mol/kg) had slightly different values though the latter had the lowest. Compound VII was predicted to be hepatotoxic, however, it's the only one predicted not to exert skin sensation.

In our study, the antioxidant and antidiabetic potential of DM leaf was explored by investigating its secondary metabolites, antioxidant, and antidiabetic activity. Among the compounds, 3-(azepan-1-yl)-1,2-benzothiazole 1,1-dioxide and caryophyllene oxide were the most antioxidant compounds. This isn't surprising considering their high abundance in the EEF which exhibited the highest anti-peroxidation by the FTC and TBA methods. Thus, there is a correlation between the *in vitro* and *in-silico* results. Moreover, 3-(azepan-1-yl)-1,2-benzothiazole 1,1-dioxide showed good drug candidacy predicted by the ADMET study. For the antidiabetic activity, caryophyllene oxide was identified as the most antidiabetic compound next to 4a-methyl-7-(propan-2-yl) octahydronaphthalen-2(1H)-one. Although the present study showed the antioxidant, antidiabetic, and ADMET of these compounds, further studies such as isolation and *in vivo* antioxidant and antidiabetic studies of the compounds will further document these properties. Additionally, structural modification of the compounds might enhance the ADMET properties of the compounds. Conclusively, DM contains several compounds that might be associated with the antioxidant and antidiabetic activity of the plant due to the observed scavenging and the *in-silico* antioxidant, antidiabetic, and ADMET study. Additionally, all the extracts possess significant antioxidant activity and some of the identified compounds might be novel sources of antioxidant and antidiabetic drugs.

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AUTHOR CONTRIBUTIONS

Concept: M.M.D., N.M.; Design: M.M.D., N.M.; Control: M.M.D., N.M.; Sources: M.M.D.; Materials: M.M.D., N.M.; Data Collection and/or Processing: M.M.D., N.M.; Analysis and/or Interpretation: M.M.D., N.M.; Literature Review: M.M.D.; Manuscript Writing: M.M.D.; Critical Review: M.M.D., N.M.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

WHAT DO PEOPLE PREFER TO SUPPORT DIABETES TREATMENT IN TURKIYE? A STUDY ON OLIVE LEAF AND DIABETES

TÜRKİYE'DE DİYABET TEDAVİSİNE DESTEK OLMAK İÇİN İNSANLAR NE TERCİH EDİYOR? ZEYTİN YAPRAĞI VE DİYABET ÜZERİNE BİR ARAŞTIRMA

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ABSTRACT

Objective: *A questionnaire study was conducted to evaluate the applications of plants and herbal products consumed by diabetic patients for the treatment of chronic health conditions. Evaluation of the questionnaire confirmed that olive leaf is one of the most used herbs in the treatment of diabetes, in line with its traditional use in the literature. In vitro biological activity studies were performed to determine whether different olive leaf samples have antidiabetic effects. Additionally, the major component oleuropein was quantitively determined in the samples.*
Material and Method: *The established survey was firstly approved by the ethics committee at Gazi University then the survey was conducted at the University Hospital, Department of Endocrinology between January 2021, and July 2021. Based on the result of the survey, pharmacognostic analyses, chromatographic analyses, and inhibition on diabetes-related enzymes (α-amylase, α-glucosidase, and aldose reductase) were performed on the samples of olive leaves collected from nature, obtained from herbalists, markets and pharmacies.*
Result and Discussion: *Evaluation of the survey revealed that the patients mostly used cinnamon (29.3%) and olive leaves (21.7%) for the treatment of diabetes, and these plants were generally obtained from herbalists (51.7%). The study findings showed that aqueous and ethanolic extracts prepared from olive leaf samples contained 190.3-374.3 mg/g oleuropein. The amount of oleuropein in the ready-made olive leaf extract from herbalists was found to be much lower (50.9 mg/g) than the other olive leaf extracts. When the enzyme inhibition activity assays were evaluated, it was determined that all olive leaf samples had inhibitory effects on α-amylase, α-glucosidase, and aldose reductase enzymes. All olive leaf samples, including teas prepared by the public at home with water, were found to have capacity to decrease the blood level in other words antidiabetic activities in vitro. The oleuropein contents detected in this study once again revealed the importance of meticulous examination in herbal products.*
Keywords: *Aldose reductase, α-amylase, α-glucosidase, high pressure liquid chromatography, Olea europea*

ÖZ

Amaç: *Diyabet hastalarının tükettikleri bitki ve bitkisel ürünlerin kronik sağlık durumlarının tedavisine yönelik uygulamalarının değerlendirilmesi amacıyla bir anket çalışması yapılmıştır. Anket sonuçları, literatürdeki geleneksel kullanımına paralel olarak zeytin yaprağının diyabet tedavisinde en çok kullanılan bitkilerden biri olduğunu doğrulamıştır. Farklı zeytin yaprağı*

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örneklerinin antidiyabetik etkisinin olup olmadığının belirlenmesi amacıyla in vitro biyolojik aktivite çalışmaları yapılmış ve ana bileşen oleuropein kantitatif olarak belirlenmiştir.

Gereç ve Yöntem: *Anket için öncelikle Gazi Üniversitesi Etik kurulu tarafından onay alınmış, Ocak 2021-Temmuz 2021 tarihleri arasında Gazi Üniversitesi Endokrinoloji Bölümü'nde uygulanmıştır. Çalışmaya 18 yaş üstü Tip 1 ve Tip 2 diyabetli 200 hasta dahil edilmiştir. Anket sonuçlarından yola çıkarak, doğadan toplanan, aktarlardan, marketlerden ve eczanelerden temin edilen zeytin yaprağı örnekleri üzerinde farmakognozik analizler (makroskopik ve mikroskobik analiz, toplam kül, kuruma kaybı), kromatografik analizler (ince tabaka kromatografisi ve yüksek basınçlı sıvı kromatografisi analizleri) ve diyabetle ilişkili enzimlerin (α-amilaz, α-glukosidaz ve aldoz redüktaz) inhibisyonu çalışmaları yapılmıştır.*

Sonuç ve Tartışma: *Anketin değerlendirilmesinde hastaların diyabet tedavisi için en çok tarçın (%29.3) ve zeytin yaprağını (%21.7) kullandığı ve bu bitkilerin genellikle aktarlardan (%51.7) temin edildiği görülmüştür. Çalışma bulguları, zeytin yaprağı örneklerinden hazırlanan sulu ve etanol ekstraktlarının 190.3-374.3 mg/g oleuropein içerdiğini göstermiştir. Aktarlardan alınan hazır zeytin yaprağı ekstraktındaki oleuropein miktarı diğer zeytin yaprağı ekstraktlarına göre çok daha düşük (50.9 mg/g) bulunmuştur. Enzim inhibisyon aktivite testleri değerlendirildiğinde, tüm zeytin yaprağı örneklerinin α-amilaz, α-glukosidaz ve aldoz redüktaz enzimleri üzerinde inhibitör etkiye sahip olduğu belirlenmiştir. Halk tarafından evde su ile hazırlanan çaylar da dahil olmak üzere tüm zeytin yaprağı örneklerinin in vitro kan seviyesini düşürme yani antidiyabetik aktiviteye sahip olduğu tespit edilmiştir. Bu çalışmada tespit edilen oleuropein içerikleri bitkisel ürünlerde titiz incelemenin önemini bir kez daha ortaya koymuştur.*

Anahtar Kelimeler: *Aldoz redüktaz, α-amilaz, α-glukozidaz, Olea europea, yüksek performanslı sıvı kromatografisi*

INTRODUCTION

Diabetes is a chronic metabolic disease characterized by high blood glucose levels, which occurs because of insufficient insulin production from the pancreas or inability to use the produced insulin effectively in the body [1]. If the blood glucose level is not controlled, it can lead to increased morbidity and mortality with serious complications.

In many countries and Türkiye various plants and plant products are used in the treatment of diabetes due to their antidiabetic effects [2-4]. In a study conducted with 453 Type 2 diabetes patients in Nigeria, 67.3% of the patients were found to use only herbal medicine, and 35.4% of them used herbal medicines together with conventional medicines. As a result of that study, *Vernonia amygdalina* Delile, *Moringa oleifera* Lam., *Ocimum gratissimum* L., *Picralima nitida* T. Durand & H. Durand plants and mixtures containing these plants were determined to be among the most preferred plants. The data showed that herbal medicine use was associated with age, education level, occupation, duration of diabetes mellitus symptoms, diabetes management style, positive history of diabetes, and presence of diabetes complications [4]. In another study conducted in Nigeria, Aloe vera, garlic, and ginger were determined to be used differently from these plants [5].

In Thailand, more than half (61%) of diabetic patients, who applied to endocrine clinic reported that they used herbal products. Patients mostly used turmeric, bitter gourd, reishi mushroom, ginseng, and cinnamon [6]. In the USA, it was reported that adults with diabetes preferred herbal treatments (56.9%) the most among complementary and alternative medicine applications [7]. In eastern Morocco, more than half (54.8%) of 279 diabetes patients used herbal supplements. The most used are *Salvia officinalis* L., *Trigonella feoenum graecum* L., *Olea europea* L., *Artemisia herba-alba* Asso, and *Origanum vulgare* L. plants/products [8]. In Iran, it was determined that 54% of 500 Type 2 diabetes patients used at least one plant and the most used plant was cinnamon (24%) [9]. In a study of 519 Type 2 diabetes patients in Serbia, 94.5% of women and 82.3% of men used herbal dietary supplements in addition to the prescribed treatment. While women mostly used garlic and St. John's Wort based products, men preferred ginseng and cinnamon based products [10].

In Turkiye, a study conducted on 453 adult diabetic patients, showed that 46.1% of the patients utilized complementary and alternative medicine applications. The most preferred application is the use of herbal products containing black cumin (26.6%), cinnamon (23.3%), and olive leaves (12.5%) [11]. In a study investigating the use of herbal products in 150 adult diabetic patients who applied to the

endocrine clinic, it was determined that 22% of the patients used herbal products. It was reported that the most used herbal products were cinnamon (5.3%), lemon (4.7%), pomegranate syrup (3.3%), and green tea (2.7%). Other herbal products applied included almond, yarrow, sage, olive leaf tea and black cumin oil [12]. In a study conducted with 120 Type 2 diabetes patients, it was found that 52.1% of the patients used herbal products after being diagnosed with diabetes [13]. In a study conducted with 193 adult Type 2 diabetes patients, it was determined that this rate was 30.1% and the most used were cinnamon (25.9%) and other herbal mixture products [14].

Since Turkiye is rich in plant diversity, people living in rural areas collect plants from nature and use them in line with the knowledge from the past [15]. In cities, the way to obtain plants is usually herbalists. Many herbs are offered for sale by herbalists in Turkiye, considering that they will cure [16-18]. In a study, it was determined that 142 medicinal and aromatic plant species were sold in 20 different herbalists visited in Adana and diabetes was among the uses of these plants. In the study, it was found that the herbs were sold without a standard packaging, and without a labeling system. Moreover, recommendations for the use of herbal products do not fully coincide with the literature [16]. Studies have shown that herbalists are not academically educated on medicinal plants [19], and they mostly get information via the internet [20].

The aim of this study is to conduct a survey on Type 1 and Type 2 diabetes patients to determine the plants that were used the plants that were used among the public for the treatment of diabetes. Moreover, pharmacognostically examine the plants/plant products according to the survey results. As a result of the survey, olive leaf was determined, as one of the most used plants for diabetes in Turkiye. The comparative analyses were conducted on the olive leaf plant samples collected from nature, sold in herbalists and pharmacies. Pharmacognostic analyses (macroscopic and microscopic analysis, total ash assay, loss on drying), chromatographic analyses (thin layer chromatography and high-performance liquid chromatography assays), and *in vitro* inhibition activity assays of diabetes-related enzymes (α-amylase, α-glucosidase, and aldose reductase) were performed on the samples.

The olive leaf is the leaf of the *Olea europaea* L. plant from the Oleaceae family. Although *O. europaea* is a widely distributed plant all over the world, it is mostly grown in Mediterranean countries due to its growing conditions. Two varieties of olives present in Turkiye: *O. europaea* L. var. *europaea* Zhukovsky and *O. europaea* L. var. *sylvestris* (Miller) Lehr. which were commercially and traditionally mostly produced in Aegean and Marmara regions such as Aydın, Balıkesir, Canakkale, Hatay, Izmir, Manisa, Mersin and Mugla [21,22]. Traditionally, different parts of the plant have been used for stomach and intestinal diseases, oral hygiene, hypertension, diabetes, bronchial asthma, diarrhea, urinary tract infections, hemorrhoids, and rheumatism [23,24]. The antihypertensive, antihypercholesterolemic, cardioprotective, antidiabetic, antimicrobial, antioxidant, cytotoxic, and hepatoprotective activities of the plant and its components have been demonstrated by scientific studies [25]. In the treatment of diabetes, the leaves are consumed by brewing (infusion) and/or boiling (decoction) [26-30]. The main phytochemical components of *O. europaea* are phenolics and lipids. Phenolic components are phenolic acids (ferulic acid, gallic acid, caffeic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, sinapic acid, syringic acid, and vanillic acid), flavonoids (chrysoeriol and luteolin), phenolic alcohols (hydroxytyrosol and tyrosol), and secoiridoids (oleuropein and verbascoside) [31].

α-Amylase and α-glucosidase enzymes are involved in the breakdown of starch into small monosaccharides. α-Amylase can convert starch to 60% maltose, cleaving α-(1,4) bonds, but not α-(1,6) bonds [32]. α-Glucosidase hydrolytically cleaves disaccharides (maltose and sucrose) into monosaccharides (glucose and fructose) [33]. Inhibition of these enzymes prevents postprandial hyperglycemia by delaying the absorption and digestion of carbohydrate molecules in the gastrointestinal tract. Aldose reductase is involved in the first step of the polyol pathway in glucose metabolism. It catalyzes the conversion of glucose to sorbitol. In diabetic patients, high blood glucose activates the polyol pathway and turns into sorbitol [34]. Sorbitol accumulation causes diabetic complications such as cataracts, nephropathy, neuropathy, and retinopathy. Inhibition of the aldose reductase enzyme has been shown to prevent diabetes complications, especially cataracts and retinopathy [35].

MATERIAL AND METHOD

Materials

A total of four different olive leaf samples were used, two of which were the product containing leaves and two of which were olive leaves. The first of the leaf samples (OLE-N) were collected by the researchers from a 10–15-year-old olive tree in Dortyol, Hatay. The other leaf sample (OLE-H) was obtained from Ankara's best-known herbalist, in a packaged form. One of the samples (EH) containing olive leaves is olive leaf extract in liquid form and was purchased from an herbalist in Ankara. The other olive leaf sample (CP) is a product in capsule form containing standardized olive leaf extract and was obtained from the pharmacy. The definitions of the materials are presented in the tables as follows:

O: Oleuropein;
OLE-N.I: Olive leaf samples collected from nature- infusion extract;
OLE-N.D: Olive leaf samples collected from nature- decoction extract;
OLE-N.E: Olive leaf samples collected from nature- ethanolic extract;
OLE-H.I: Olive leaf samples obtained from herbalists- infusion extract;
OLE-H.D: Olive leaf samples obtained from herbalists- decoction extract;
OLE-H.E: Olive leaf samples obtained from herbalists- ethanolic extract;
CP: Capsule from pharmacy;
EH: Olive leaf extract from herbalist

Survey Study

The survey study is a descriptive cross-sectional study. The study was approved by Gazi University Ethics Committee (E-77082166-604.01.02-35354). It was carried out with diabetes patients who applied to Gazi University Endocrinology Department between January 27, 2021, and July 27, 2021. The study included 200 patients. While patients over the age of 18 with a definite diagnosis of Type 1 or Type 2 diabetes were included, patients with gestational diabetes and pre-diabetes were not included in the study. Questionnaire forms were filled by the researchers through face-to-face interviews.

The questionnaire form was prepared by scanning the relevant literature. The form consists of 38 questions, which is divided into three sections. The first part consisting of 11 questions asking demographical data, patient information; the second part consisting of 9 questions about disease information; the last part consists of 18 questions, about the use of plants / herbal products. Data from the study were analysed with the Statistical Package for the Social Sciences (SPSS) program. The frequency of participant responses is shown in the tables and figures. Chi-square tests performed and crosstabs created to correlate responses. Significance was evaluated at the 95% confidence level, $p<0.05$.

Pharmacognostic Analyses

Macroscopic analysis, microscopic analysis and total ash amount determination, loss in drying experiments were performed on olive leaf samples collected from nature and obtained from herbalists. Experiments were carried out in accordance with the '*Oleae folium*' monograph in European Pharmacopoeia 8.0.

The general appearance, size and color of the samples were determined within the scope of macroscopic analysis. For microscopic analysis, olive leaf samples collected from nature and obtained from herbalists were pulverized and colors saved. Later, examined under a microscope (Leica DM500 binocular microscope, objectives x10 and x40) with chloralhydrate solution. For total ash, empty crucibles were heated in a muffle furnace at 600ºC, cooled in a desiccator and brought to constant weight. Samples weighing around 1.00 g were placed in empty crucibles and burned in a muffle furnace at 600ºC. The crucibles removed from the muffle furnace were taken to the desiccator, cooled, and weighed after constant weight. The difference between the weighings before and after combustion was calculated. 3 parallel experiments were performed for each sample. For loss on drying, samples weighing around 1.0 g were taken into cups and dried in an oven at 105 ºC for 2 hours. At the end of the period, the removed cups from the oven were cooled in the desiccator, brought to a constant weight, and weighed. The difference between the weighings before and after drying was calculated. 3 parallel

experiments were performed for each sample.

Preparation of Extracts from Olive Leaf Samples

Ethanolic and aqueous extracts of *O. europaea* leaves were prepared from the leaf samples collected from Hatay-Dortyol and purchased from the herbalist. Olive leaves were ground with a grinder in large and small sizes. For the ethanolic extract, 500 ml of pure ethanol was added to 50 g of the samples and macerated for 3 days at room temperature by shaking on an orbital shaker. At the end of the maceration, the extracts were filtered and concentrated in a rotavapor under reduced pressure at 40-45°C. For infusion, 450 ml of boiling distilled water was added to 50 g of the samples, shaken, and filtered. For decoction, 450 ml of room temperature distilled water was added to 50 g of the samples and boiled. Aqueous extracts were concentrated using a lyophilizer. Olive leaf extract obtained from the herbalist as a ready-made liquid extract was first concentrated in the rotavapor. The concentrated semi-solid extract was dissolved with methanol and used in the analysis. Olive leaf extract purchased from the pharmacy as a capsule was prepared by dissolving the capsule contents with methanol.

Chromatographic Analysis

Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out in accordance with the '*Oleae folium*' monograph in European Pharmacopoeia 8.0.

Test solution: Extracts obtained from olive leaves, prepared extract and capsule contents were dissolved with methanol and applied.

Reference solution: Oleuropein dissolved in methanol

Plate: TLC silica gel plate

Mobile phase: Distilled water: methanol: dichloromethane (1,5:15:85 v/v/v)

Application: as 10 µl strips

Drift: More than 10 cm

Drying: In Air

Detection: Vanillin reagent R was sprayed, heated at 100-105°C for 5 min and examined in daylight

HPLC Analysis

Oleuropein content in olive leaf samples was determined by HPLC. Agilent model instrument and C18 (150 ×4.6 mm, 5 µm) column were used for HPLC. The analysis conditions used for oleuropein quantification were determined by modifying the studies in the literature. The mobile phase was water containing formic acid (0.1%) and acetonitrile (85:15). The mobile phase flowed at a rate of 1 ml/min for 30 minutes. Analysis was carried out at 320 nm. LOD (the smallest detectable amount) and LOQ (the lowest concentration that can be measured with acceptable accuracy and repeatability) values for the analysis were calculated. The calibration equation was found for standard oleuropein. The oleuropein content of olive leaf samples were determined using this equation.

***In vitro* Activity**

The inhibition effects of different concentrations of olive leaf samples on α-amylase, α-glucosidase and aldose reductase enzymes were investigated.

For α-amylase enzyme inhibition assay, the experimental method was created by modifying the *in vitro* method used by Eruygur et al. 50 µl of amylase enzyme (0.06 M NaCl, in 0.02 M, pH 6.9 phosphate buffer) was added to the plant extracts at different concentrations and incubated at 37°C for 10 min [36]. Then, 50 µl of starch solution was added and incubated for 10 min. Starch solution was prepared in distilled water at a concentration of 0.05%. After incubation, 25 µl of HCl and 100 µl of lugol solution were added. The absorbance was measured spectrophotometrically at 540 nm. Buffer was used as a control instead of extract. The reference substance was acarbose. Inhibition levels of plant extracts on amylase enzyme were evaluated *in vitro* using the following equation:

$$\text{Inhibition (\%)} = [1 - (\text{Absorbance (sample)} / \text{Absorbance (control)}] \times 100$$

Absorbance (sample): The absorbance value of the samples at 540 nm
Absorbance (control): The absorbance value of the control at 540 nm

For α-glucosidase enzyme inhibition assay, the experimental method was created by modifying the *in vitro* method used by Eruygur et al [36]. 50 µl of α-glucosidase enzyme was added to the plant extracts at different concentrations and incubated at 37°C for 5 minutes. Then, 50 µl of p-nitrophenyl-α-D-glucopyranoside was added and incubated at 37°C for 30 minutes. The reference substance was acarbose. At the end of incubation, absorbances were read at 405 nm. The inhibition effect of plant extracts on the α-glucosidase enzyme was calculated as follows:

$$\text{Inhibition (\%)} = [1 - (\text{Absorbance (sample)} / \text{Absorbance (control)}] \times 100$$

Absorbance (sample): The absorbance value of the samples at 405 nm
Absorbance (control): The absorbance value of the control at 405 nm

The aldose reductase enzyme inhibition effect of the samples was determined by modifying the *in vitro* method used by Hayman and Knoshita [37]. Homogenizer obtained from rabbit lenses was used as the source of aldose reductase enzyme. Rabbit lenses were homogenized in 100 mM phosphate buffer (pH 6.9) and was prepared a 10% homogenizer. Centrifuged at 10,000 rpm at 4°C for 20 min. The supernatant was stored at -20°C and used in the aldose reductase inhibition experiment. Plant extracts, 25 µl of NADPH and 25 µl of homogenate were added to 100 µl of potassium buffer. The mixture was incubated at 37°C for 5 minutes. DL-glyceraldehyde was added at the end of the incubation. NADPH change was followed at 340 nm at 37°C for 15 min. Quercetin was used as a positive control. The inhibition effect of the samples on the aldose reductase enzyme was calculated as follows:

$$\text{Inhibition (\%)} = [1-(\Delta A \text{ sample/min} / \Delta A \text{ control/min})] \times 100$$

ΔA sample/min: Absorbance change of the samples at 340 nm in one minute
ΔA control/min: Absorbance change of the control at 340 nm in one minute

RESULT AND DISCUSSION

Survey Results

The mean age of 200 individuals participating in the study was 49.59±15.22 and ranged from 18 to 84. More than half of the participants are women (59.0%), most of them are married (79.5%) and they usually live in the city center (77.0%). Twenty eight percent of individuals are high school graduates and 26.0% are university graduates. The number of people having monthly income between 2000 and 5000 is more than half of the participants (55.0%). The individuals (55.0%) reported that they had chronic diseases other than diabetes, additionally 83.0% of them reported that they used regular drugs. While most of the respondents do not consume cigarettes and alcohol, only 16.5% of individuals exercise regularly. The demographic characteristics of the participants are shown in Table 1.

Of the individuals participating in the study were found to have the number of Type 1 diabetes patients is 27.0% and a Type 2 diabetes was determined as 73.0%. Nearly half of the patients reported that they used only oral antidiabetic on the other hand 18.5% reported that they used insulin as well as oral antidiabetics, 38.0% of the participants were treated in the hospital due to diabetes. Only, 12 patients stated that they did not use any medication for diabetes treatment. the most common complication was diabetic retinopathy (26.3 %). Diabetic neuropathy (24.2%), cardiovascular disease (12.6%) and diabetic foot (12.6%) are among other common diabetes complications. 53.0% of the participants reported that they received education on diabetes. Detailed information about individuals' diabetes diseases is included in Table 2.

Herbal and herbal product usage information of the participants is shown in Table 3. Only 39.0% of individuals reported that they used plants/herbal products to support diabetes treatment. The most used herbs were cinnamon (29.3%), olive leaves (21.7%) and black cumin (16.8%). Garlic, nettle, rosehip, blueberry, ginseng, bitter melon, mahaleb and fenugreek are plants used to support diabetes

treatment.

Table 1. The demographic characteristics of the participants

	Number of people (n)	Percentage (%)
Gender		
Female	118	59.0
Male	82	41.0
Marial status		
Married	159	79.5
Unmarried	41	20.5
Education level		
Illiterate	11	5.5
Literate	7	3.5
Primary school graduate	42	21.0
Secondary school graduate	23	11.5
High school graduate	56	28.0
University graduate	52	26.0
Postgraduate (Master's/PhD)	9	4.5
Monthly income		
2000 or less	31	15.5
2000-5000	110	55.0
5000-10000	44	22.0
10000-15000	12	6.0
15000 and higher	3	1.5
Residential area		
City center	154	77.0
District	41	20.5
Other	5	2.5
Chronic disease other than diabetes		
Yes	110	55.0
No	90	45.0

Table 2. Information about the diabetes diseases of the participants

	Number of people (n)	Percentage (%)
Diabetes type		
Type 1	54	27.0
Type 2	146	73.0
Diabetes treatment		
Does not use medication	12	6.0
Only oral antidiabetic	96	48.0
Only insulin	55	27.5
Both oral antidiabetics and insulin	37	18.5
Have you been hospitalized due to diabetes?		
Yes	76	38.0
No	124	62.0
Do you have a complication due to diabetes?		
Yes	54	27.0
No	146	73.0

Table 2 (*continue*). Information about the diabetes diseases of the participants

	Number of people (n)	Percentage (%)
Which of the chronic complications of diabetes do you have?		
Diabetic retinopathy	25	26.3
Diabetic neuropathy	23	24.2
Cardiovascular disease	12	12.6
Diabetic foot	12	12.6
Diabetic nephropathy	11	11.6
Peripheral vascular disease	9	9.5
Liver disease	2	2.1
Cerebrovascular attack	1	1.1
Have you been trained in diabetes?		
Yes	106	53.0
No	94	47.0

Unlike these herbs, two patients reported that they mixed ginger and turmeric with yogurt for their diabetes. While 38.4% of the patients stated that they consumed these herbs every day, the rate of patients who consumed them 1-3 days a week was 26.0 %. About one-third (31.6%) of the patients using the herbs used it for one to three months. Although some participants used prescription and herbal medicines together, only 9.5% reported such use to a healthcare professional. Nearly half of the patients who preferred to use herbs determined the amount of the herbs by eye. Most patients (94.9%) did not change the dose of their current medication while using the herb. Diabetes patients learned from their neighbors and friends (28.9%) that they could use herbs for support, and more than half (51.7%) obtained herbs from herbalists and spice shops.

Table 3. Herbal and herbal product usage information of the participants

	Number of people (n)	Percentage (%)
Do you use herbs/herbal products to support diabetes treatment?		
Yes	78	39.0
No	122	61.0
Which herbs/herbal product did you use to support diabetes treatment?		
Cinnamon	54	29.3
Olive leaf	40	21.7
Black cumin	31	16.8
Garlic	14	7.6
Dead nettle	8	4.3
Rosehip	7	3.8
Blueberry	5	2.7
Ginseng	4	2.2
Bitter melon	4	2.2
Mahaleb	4	2.2
Fenugreek	2	1.1
Other	11	6.0
What is the frequency of your use of herbs/herbal products to support diabetes treatment?		
Every day	28	38.4
1-3 days a week	19	26.0
4-6 days a week	9	12.3
Biweekly	10	13.7
Sometimes	7	9.6

Table 3 (*continue*). Herbal and herbal product usage information of the participants

	Number of people (n)	Percentage (%)
How long did you use a herbs/herbal product for supporting diabetes treatment?		
Less than one month	22	28.9
One-three months	24	31.6
Three-twelve months	8	10.5
More than one year	22	28.9
How did you adjust the dose of the herbs/herbal product you use to support diabetes treatment?		
Sense of proportion	36	46.2
Teaspoon	20	25.6
Handful/ pinch	11	14.1
Tablet/capsule/pill	6	7.7
Sensitive scales	2	2.6
Other	3	3.8
Where do you get the plant/herbal product you use for supporting diabetes treatment?		
Herbalist, Spice	62	51.7
Village/Country	19	15.8
Pharmacy	15	12.5
I gathered it myself	8	6.7
Market, supermarket	8	6.7
Internet, television	7	5.8
Other	1	0.8
From whom/where did you learn that the herbs/herbal products you use can be used in your disease?		
Neighbor, friend	37	28.9
Internet, television	35	27.3
Relatives, family elders	29	22.7
Pharmacist	16	12.5
Doctor	5	3.9
Health personnel	4	3.1
Other	2	1.6
Have you made any changes in the dosage of the current medications you use while using herbs/herbal products for support of diabetes treatment?		
Yes	4	5.1
No	75	94.9
Do you think that side effects occur in your body depending on the herbs/herbal product you use to support diabetes treatment?		
Yes	5	6.3
No	74	93.7
Have you shared with your doctor that you are using herbs/herbal products?		
Yes	19	24.1
No	60	75.9

Patients were also asked about diabetes-related wounds and diabetic foot, one of the common and important complications of diabetes (Table 4). Most of the patients (80.3%) reported that they would consult a doctor in case of inflamed wounds on their feet. The patients who stated that they could use herbs/herbal products in case of foot wounds constituted only 9.2% of the respondents. Approximately one-third (30.3%) of patients with diabetes-related wounds used herbs/herbal products to treat their wounds. The most preferred ones are St. John's Wort oil and olive oil. Patients generally used the herbals

they preferred every day (40.9%) and for less than one month (68.2%).

Table 4. Questions about diabetic wound

Question	Number of people (n)	Percentage (%)
What do you do if you have an inflamed wound on your foot?		
I will go to doctor	192	80.3
I apply/use herbs/herbal products	22	9.2
I apply vaseline	14	5.9
I apply ice to my feet	5	2.1
Other	6	2.5
Do you have sores on your feet, mouth or any part of your body?		
Yes	43	21.5
No	157	78.5
Have you used herbs/herbal products to treat wounds on your body?		
Yes	23	30.3
No	53	69.7
Which herbs/herbal products did you use to treat the wounds on your body?		
St. John's Wort oil	16	37.2
Olive oil	10	23.3
Aloe vera gel	7	16.3
Rosemary oil	4	9.3
Calendula flower	3	7.0
Bitter melon	1	2.3
Pomegranate peel	1	2.3
Coconut oil	1	2.3
What is the frequency of use of herbs/herbal products for your wounds on your body?		
Every day	9	40.9
1-3 days a week	6	27.3
Biweekly	5	22.7
Other	2	9.1
How long did you use the plant/herbal product for your wounds on your body?		
Less than one month	15	68.2
One-three months	6	27.3
More than one year	1	4.5

As a result of the cross-statistical analysis, the characteristics of the patients who used the plant for diabetes diseases were determined (Table 5). Gender, type of diabetes and number of years with diabetes were effective in plant use ($p<0.05$) (Figure 1). Women and Type 2 diabetes patients applied to plants more. Patients turned to plants in the first years after the diagnosis of diabetes, and interest in plants decreased in the following years ($p=0.05$). Individuals who are married, high school graduate, income between 2000-5000, living in the city center, using only oral antidiabetic and without diabetes-related complications used plants more. On the other hand, these cases were not found statistically significant ($p>0.05$). The distribution of the use of plants determined to be used because of the survey according to diabetes type is given in Table 6. It has been determined that cinnamon, olive leaves and black cumin, which are the most used plants, are often preferred by Type 2 diabetes patients.

Table 5. Characteristics of patients using plants for the treatment of diabetes

		Number and percentage of people using plants n (%)	*p value*
Gender	Female	54(69.2%)	$p<0.05$
	Male	24(30.8%)	
Marital status	Married	67(85.9%)	$p>0.05$
	Unmarried	11(14.1%)	
Education level	Illiterate	1(1.3%)	$p>0.05$
	Literate	4(5.1%)	
	Primary school graduate	19(24.4%)	
	Secondary school graduate	9(11.5%)	
	High school graduate	22(28.2%)	
	University graduate	18(23.1%)	
	Postgraduate (Master's/PhD)	56.4(%)	
Monthly income	0-2000	11(14.1%)	$p>0.05$
	2000-5000	41(52.6%)	
	5000-10000	20(25.6%)	
	10000-15000	6(7.7%)	
	15000 and higher	0	
Residential area	City center	55(70.5%)	$p>0.05$
	District	21(26.9%)	
	Other	2(2.6%)	
Diabetes type	Type 1	15(19.2%)	$p<0.05$
	Type 2	63(80.8%)	
Diabetes duration	0-5 years	24(30.8%)	$p=0.05$
	5-10 years	23(29.5%)	
	10-15 years	12(15.4%)	
	15-20 years	9(11.5%)	
	20-25 years	4(5.2%)	
	25-30 years	6(7.7%)	
Diabetes treatment	Does not use medication	2(2.6%)	$p>0.05$
	Only oral antidiabetic	44(56.4%)	
	Only insulin	16(20.5%)	
	Both oral antidiabetics and insulin	16(20.5%)	
Presence of complications	Yes	24(30.8%)	$p>0.05$
	No	54(69.2%)	

Table 6. Distribution of the use of plants determined to be used as a result of the survey according to diabetes type

	Diabetes type n(%)	
Plant name	**Type 1**	**Type 2**
Cinnamon (n=54)	13(24.1%)	41(75.9%)
Olive leaf (n=40)	6(15.0%)	34(85.0%)
Black cumin (n=31)	5(16.1%)	26(83.9%)
Garlic (n=14)	4(28.6%)	10(71.4%)
Dead nettle (n=8)	1(12.5%)	7(87.5%)
Rosehip (n=7)	1(14.3%)	6(85.7%)
Blueberry (n=5)	1(20.0%)	4(80.0%)
Ginseng (n=4)	4(100.0%)	0
Bitter melon (n=4)	1(25.0%)	3(75.0%)
Mahaleb (n=4)	0	4(100.0%)
Fenugreek (n=2)	1(50.0%)	1(50.0%)

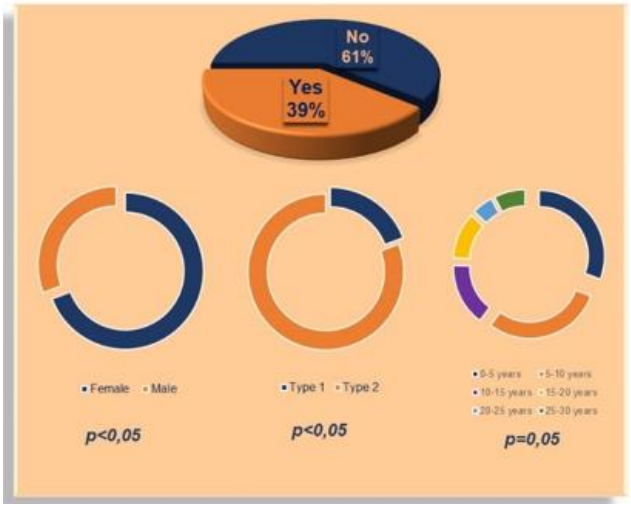

Figure 1. Relationship between plant use and gender, type of diabetes, number of years with diabetes

Pharmacognostic Analysis Results

Olive leaf samples collected from nature and purchased from the herbalist have similar characteristics in terms of general appearance (Figure 2). The leaves have a long thin appearance of 5-6 cm long, 0,5-1 cm wide. The leaves are lanceolate. The upper surface of the leaves is dark green, the lower surface is grayish and has a hairless and skinny structure. The leaf edges are curled due to drying.

It was determined that the colors of the powdered olive leaf samples for microscopic analysis were yellowish green. As stated in the European Pharmacopoeia 8.0, abundant peltate trichomes were observed in the microscope examination of the samples examined with chloralhydrate. Other elements detected microscopically are epiderma and parenchymatic parts, and stone cells like sclerenchyma bundles. Microscope images are presented in Figure 3.

The total ash content and the loss on drying results of olive leaf samples are presented in Table 7. According to European Pharmacopoeia 8.0, the total ash content of olive leaf samples should be at most 9%, and the loss on drying of olive leaf samples should be at most 10%.

Figure 2. Olive leaf sample (A. Olive leaf from natura, B. Olive leaf from herbalist)

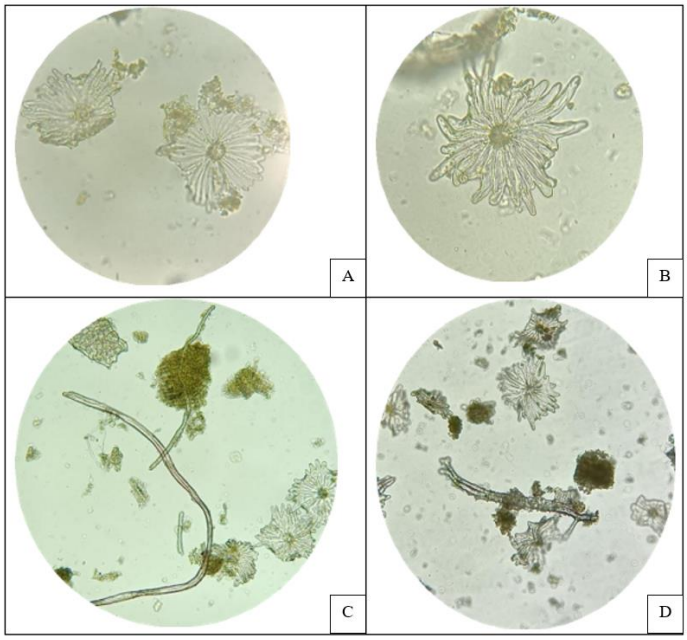

Figure 3. Peltate trichomes (A, B), and stone cells like sclerenchyma bundle found together with parenchymatic fragments (C, D) detected in olive leaf samples

Table 7. Analysis findings of total ash and loss on drying amount of olive leaf samples

Sample	Total ash content±standard deviation	Loss on drying+ standard deviation
Olive leaf samples collected from nature (OLE-N)	5.57±0.00	3.57±0.07
Olive leaf samples obtained from herbalists (OLE-H)	6.32±0.16	4.69±0.31

Chromatographic Analysis

Thin Layer Chromatography Results

Brownish oleuropein stains were detected because of the reaction developed by spraying the vanillin reagent on the plate after the drift on the plate was completed. The resulting image of the plaque is shown in Figure 4.

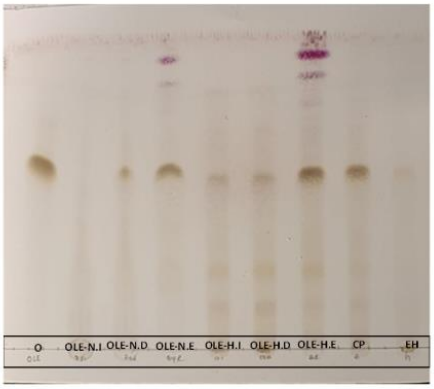

Figure 4. TLC plate image of olive leaf samples

*O: Oleuropein; OLE-N.I: Olive leaf samples collected from nature- infusion extract; OLE-N.D: Olive leaf samples collected from nature- decoction extract; OLE-N.E: Olive leaf samples collected from nature- ethanolic extract; OLE-H.I: Olive leaf samples obtained from herbalists- infusion extract; OLE-H.D: Olive leaf samples obtained from herbalists- decoction extract; OLE-H.E: Olive leaf samples obtained from herbalists- ethanolic extract; CP: Capsule from pharmacy; EH: Olive leaf extract from herbalist

HPLC Analysis

The major component oleuropein was detected by HPLC analysis. The equation to be used for oleuropein quantification was found to be y = 0.6576x-2.6542 (R^2 = 0.99). LOD and LOQ values determined as 1.63 and 4.94 respectively.

The results of oleuropein assay analysis of olive leaf samples are shown in Table 8 and Figure 5. As a result of the analysis, the amount of oleuropein in olive leaf extracts varied between 19-38%. The amount of oleuropein in the ethanolic extracts of olive leaves was higher than in the aqueous extracts. Olive leaf capsules obtained from the pharmacy have higher oleuropein content than olive leaves prepared by infusion method. Compared to olive leaf extracts, the oleuropein content of the liquid olive leaf extract obtained from herbalists is quite low.

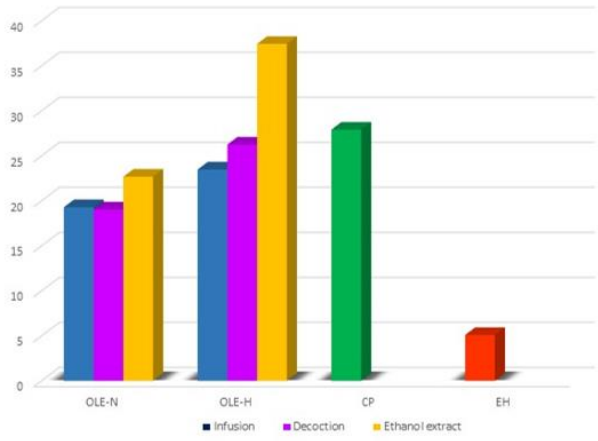

Figure 5. Percent oleuropein content of olive leaf samples

Table 8. Results of oleuropein assay analysis of olive leaf samples

Sample name		The amount of ppm oleuropein found in 100 ppm extract	mg amount of oleuropein (mg/g) in 1 gram extract
Olive leaf samples collected from nature (OLE-N)	Infusion	19.29±1.96	192.90±19.62
	Decoction	19.03±3.05	190.31±30.54
	Ethanolic extract	22.67±4.67	226.75±46.70
Olive leaf samples obtained from herbalist (OLE-H)	Infusion	23.47±6.04	234.76±60.47
	Decoction	26.24±2.98	262.45±29.86
	Ethanolic extract	37.42±4.20	374.29±42.09
Capsule from pharmacy (CP)		27.90±3.52	279.04±35.20
Olive leaf extract from herbalist (EH)		5.09±1.84	50.95±18.45

In vitro Enzyme Inhibition Effects

All the olive leaf samples showed high α-amylase enzyme inhibition comparable to the reference substance. Analyses are shown in Table 9. Extracts of olive leaves collected from nature and obtained from herbalists exhibited similar degree of inhibition of α-amylase enzyme. Ethanolic extracts provided higher inhibition than aqueous extracts prepared with different techniques. The olive leaf capsule obtained from the pharmacy and the olive leaf extract obtained from the herbalist in liquid form showed similar inhibition to the aqueous extracts.

The results of α-glucosidase enzyme inhibition of olive leaf samples are shown. According to the results of the analysis, all the extracts exhibited very high α-glucosidase enzyme inhibition. Although the solvent used in the preparation of the extracts affected the inhibition rate, large differences were not detected between the results. In addition to the extracts, the capsule obtained from the pharmacy and the liquid extract obtained from the herbalist were also found to be effective on the α-glucosidase enzyme.

The aldose reductase enzyme inhibition findings of olive leaf samples are given Although the extracts of olive leaf samples provided lower inhibition than quercetin, the activities of especially ethanolic extracts were high enough to compare with quercetin. While the lowest inhibition was observed in the infusion extracts of the samples, the activity of the capsule obtained from the pharmacy and the extract obtained from the herbalist on aldose reductase enzyme was also found to be lower than the infusions of the extracts.

Results showed that olive leaf inhibited enzymes associated with diabetes. The mechanism of action of olive leaf on diabetes is presented in Figure 6.

Table 9. Results of enzyme inhibition assays of olive leaf samples

		α-amylase enzyme inhibition (%Inhibition+Standard deviation)	*α*-glucosidase enzyme inhibition (%Inhibition+Standard deviation)			Aldose reductase enzyme inhibition (%Inhibition+Standard deviation)	
Sample name		**0.625 µg/ml**	**2.5 µg/ml**	**5 µg/ml**	**10 µg/ml**	**25 µg/ml**	**50 µg/ml**
Olive leaf samples collected from nature (OLE-N)	Infusion	41.04± 2.57	58.66±0.54	74.04±0.20	82.43±2.83	41.38±0.12	76.02±0.36
	Decoction	48.86±1.77	87.63±0.81	92.97±0.18	95.66±0.38	55.73±0.65	77.40±0.11
	Ethanolic	50.11± 1.42	90.54±0.54	95.07±2.13	95.19±0.21	66.49±0.0	80.90±1.35
Olive leaf samples obtained from herbalists (OLE-H)	Infusion	34.34±0.86	69.47±2.55	71.79±1.50	86.10±1.26	45.50±0.18	68.55±0.26
	Decoction	47.29±1.70	89.48±0.05	94.67±0.13	96.03±1.04	56.75±1.94	75.73±0.01
	Ethanolic	49.35± 2.17	89.34±1.25	95.21±0.22	97.10±0.01	79.95±0.63	90.69±0.80
Capsule from pharmacy (CP)		41.29±3.22	84.19±1.46	90.55±2.47	95.47±0.74	42.64±1.45	71.73±0.70
Olive leaf extract from herbalist (EH)		33.15± 1.05	83.07±0.16	91.18±0.29	95.59±0.13	38.89±0.95	69.88±2.77
Reference substance		21.80± 3.40 (Acarbose)	88.37±1.72 (Acarbose)	96.15±0.93 (Acarbose)	99.99±0.01 (Acarbose)	87.46±0.01 (Quercetin)	98.87±0.10 (Quercetin)

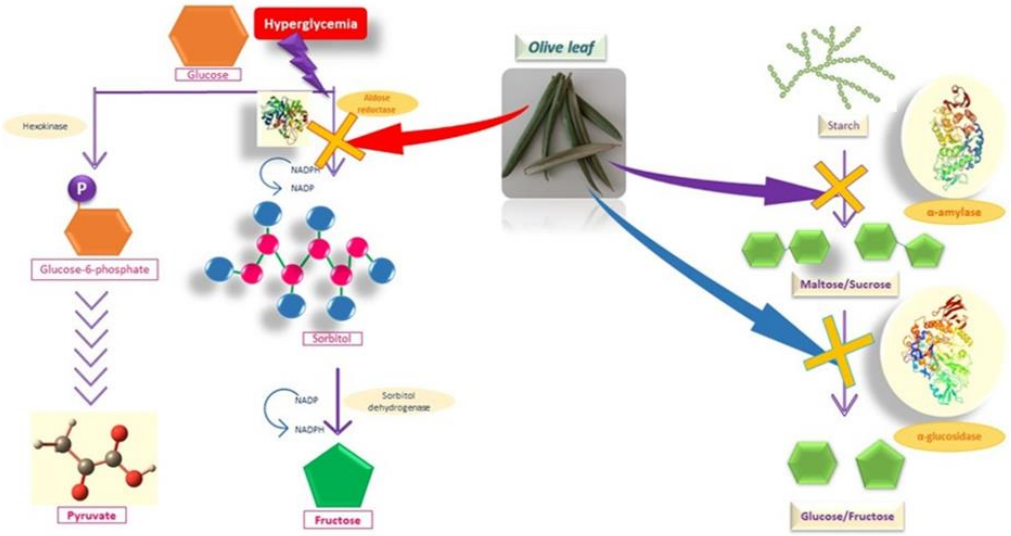

Figure 6. The mechanism of action of olive leaf on diabetes

Diabetes is a chronic disease that affects the whole world, the incidence of which is increasing day by day. After choosing this common disease as a subject, the plants to be used were determined by a survey conducted with diabetes patients. Based on the data of the survey study, the plants and the analyses to be made on them were decided.

Type 2 diabetes, which is the most common type of diabetes among all diabetes types, is also the most detected type among the survey study participants. This may be due to factors such as modern lifestyle, dietary habits, more limited physical work due to increased mental workloads, and sedentary life. The fact that most of the participants (83.5%) do not exercise regularly proves these possibilities.

As a result of the survey study, it was determined that the patients who use plants for the treatment of diabetes mostly prefer cinnamon, olive leaves and black cumin. These plants preferred by patients showed similarities with previous survey studies [9-12]. Analyses showed that there was a significant relationship between plant use and gender, type of diabetes, and duration of diabetes. Like previous studies, women preferred plants more than men in this survey study [10]. The fact that diabetic patients prefer plants more in the first years of their diabetes made us think that plants are the first-choice treatment. On the other hand, it has been determined that individuals who do not have complications prefer plants more. Considering that diabetes complications occur over time because of high blood sugar values, the survey data are compatible among themselves.

The reason for the patients who did not prefer plants was that they thought that they were ineffective. On the other hand, patients using herbal medicine mostly did not inform their doctors about their use, determined the number of herbs they used with the rule of thumb, and did not change the doses of their conventional drugs. The reason why patients cannot share their herbal use with their doctors may be because they think that herbs are completely harmless or because they are afraid of a negative attitude from the doctor. But the common point that can be deduced from all these data diabetes patients do not have enough correct information about herbs/herbals. The fact that diabetic patients learned the use of herbs from their neighbors and friends rather than from health professionals revealed that not only the patients but also the individuals around them should be educated about herbs. Similarly, the fact that herbs are frequently procured from herbalists/spices has once again revealed that herbalists/spices need to be under stricter control.

According to the results of the analysis, the leaves of the olive plant are the second most used plant for the treatment of diabetes and the oil obtained from the olive plant was used in patients with diabetes wounds as complications. In addition to these, the olive plant was chosen as the study subject because it grows in Turkiye. Since the plants used by the patients are mostly obtained from herbalists,

pharmacognostic analyses such as macroscopic, microscopic, TLC, total ash amount determination, and loss on drying were carried out on both samples collected from nature and obtained from the herbalist.

When the pharmacognostic analysis results of olive leaf samples were evaluated, it was determined that olive leaf samples collected from nature and obtained from herbalists had similar macroscopic and microscopic results, and these results were in accordance with the '*Oleae folium*' monograph in the European Pharmacopoeia 8.0. Similarly, the determination of the total ash amount of the samples and the loss findings on drying are below the maximum values specified in the monograph and are following the pharmacopoeia.

Oleuropein content of olive leaf samples were determined to be between 19-38%. It is known that the oleuropein content of olive leaves varies depending on factors such as collection time. Olive leaf methanol extract was found to contain 40.33% oleuropein in the study of Hayes et al [38]. The oleuropein amounts of the olive leaf samples in this study are not contrary to the literature findings. On the other hand, the oleuropein content of the liquid olive leaf extract obtained from herbalists is lower than a quarter of the extracts prepared by the infusion method of other samples.

Chigurupati et al. investigated the activity of *O. europaea* leaves extracted using ethanol on α-amylase and α-glucosidase enzymes [39]. As a result of the analyses, the IC_{50} value of the reference substance acarbose for the α-amylase assay was determined as 20.06±0.19 µg/ml and the IC_{50} value of the ethanolic extract was 37.99 µg/ml. In the analysis results of Ahamad et al., olive leaf extract showed inhibitory activity close to acarbose (acarbose IC_{50}: 91.04±2.16 µg/ml and extract IC_{50}: 121,8±3,18 µg/ml) [40]. Javed et al. reported that olive leaf extract also has inhibitory effect on α-glucosidase enzyme, but this effect is lower than amylase (Acarboz IC_{50}: 116.5±2.17 µg/ml and extract IC_{50}: 165.04±5.27 µg/ml). Like this study, olive leaves showed lower but similar activity than acarbose in our study.

When the literature was examined, only two studies were found about the aldose reductase enzyme inhibition effect of olive leaves. Elimam et al. reported that, methanolic (70%) extract of olive leaf showed an inhibitory effect on the aldose reductase with an IC_{50} value of 65 µg/ml [41]. However, Elimam et al. did not use a reference substance in their study and therefore did not determine the effect of olive leaf compared to the reference substance. Considering that the results of *in vitro* experiments show serious differences according to the working conditions, it would not be correct to make a comparison with this study. Papoti et al. prepared infusion, decoction, and ethanolic extracts of olive leaf in their studies but gave results only for infusion extract (IC_{50}: 26 ± 1 µg/ml). Unlike our study, Papoti et al. used sorbinil, a chemical aldose reductase inhibitor, as a reference substance and found that olive leaf had much lower activity than sorbinil [42]. In our study, the reference substance has a much higher inhibitory effect than olive leaf infusion extracts, but there is a difference in the reference substance used between these studies.

Conclusion

From a general perspective, olive leaf samples were found to have *in vitro* antidiabetic activities. Higher activity of ethanolic extracts in enzyme experiments showed that ethanolic is a good solvent for all three enzymes, and it is effective in revealing the components that cause enzyme inhibition in plant content. On the other hand, it was understood that the tea prepared by the people at home using water was not ineffective. It was determined that the extracts of the plants prepared using only ethanol or water, as well as the samples obtained from herbalists and pharmacies in different forms, had antidiabetic effects *in vitro*.

Although the ready-made liquid extract olive leaf extract showed *in vitro* enzyme inhibition levels close to the other extracts, the amount of oleuropein contained was much lower than the others. These data question the reliability of ready-made preparations obtained from outside the pharmacy. On the other hand, the extracts prepared by us from the olive leaves obtained from the most well-known herbalist in Ankara showed as strong results as the extracts prepared from the olive leaves collected from the nature. Moreover, the content determination results of these samples are like those of the samples collected from nature. The olive leaves samples obtained from the herbalist were beautiful looking, brightly colored, and carefully packaged. This showed that plant selection is also important. The preparations obtained from the pharmacy were food supplements and were not approved by the

Ministry of Health. Despite this, it showed inhibitory activity on diabetes enzymes and there was no problem with its content. This situation suggests that the products sold in pharmacies are chosen more carefully than the products sold outside the pharmacy, even though they are not approved by the Ministry of Health, and that the products offered to the patient/consumer by health workers, namely pharmacists, may be more reliable. Although our current study is sufficient to contribute to the literature and to create a general impression about the importance of the issue with data, it can be expanded with more examples.

AUTHOR CONTRIBUTIONS

Concept: M.M., U.K.C.; Design: M.M., U.K.C.; Control: U.K.C.; Sources: M.M., U.K.C.; Materials: M.M., U.K.C.; Data Collection and/or Processing: M.M., U.K.C.; Analysis and/or Interpretation: M.M., U.K.C.; Literature Review: M.M., U.K.C.; Manuscript Writing: M.M., U.K.C.; Critical Review: M.M., U.K.C.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The study was approved by Gazi University Ethics Committee (E-77082166-604.01.02-35354).

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

CYTOTOXICITY SCREENING AND ANTIOXIDANT CAPACITY ASSESSMENT OF THE INNER PERIANTH SEGMENTS OF 14 RUMEX SPECIES GROWN IN TÜRKİYE

TÜRKİYE'DE YETİŞTİRİLEN 14 RUMEX TÜRÜNÜN İÇ PERİANT SEGMENTLERİNİN SİTOTOKSİSİTE TARAMA VE ANTİOKSİDAN KAPASİTE DEĞERLENDİRMESİ

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ABSTRACT

Objective: *Breast cancer is one of the most prevalent cancer types worldwide. Antioxidant sources may prevent the occurrence of cancer. Natural sources rich in phenolics, thus, may provide alternate agents in the management of breast cancer. Rumex species are widely distributed in Turkish flora. Emerging evidence has pointed out the antitumoral property of Rumex species on a variety of cancer cells. In the present study, we propose to test the ethanolic extracts of the inner perianth segments of 14 Rumex species on four breast cancer cells with different origins. We also demonstrated their toxicity on healthy cells.*
Material and Method: *We performed the resazurin reduction assay to examine the cytotoxicity and toxicity. Furthermore, we determined the phenolic contents of the extracts as an indicator of their antioxidant profile and ascertained their antioxidant activities by DPPH radical, ABTS radical cation scavenging activity and cupric ion-reducing antioxidant capacity assays.*
Result and Discussion: *The ethanolic extracts of the inner perianth segments of Rumex species exhibited remarkable cytotoxicity profiles neither on breast cancer cells nor on healthy H9c2 rat myoblastoma cells. However, they usually displayed strong antioxidant activities due to possessing high phenolic content.*
Keywords: *Antioxidant, breast cancer, cytotoxicity, rumex, total phenol*

ÖZ

Amaç: *Meme kanseri dünya çapında en yaygın görülen kanser türlerinden biridir. Antioksidan kaynaklar kanserin oluşumunu önleyebilir. Dolayısıyla fenolikler açısından zengin doğal kaynaklar meme kanseri tedavisinde alternatif ajanlar sağlayabilir. Rumex türleri Türkiye florasında geniş bir dağılım göstermektedir. Bilimsel çalışmalar Rumex türlerinin çeşitli kanser hücreleri üzerindeki antitümöral özelliğine işaret etmektedir. Bu çalışmada, 14 Rumex türünün iç periant segmentlerinin etanolik ekstrelerinin farklı kökenlere sahip dört meme kanseri hücresi üzerinde test edilmeleri amaçlanmıştır. Ayrıca sağlıklı hücreler üzerindeki toksisiteleri de değerlendirilmiştir.*
Gereç ve Yöntem: *Sitotoksisite ve toksisiteyi incelemek için resazurin redüksiyon yöntemi kullanılmıştır. Ayrıca; antioksidan profillerinin bir göstergesi olarak ekstrelerin fenolik içerikleri belirlenmiş ve DPPH, ABTS radikali süpürücü aktivite ve bakır iyonu redükleyici antioksidan kapasite yöntemleri ile antioksidan karakteristikleri belirlenmiştir.*

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Sonuç ve Tartışma: *Rumex türlerinin iç periant segmentlerinin etanolik ekstreleri, sağlıklı H9c2 sıçan miyoblastoma hücreleri üzerinde toksik olmamasına rağmen, meme kanseri hücreleri üzerinde dikkate değer sitotoksisite profilleri sergilememiştir. Ancak; genellikle yüksek fenolik içeriğe sahip güçlü antioksidan aktiviteler sergilemektedirler.*
Anahtar Kelimeler: *Antioksidan, meme kanseri, rumex, sitotoksisite, total fenol*

INTRODUCTION

Rumex L. (Polygonaceae family) comprises more than 200 species and is widely distributed worldwide, in the northern hemisphere in particular. The genus mostly consists of perennial herbs with strong roots, paniculate inflorescences, and triangular fruits that are coated in the ampliate inner perianth [1,2]. Scientific literature has pointed out that the plants involved in the genus *Rumex* have been used either traditionally as edible plants or for curing several diseases worldwide [1-6]. Emerging data has further revealed the pharmacological activities of *Rumex* extracts or their containing compounds in preclinical experiments. The studies focusing on the determination of the phytochemical composition of *Rumex* species indicated the presence of hundreds of phytochemicals from different chemical classes including anthraquinones, flavonoids, alkaloids, lignans, naphthalenes, stilbenes, tannins and terpenes [1,2].

The Turkish Plant Data Service (TÜBİVES), a biodiversity database of the plants in Türkiye, reported that 31 *Rumex* taxa exist in the flora of Türkiye [7-9]. To date, a number of *Rumex* species grown in Türkiye have been investigated by our group. We have contributed to the scientific knowledge in terms of their phytochemical or biological activity profiles [10-21].

Breast cancer is a life-threatening condition occurring in every country in the world. It is the most prevalent cancer type globally and is reported to cause 685 000 deaths in 2020. Surgery, radiation therapy, hormonal therapies, chemotherapy or targeted biological therapies are currently applied treatment options in breast cancer. Chemotherapy regimens are decided based on the cancer type (i.e., Cancers express estrogen receptor (ER) or progesterone receptor (PR), or overexpress the human epidermal growth factor receptor 2 (HER-2)/neu oncogene… etc.) [22]. Nature provides an indispensable source of new agents that can be used against numerous cancers. Newman and Cragg (2020) stated that only 29% of small molecule drugs approved between 1981 and 2019 were totally synthetic drugs while others are inclusive of natural products, their mimics or derivatives [23]. In the meantime, oxidative stress is involved in the occurrence of many diseases including cancer [24,25]. In fact, cancer initiation and progression have been associated with oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation [26]. Natural products are antioxidant sources due to possessing phenolics-rich phytochemical constituents [27]. Considering the association of cancer with oxidative stress, the investigation of cytotoxic and antioxidant profiles of natural products holds importance. In the 1920s, Essiac tea which is a blend of different herbs, including *Rumex acetosella* L. was promoted as a natural cancer treatment. This information indicates that *Rumex* species may have anticancer potential [28].

In the present study, we examined the cytotoxic profiles of the ethanolic inner perianth extracts of 14 *Rumex* species on breast cancer cells with different origins (i.e., they may include any of ER, PR, HER2/neu or not) and their toxicity profiles on healthy H9c2 rat myoblastoma cells. We further investigated the antioxidant properties of these extracts. Thus, we aim to provide a general overview about the potential of *Rumex* inner perianth extracts against breast cancer.

MATERIAL AND METHOD

Plant Materials

Aerial parts (including inner perianth segments, leaves, and roots) of 14 *Rumex* species, four of which are endemic to Türkiye, Polygonaceae, were collected from different locations of Türkiye in May to July 2018 and May to July 2019. The plants were identified by Prof. Dr. L. Ömür Demirezer and Dr. Pharm. Filiz Boyalı. Voucher specimens have been kept in the Herbarium of Hacettepe University, Faculty of Pharmacy, Ankara, Türkiye (HUEF) under associated HUEF codes. The detailed information

of collected plant samples is indicated below (Table 1).

Table 1. The location and altitude of the places where the investigated *Rumex* species were collected along with their herbarium numbers

Plant taxa	Collection date	Collection site	Altitudes	Herbarium number
Rumex alpinus L.	08.07.2019	Artvin	1100 m	HUEF 22051
Rumex amanus Rech.*	06.06.2019	Muğla-Marmaris Söğüt Village	0 m	HUEF 19043
Rumex caucasicus Rech.	31.05.2019	Uşak-İzmir Road	900m	HUEF 19046
Rumex chalepensis Mill.	02.06.2019	Uşak Kemalöz District	900 m	HUEF 19049
Rumex conglomeratus Murray	12.06.2018	Bursa İznik Gölü Area	85 m	HUEF 19036
Rumex crispus L	04.06.2019	Uşak Kemalöz District	900 m	HUEF 19045
Rumex cristatus DC.	31.05.2019	The area between İzmir-Kemalpaşa and Manisa-Turgutlu	68 m	HUEF 19050
Rumex grasilescens Rech.*	12.06.2018	The area between Bilecik and Kütahya, İnönü Junction	833 m	HUEF 19047
Rumex hydrolapathum Huds.	15.07.2019	Bolu Abant Lake Area	1350 m	HUEF 22057
Rumex sanguineus L.	06.06.2019	Muğla-Marmaris Söğüt Village	0m	HUEF 19051
Rumex tmoleus Boiss.*	13.05.2019	İzmir-Ödemiş, Bozdağ- Ovacık Village	1170 m	HUEF 19037
Rumex obtusifolius subsp. *subalpinus* L.	16.06.2019	Ankara Çayyolu Necatibey District	1042 m	HUEF 19086
Rumex olympicus Boiss.*	13.05.2018	İzmir-Ödemiş, Bozdağ	1190 m	HUEF 22052
Rumex pulcher L.	13.05.2018	İzmir-Ödemiş, Birgi	325 m	HUEF 19039

* Endemic plant

Cell Culture

The cell lines used in this study, their origins and maintenance conditions were previously reported [29]. Various breast cancer cells with different origins (MCF-7, MDA-MB-231, MDA-MB-468 and SKBR-3) and healthy rat cardiomyoblast cells (H9c2) were used for experimental studies.

Chemicals

Gallic acid was purchased from Merck, Türkiye. 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,9-dimethyl-1,10-phenanthroline (neocuproine), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid, quercetin, Folin-Ciocalteu's phenol reagent, potassium persulfate, sodium phosphate, ammonium molybdate, sodium nitro- prusside dihydrate, sulfanilamide, N-(1-naphthyl) ethylenediamine and other chemicals were purchased from Sigma, Türkiye.

Doxorubicin was obtained from Cayman, Türkiye.

Methods

Extracts Preparation

Air-dried and powdered inner perianth segments of the plant substances (5 g) were extracted with ethanol (3 x 50 ml) in a water bath at 40°C, concentrated to dryness under decreased pressure and lyophilized in vacuo. The extracts were dissolved in ethanol to prepare the required concentrations for antioxidant studies. For cytotoxicity studies, the extracts were dissolved in dimethyl sulfoxide (DMSO)

to prepare 10 mM stock solutions as an initial step, then the required concentrations were prepared via stock solution.

Resazurin Reduction Assay

We performed the resazurin reduction assay in order to examine the cytotoxicity of the extracts. The assay depends on the reduction of resazurin to resorufin by viable cells [30]. When non-viable cells lose their metabolic capacity hindering resorufin generation from resazurin, they do not show a blue staining. Briefly, aliquots of 5×10^5 adherent cells were seeded in 96-well-plates and were allowed to attach overnight. In the following, the cells were incubated with or without adding variable concentrations of the test substance to obtain a final volume of 200 µl/well. After 72 h incubation and combining resazurin (Sigma-Aldrich, Türkiye) for 4 h, staining was determined by an Infinite 200 M Plex plate reader (Tecan, Türkiye) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was independently performed thrice, with six parallel replicates each. The protocol has been recently reported [31].

Ascertaining Total Phenolic Contents

Phenolic contents of the inner perianth segments of plants were detected via Folin-Ciocalteau's reagent according to the protocol applied by Arıtuluk et al. (2006) with slight modification [32]. Folin-Ciocalteau's reagent was diluted with distilled water and then this solution was mixed either with the extract or with different concentrations of the reference compound. The mixture was mixed and sodium carbonate (Na_2CO_3) solution was joined. The reaction mixture was maintained at room temperature for 2 hours in darkness, then absorbance was determined at 765 nm. The assay was examined thrice. We used gallic acid as a reference. The total phenolic contents of the inner perianth segments were expressed as gallic acid equivalents (mg/g extract).

Antioxidant Assays

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The previously described method was applied to test DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity [19,33]. Ascorbic acid was used as a positive control.

ABTS (2,2′-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid) Radical Scavenging Activity

The previously described method with slight modifications was performed according to the previously described method to examine Trolox equivalent antioxidant capacity (TEAC) [19,34]. Trolox was used as a positive control.

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

The assay was applied according to the method described by Arıtuluk et al. (2006) with slight modification [32]. Gallic acid was used as a reference. Results were represented as gallic acid equivalents (mg/g extract).

Statistical Analysis

The resazurin reduction assay was carried out independently and performed thrice, with six parallel replicates each. Determination of phenolic content and antioxidant assays were conducted independently and performed thrice. Microsoft Excel was used for the generation of the graphs. The results were expressed as ± standard deviations (SD).

RESULT AND DISCUSSION

Cytotoxicity of the Ethanolic Extracts of *Rumex* Inner Perianth Segments Towards a Variety of Breast Cancer Cells

Our initial aim was to test the ethanolic extracts of *Rumex* inner perianth segments on assorted breast cancer cells with different origins. Therefore, as a preliminary evaluation, we tested all the

extracts at 30 µg/ml to see if they exhibited sufficient cytotoxicity deserving further detailed cytotoxic studies. The extracts were applied to various breast cancer cells including MDA-MB-231, MDA-MB-468, MCF-7 and SK-BR-3. The ethanolic extract of the *R. sanguineus* inner perianth segments exhibited the best cytotoxicity on SK-BR-3 cells among all the extracts with 57.10 ± 1.97% cell viability at 30 µg/ml, while others have relatively poor cytotoxic activity.

The cell viability percentages of the breast cancer cells under the treatment of the ethanolic extracts of *Rumex* inner perianth segments are shown in Table 2 and Figure 1 below.

Table 2. The effects of ethanolic extracts of *Rumex* fruits on breast cancer cells at 30 µg/ml

The ethanolic extracts of *Rumex* fruits	**The cell viability % on MDA-MB-231**	**The cell viability % on MDA-MB-468**	**The cell viability % on MCF-7**	**The cell viability % on SK-BR-3**
Rumex alpinus L.	86.35 ± 7.61	82.25 ± 5.82	84.66 ± 0.91	95.05 ± 13.78
Rumex amanus Rech.*	84.70 ± 9.63	86.73 ± 9.23	79.01 ± 11.18	86.31 ± 7.08
Rumex caucasicus Rech.	81.96 ± 8.16	74.55 ± 4.65	81.54 ± 9.57	81.48 ± 5.51
Rumex chalepensis Mill.	84.47 ± 6.37	80.96 ± 5.64	84.82 ± 10.40	69.76 ± 8.60
Rumex conglomeratus Murray	88.04 ± 12.18	77.47 ± 9.30	100.15 ± 7.85	76.78 ± 11.02
Rumex crispus L.	79.56 ± 7.78	70.91 ± 6.22	79.15 ± 5.42	65.01 ± 3.50
Rumex cristatus DC.	80.18 ± 12.08	73.82 ± 6.85	78.22 ± 10.88	69.03 ± 5.94
Rumex grasilescens Rech.*	85.36 ± 6.98	77.30 ± 2.54	73.12 ± 15.76	74.51 ± 8.41
Rumex hydrolapathum Huds.	80.80 ± 6.93	80.88 ± 7.43	86.96 ± 4.95	73.31 ± 8.23
Rumex sanguineus L.	80.20 ± 9.52	79.32 ± 5.52	74.87 ± 9.32	57.10 ± 1.97
Rumex tmoleus Boiss.*	80.43 ± 3.71	84.04 ± 8.02	88.78 ± 8.20	83.58 ± 9.14
Rumex obtusifolius subsp. *subalpinus* L.	84.35 ± 2.70	73.55 ± 7.05	77.97 ± 4.04	96.05 ± 12.13
Rumex olympicus Boiss.*	85.55 ± 8.63	79.36 ± 11.51	82.51 ± 3.17	83.56 ± 3.60
Rumex pulcher L.	83.99 ± 7.88	82.07 ± 5.01	90.68 ± 7.00	78.58 ± 3.32

* Endemic plant

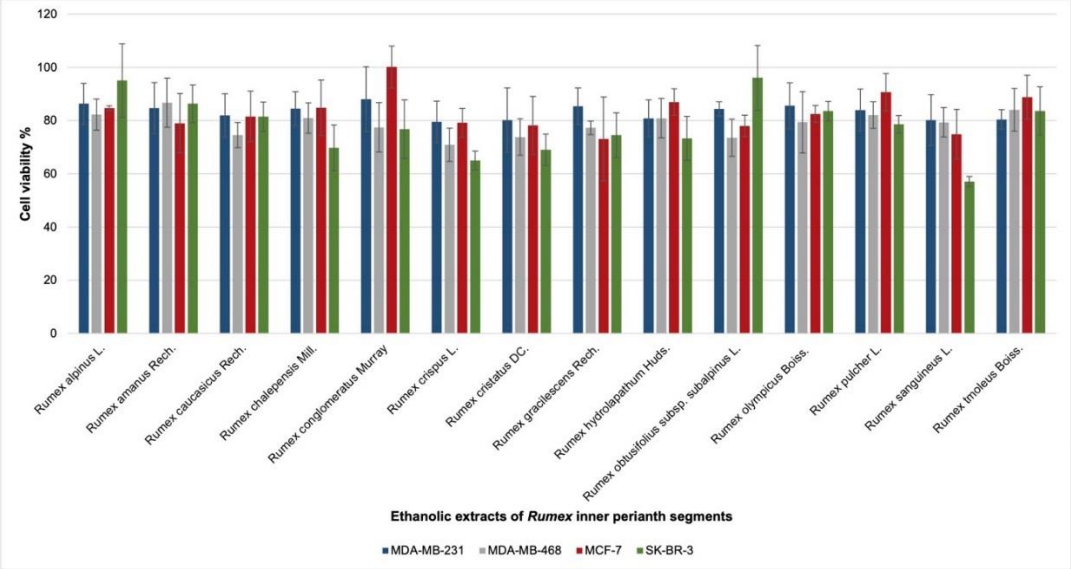

Figure 1. The effects of the ethanolic extracts of *Rumex* inner perianth segments on a variety of cancer cells at 30 µg/ml

Toxicity of the Ethanolic Extracts of *Rumex* Inner Perianth Segments in Normal Cells

We also investigated the toxicity of the ethanolic extracts of *Rumex* inner perianth segments on healthy rat cardiac H9c2 myoblastoma cells. Assessing their toxicity at 30 µg/ml, we observed that the extracts did not exert remarkable toxicity on H9c2 cells (Figure 2). Cardiotoxicity is one of the most common and serious side effects of clinically used chemotherapeutics such as doxorubicin [35-37]. Therefore, our assumption was cytotoxic agents with low toxicity on healthy cells may keep promise either as potential drug leads or as a part of drug combination regimens. Nearly all the tested extracts possessed low toxicity confirming their safety profile in comparison to clinically used doxorubicin which killed nearly half of the cells at the lowest concentrations tested (0.003 µM) (Figure 2).

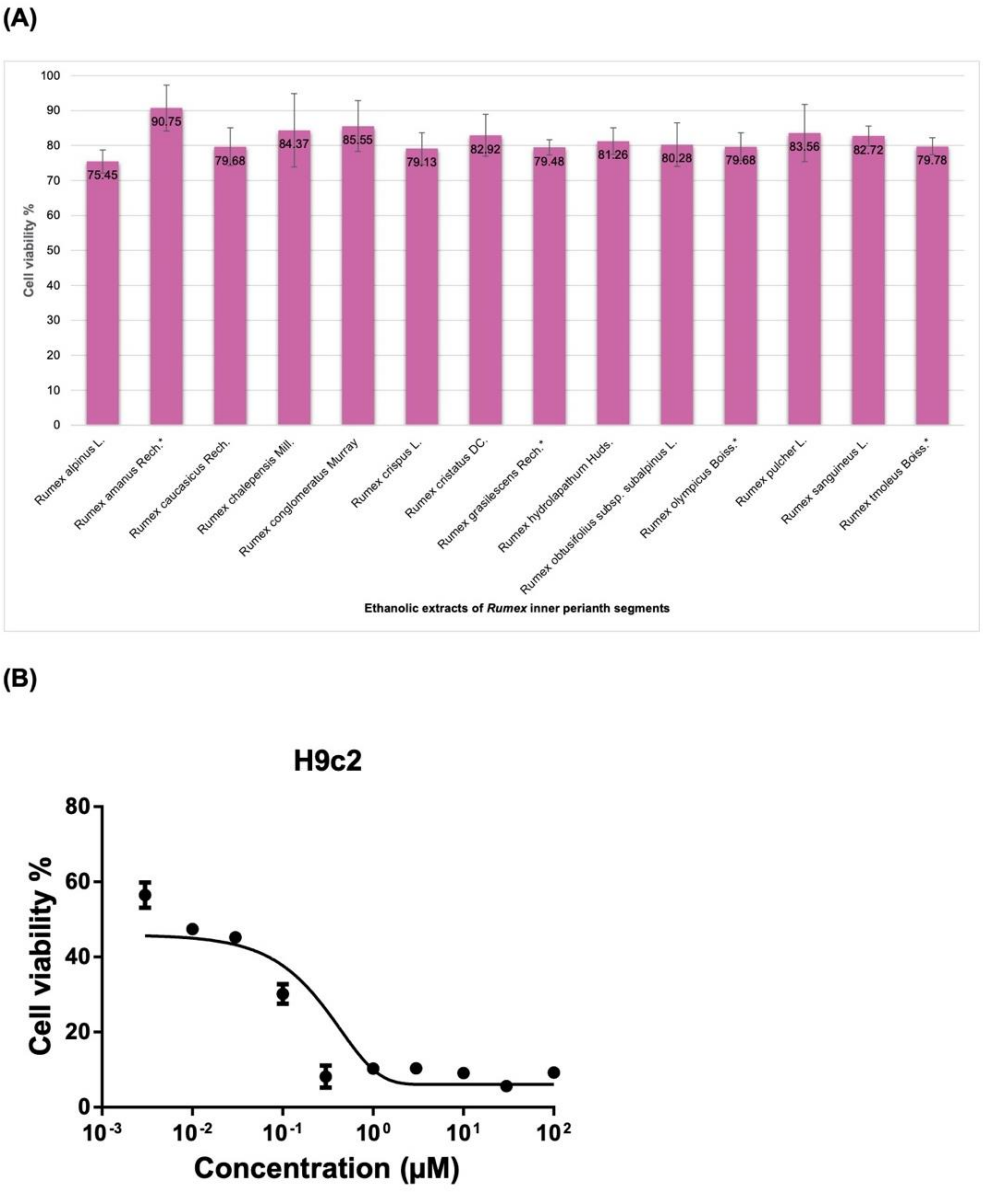

Figure 2. The effects of **(A)** the ethanolic extracts of *Rumex* inner perianth segments on H9c2 rat cardiac myoblastoma cells at 30 µg/ml **(B)** doxorubicin on H9c2 rat cardiac myoblastoma cells at various concentrations

Ascertaining Total Phenolic Contents

We investigated the total phenolic contents of the extracts. Total phenolic contents were estimated according to the equation (y=0.0042x+0.5131, R^2=0.9957) acquired from the calibration curve of gallic acid. The results were expressed as mg gallic acid equivalent (GAE)/g extract). The amount of total phenolics in the extracts ranged from 54.60 to 747.05 mg GAE/g extracts (Figure 3). The highest total phenolic grades have been observed in *R. conglomeratus*, while the lowest activity was detected in *R. alpinus*, confirming the outcomes of DPPH, ABTS and CUPRAC tests.

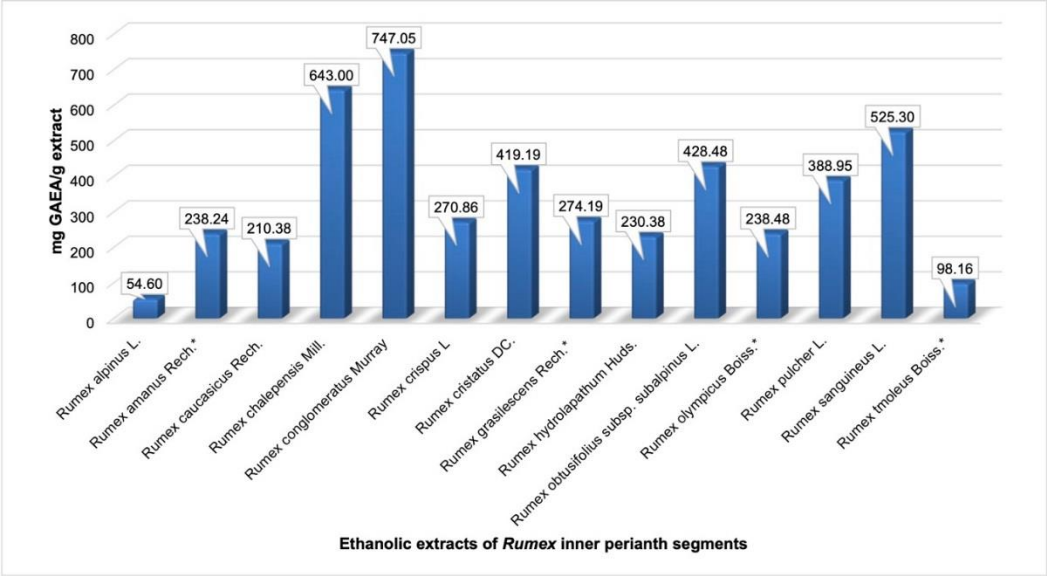

Figure 3. Total phenolic content of the ethanolic extracts of *Rumex* inner perianth segments (mg GAE/g extracts)

Antioxidant Assays

DPPH Radical Scavenging Activity

The assay is a method in which antioxidants donate hydrogen atoms resulting in the formation of reduced form of DPPH radical. DPPH generates purple/violet color in an alcohol solution and discolors the shades of yellow color in the presence of antioxidants [38]. The DPPH radical scavenging activities of the ethanolic extracts of *Rumex* inner perianth segments were tested at 6.25, 12.5, 25, 50 and 100 µg/ml concentrations. All the extracts scavenged DPPH radical dose-dependently. 50% radical scavenging capacities (RC_{50}), the concentration in which the extracts scavenge 50% of the DPPH radical, were stated in Figure 4.

The plant extract with higher antioxidant activity possessed a lower RC_{50} value in comparison to those with lower antioxidant activity. Almost all the extracts except for the ethanolic extract of *R. alpinus* inner perianth segments (RC_{50}: 75.07 µg/ml) efficiently scavenged DPPH radical with low RC_{50} values similar to that of reference compound ascorbic acid. Furthermore, the ethanolic extracts of *Rumex obtusifolius* subsp. *subalpinus* (RC_{50}: 7.83 µg/ml), *R.crispus* (RC_{50}: 10.91 µg/ml), *R. conglomeratus* (RC_{50}: 12.64 µg/ml) *R. pulcher* (RC_{50}: 13.02 µg/ml) and *R. olympicus* (RC_{50}: 14.06 µg/ml) inner perianth segments possessed higher DPPH radical scavenging activity than that of ascorbic acid with the RC_{50} of 14.44 µg/ml, emphasizing the importance of *Rumex* inner perianth segments as potential antioxidant agents.

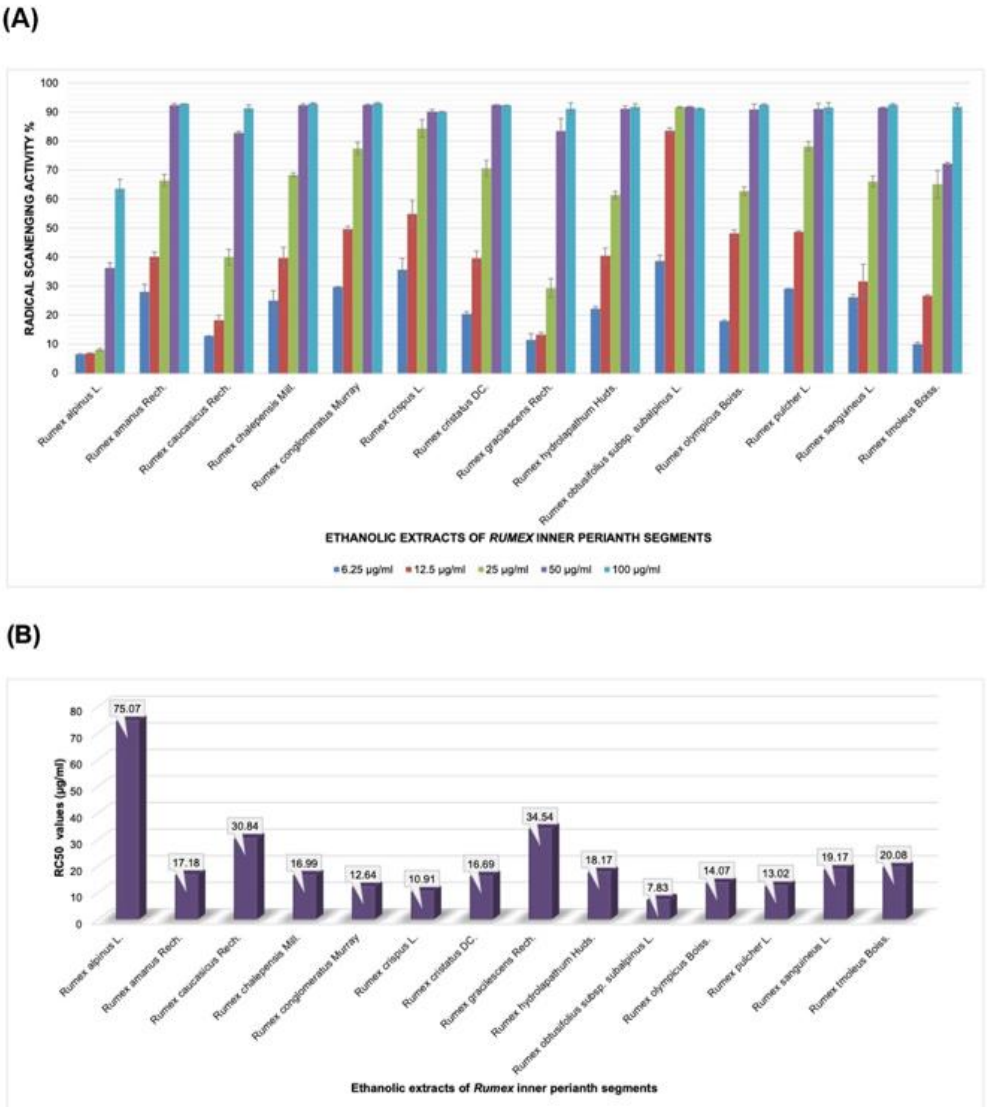

Figure 4. DPPH radical scavenging activity % of the ethanolic extracts of *Rumex* inner perianth segments **(A)**. RC_{50} values of the ethanolic extracts of *Rumex* inner perianth segments (**B**)

ABTS Radical Cation Scavenging Activity

ABTS radical cation scavenging activity assay is a method that depends on the neutralization of the ABTS radical cation in the existence of antioxidants and is widely used for the preliminary determination of antioxidant capacities of natural products [39,40]. ABTS radical cation scavenging activity of the ethanolic extracts of *Rumex* inner perianth segments was ascertained in reference to the equation ($y=1.8032x+1.7793$, $R^2=0.9845$) of the Trolox calibration curve. The results are demonstrated in terms of TEAC in Figure 5. A greater antioxidant activity of the extract was indicated with higher TEAC values. Therefore, evaluating TEAC of the extracts, specifically the ethanolic extracts of *R. conglomeratus* and *R. sanguineus* possessed the highest TEAC with the values of 2154.08 and 2036.94 mg Trolox/g extract, respectively. Similar to DPPH assay, *R. alpinus* possessed the lowest TEAC 393.63 mg TE/g extract). The rest of the extracts exhibited identical ABTS radical cation scavenging activities with similar TEAC values (Figure 5).

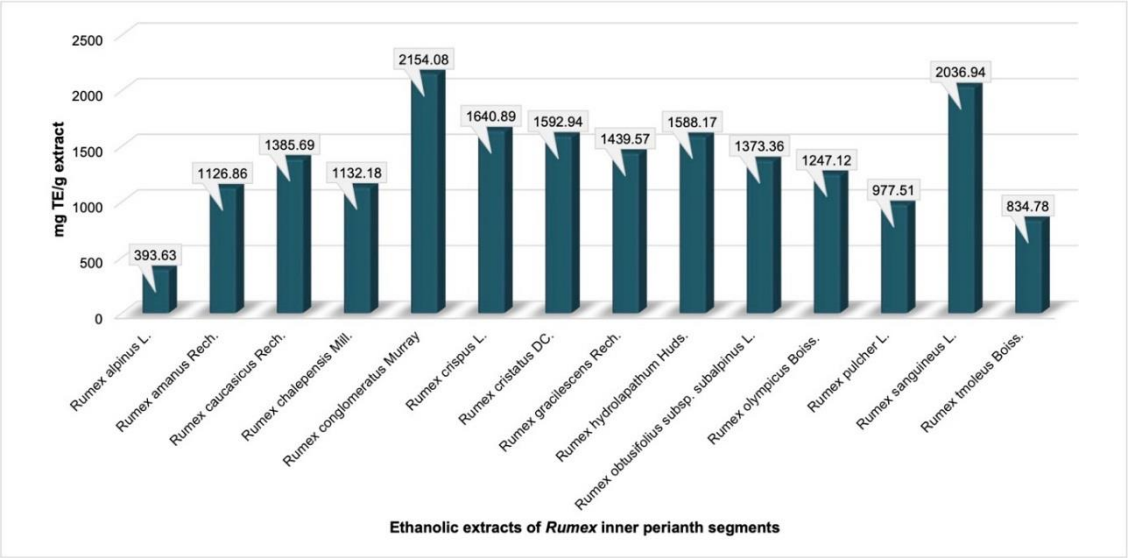

Figure 5. ABTS radical cation scavenging activity of the ethanolic extracts of *Rumex* inner perianth segments (mg TE/g extract)

CUPRAC Assay

Antioxidants do not only scavenge free radicals by donating electrons but also ease higher valent atoms to their lower valence state (e.g. iron, copper… etc.). The redox potential of an antioxidant gets an interest and is involved in the activity [41]. In the case of CUPRAC assay, the reducing power of antioxidants to convert cupric (Cu^{+2}) to cuprous (Cu^{+1}) ion is measured [42]. Cupric ion-reducing antioxidant capacities of the extracts were unraveled according to the equality (y=0.0099x+0.4928, R^2=0.9939) as gallic acid equivalent (mg/g extract). The results are shown in Figure 6. The CUPRAC values of the extracts ranged from 16.57 to 809.49 mg GAE/g extract. *R. obtusifolius* subsp. *subalpinus* displayed the highest antioxidant capacity, which was rather like DPPH radical scavenging activity outcomes. On the other hand, the ethanolic extracts of *R. alpinus* inner perianth segments did not exhibit any antioxidant activity, and the ethanolic extracts of *R. amanus* inner perianth segments displayed slight cupric ion-reducing antioxidant capacity (16.57 mg GAE/g extract).

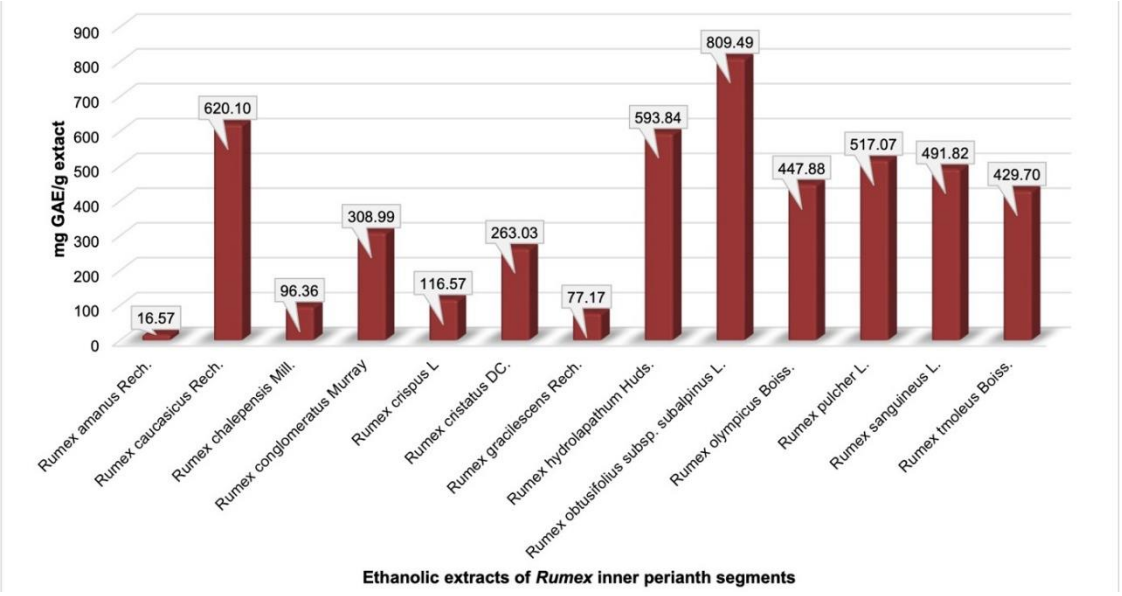

Figure 6. CUPRAC (Cupric ion reducing antioxidant capacity) of the ethanolic extracts of *Rumex* inner perianth segments (mg GA/g extract)

According to the National Cancer Institute USA (NCI), botanicals are taken into consideration as efficient cytotoxic agents if their IC_{50} values are below 20 μg/ml upon 72 h incubation [43,44]. Besides, NCI suggests that botanicals yielding IC_{50} values around or below 30 μg/ml should be subjected to purification to obtain cytotoxic molecules [44,45]. To be on the safe side, we set 30 μg/ml as a cut-off value for determining the extracts with effective cytotoxicity worth further investigations.

Unfortunately, nearly all the extracts studied within the context of our study showed no remarkable cytotoxic activity despite becoming relatively safe agents based on the preliminary evaluation of resazurin data.

Phenolic compounds (PCs) are extensively distributed phytochemicals in plants. They are secondary metabolites biosynthesized through the shikimic acid and phenylpropanoid pathways [46]. PCs exhibit various biological properties and, their dietary intake supplies beneficial effects on health [47]. Many studies emphasized their contribution to antioxidant activity [48]. Oxidative stress arises from the imbalance between the formation and the accumulation of reactive oxygen species (ROS) and is the underlying cause of many diseases [49]. Therefore, scavenging ROS by plants rich in phenolics may be a rational strategy to prevent or treat many disorders. Therefore, we also assessed the presence of phenolics and the antioxidant properties of the extracts.

The outcomes of this research demonstrated that generally, all the extracts have antioxidant activity. Among these *R. conglomeratus* possessing the highest total phenolic contents comes to the forefront also by exhibiting one of highest antioxidant activity by DPPH radical and ABTS radical cation scavenging activities. On the other hand, the ethanolic extract of *R. alpinus* inner perianth segments exhibited the lowest total phenolic and antioxidant properties in all.

Our study also holds importance in that 4 endemic species (*R. amanus*, *R. grasilescens*, *R. tmoleus*, *R. olympicus*) were investigated and assessed for the first time in terms of their antioxidant and cytotoxic profiles.

In this research, we evaluated the cytotoxic and antioxidant profiles of 14 *Rumex* species grown in Türkiye. We selected a variety of breast cancer cells with different origins to test the cytotoxicity of the ethanolic extracts of *Rumex* inner perianth segments. A number of investigations have been carried out to determine the cytotoxic potential of assorted *Rumex* species on various cancer cells. Emerging evidence has pointed out the cytotoxic activity of some *Rumex* species on assorted cancer cell lines to date. Ahmad et al. (2016) stated the potent cytotoxicity of the chloroform fraction of *R. hastatus* on HeLa and NIH/3T3 cells, emphasizing the importance of the fraction for further studies to isolate the potential cytotoxic compounds [49]. In another study, the dichloromethane extract of *R. crispus* effectively inhibited the cell viability of MCF-7 cell line [50]. Li et al. (2022) compiled the current literature data and mentioned the antitumor properties of various *Rumex* species such as *R. acetosa*, *R. thyrsiflorus, R. crispus, R. rothschildianus …etc.* on a variety of cancer cells [2].

The present study assessed the cytotoxic and antioxidant profiles of 14 *Rumex* species grown in Türkiye. We selected a variety of breast cancer cells with different origins to test the cytotoxicity of the ethanolic extracts of *Rumex* inner perianth segments. A number of investigations have been examined to determine the cytotoxic potential of assorted *Rumex* species on assorted cancer cells. Emerging evidence has pointed out the cytotoxic activity of some *Rumex* species on assorted cancer cell lines to date. Ahmad et al. (2016) stated the potent cytotoxicity of the chloroform fraction of *R. hastatus* on HeLa and NIH/3T3 cells, emphasizing the importance of the fraction for further studies to isolate the potential cytotoxic compounds [49]. In another study, the dichloromethane extract of *R. crispus* effectively inhibited the cell viability of MCF-7 cell line [50]. Li et al. (2022) compiled the current literature data and mentioned the antitumor properties of various *Rumex* species such as *R. acetosa*, *R. thyrsiflorus, R. crispus, R. rothschildianus …*etc. on a variety of cancer cells [2].

Despite the presence of various studies in the current literature data, no research is available comparing the cytotoxic activity and antioxidant profiles of *Rumex* inner perianth segments. Though there are few studies unraveling the cytotoxic activity of a particular *Rumex* plant of interest, they do not provide sufficient data to come to a general conclusion about the cytotoxicity of *Rumex* inner perianth segments on breast cancer cells. Our study holds importance in that 14 *Rumex* inner perianth segments were tested on four breast cancer cells with different origins. To be more precise, we selected

the breast cancer cells based on their origin (i.e., whether they carry ER, PR, or HER2/neu or not), and tested a series of *Rumex* inner perianth segments on those cells. Thus, unlike the existing data in the literature, which includes assorted extracts or subfractions of some *Rumex* species, we intended to give a general concept about the cytotoxic evaluation of the ethanolic extracts of 14 different types of *Rumex* inner perianth segments on breast cancer cells. Further, we also examined and compared 14 different types of *Rumex* inner perianth segments in terms of their antioxidant activity. Considering that antioxidant activity is involved in the pathogenesis of many diseases including cancer, our research demonstrated the potential of *Rumex* inner perianth segments as probable complementary and alternative agents, which can be used in cancer therapy as combination regimens.

The phytochemical compositions of the plant directly contribute to their activity. The description and identification of the plant extracts are needed for a detailed investigation of *Rumex* species of particular interest. In the HPLC studies performed on the inner perianth with fruit in our previous doctoral thesis, especially in *R. crispus* and *R. conglomeratus* species, hyperoside from flavonoids was found to be high. It was determined that the inner perianth of *R. tmoleus* and *R. tuberosus* is rich in frangulin B and carries a higher rate than all other species. Frangulin B was found as the main ingredient in the inner perianth segments of the subgenus *Rumex* [51]. Our group has been focusing and specializing on *Rumex* species, their phytochemical composition, and their biological activities for 30 years. Our research is still ongoing. We are also about to publish new findings on the cytotoxicity of *Rumex* roots and *Rumex*-containing constituents as well as their relevant modes of action. In the present study, we give an overview of the cytotoxic and antioxidant activities of the ethanolic inner perianth segment extracts of 14 *Rumex* species for the first time. We believe our findings will make a significant contribution to the assessment and comparison of the bioactivities of *Rumex* inner perianth segments.

To conclude, the ethanolic extracts of 14 *Rumex* inner perianth segments grown in Türkiye were examined in terms of their cytotoxicity on breast cancer, toxicity on H9c2 healthy rat myoblastoma cells, and phenolic contents and antioxidant properties for the first time. They did not exhibit remarkable cytotoxicity profiles on breast cancer cells despite being safe on healthy cells. On the other hand, they generally possessed rich phenolic content and high antioxidant capacity.

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AUTHOR CONTRIBUTIONS

Concept: N.Ö., L.Ö.D.; Design: N.Ö., L.Ö.D.; Control: N.Ö., L.Ö.D.; Sources: L.Ö.D.; Materials: F.B., L.Ö.D.; Data Collection and/or Processing: N.Ö., Y.E., F.B.; Analysis and/or Interpretation: N.Ö., Y.E.; Literature Review: N.Ö., F.B.; Manuscript Writing: N.Ö.; Critical Review: N.Ö., Y.E., F.B., L.Ö.D.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PREPARATION OF LIPID NANOCARRIER FORMULATIONS AND CYTOTOXICITY STUDIES OF DONEPEZIL

DONEPEZİL'İN LİPİD NANO TAŞIYICI FORMÜLASYONLARININ HAZIRLANMASI VE SİTOTOKSİSİTE ÇALIŞMALARI

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ABSTRACT

Objective: *Our research endeavors to discover innovative formulations for the pharmaceutical component of radiopharmaceuticals, which are used to diagnose Alzheimer's disease. Our approach involves the incorporation of Donepezil, a proven active ingredient, into lipid-based nanocarrier systems. Additionally, we have conducted a comprehensive study on the cytotoxicity of Donepezil as a vital aspect of our research.*
Material and Method: *Two distinct techniques were employed in creating nanocarrier formulations: emulsion and sonication. Malvern Zeta Sizer measurements were conducted to assess the properties of the prepared formulations. In addition, the cell proliferation kit II (XTT) was used to evaluate the cytotoxicity of the active ingredient Donepezil.*
Result and Discussion: *Formulations with particle sizes ranging from 100-200 nm have been selected based on the results of characterization studies. Cytotoxicity assays have shown that amounts of Donepezil (50, 100, 500, 1000, 2000, and 5000 µg/ml) are biocompatible. These findings confirm the optimal formulation parameters for producing high-quality Donepezil-based pharmaceutical products. The characterization studies of the prepared formulations have shown that they have the potential to be used in the diagnosis of Alzheimer's disease.*
Keywords: *Characterization studies, cytotoxicity studies, donepezil, drug delivery systems*

ÖZ

Amaç: *Araştırmamızın amacı Alzheimer hastalığının teşhisinde kullanılan radyofarmasötiğin farmasötik bileşeni için yenilikçi formülasyonlar geliştirmektir. Bu hedefi gerçekleştirmek için lipit bazlı nanotaşıyıcı sistemler geliştirdik ve Donepezil aktif bileşen olarak lipid bazlı nanotaşıyıcı sistemlere dahil edildi. Ayrıca çalışmamızın önemli bir parçası olarak Donepezilin sitotoksisitesine ilişkin değerlendirmeler yapıldı*
Gereç ve Yöntem: *Nanotaşıyıcı formülasyonların oluşturulmasında emülsiyon ve sonikasyon olmak üzere iki farklı teknik kullanıldı. Hazırlanan formülasyonların özelliklerini değerlendirmek için Malvern Zeta Sizer ölçümleri yapıldı. Ayrıca Donepezil etken maddesinin sitotoksisitesini değerlendirmek için hücre proliferasyon kiti (XTT) kullanıldı.*
Sonuç ve Tartışma: *Karakterizasyon çalışmalarının sonuçlarına göre partikül boyutları 100-200 nm arasında değişen formülasyonlar seçilmiştir. Sitotoksisite analizleri, Donepezil'in 50, 100, 500, 1000, 2000 ve 5000 µg/ml aktif madde konsantrasyonlarının biyolojik olarak uyumlu olduğunu göstermiştir. Bu bulgular, yüksek kaliteli Donepezil bazlı farmasötik ürünler üretmek için en uygun formülasyon parametrelerini doğrulamaktadır.*

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Hazırlanan formülasyonların karakterizasyon çalışmaları Alzheimer hastalığının tanısında kullanılma potansiyeline sahip olduklarını göstermiştir. Formülasyonlara eklenecek Donepezil etken maddesinin optimal miktarı belirlenmiş ve biyouyumluluk açısından uygun bulunmuştur.
Anahtar Kelimeler: *Donepezil, ilaç taşyıcı sistemler, karakterizasyon çalışmaları, sitotoksisite çalışmaları*

INTRODUCTION

Alzheimer's is a neurological condition that leads to a decline in daily activities and cognitive abilities, alongside changes in behavior and neuropsychiatric symptoms. The 2019 World Alzheimer's Prevalence study revealed that dementia affects over 50 million individuals worldwide, and this number is expected to skyrocket to 152 million by 2050. Unfortunately, the disease is often discovered in its advanced stages, particularly in developing countries [1]. Currently, the Food and Drug Administration (FDA) has approved three cholinesterase inhibitors - Galantamine, Rivastigmine, and Donepezil - which are typically prescribed for mild to moderate cases [2,3]. Donepezil is a piperidine-based medication that functions by reversibly inhibiting the acetylcholinesterase enzyme. The drug is approved by the FDA for the symptomatic treatment of mild to moderate Alzheimer's disease. It is believed that Donepezil enhances cholinergic function by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by the acetylcholinesterase enzyme [4-6]. Donepezil is essentially a non-competitive inhibitor of acetylcholinesterase [7,8]. Donepezil is a medication that is utilized for the treatment of mild to moderate Alzheimer's disease. It works by inhibiting the activity of acetylcholinesterase, which elevates the amount of acetylcholine in the brain. Acetylcholine is an essential neurotransmitter involved in cognitive function. However, Donepezil and other acetylcholinesterase inhibitors can cause adverse gastrointestinal changes and hepatotoxicity [9]. Common side effects of Donepezil include diarrhea, vomiting, insomnia, fatigue, muscle cramps, nausea, and anorexia due to increased cholinergic activity in the gastrointestinal tract following oral administration [10,11]. To address these limitations, lipid-based formulations consisting of Capryol 90, oleic acid, water, and surfactants such as Span 80, Tween 80, and Soy Lecithin have been developed [12,13]. Administered orally, these formulations are available in various forms such as orally disintegrating tablets or sustained-release formulations. Nanoparticle lipid delivery systems offer a promising platform for diagnosis and treatment, which can increase the efficacy of drugs and reduce associated side effects. Lipids are administered as simple vehicles in drug administration, but some amphiphilic lipids, such as monoglycerides, self-assemble after swelling with water. This complex phase behavior is utilized to obtain nanostructured colloidal systems, and studies on it have increased recently [14,15]. In this study, researchers prepared the pharmaceutical components of the radiopharmaceutical used in the diagnosis of Alzheimer's disease. The formulations were developed, and particle size and charge were measured to study the physicochemical properties of these formulations. The emulsion and sonication methods were employed to prepare the formulations, with the sonication method being preferred due to its practicality. The composition of lipid nanocarrier systems generally includes oils or fats, surfactants, and an aqueous phase. The study evaluated different production parameters such as formulation ratios and mixing times by making changes to the ratios of the ingredients in the composition. In addition, the cytotoxicity studies of the active ingredient Donepezil were conducted using the XTT kit. The results of the cytotoxicity studies determined the correct amounts of Donepezil to be added to the formulations. The findings of this research demonstrate the potential of lipid-based formulations as an effective treatment for Alzheimer's disease.

MATERIAL AND METHOD

Preparation of Lipid-Structured Nanocarriers

During the process of developing formulations, we created different variations with varying particle sizes and loads. To accomplish this, we prepared formulations using various compositions through both emulsion and sonication methods. In the end, we found that sonication was the preferred method due to its practicality in preparing the formulations.

The composition of lipid nanocarrier systems generally includes oils or fats, surfactants, and an aqueous phase. While developing formulations;

• As the oil phase; oleic acid, Capryol 90

• Bidistilled water and water: acetone: ethanol (5:2.5:2.5 h/h) as the water phase

• Studies were carried out with Span 80, Tween 80, and Soy Lecithins (Lipoid S 70, Lipoid S 100) as surfactants.

By making changes in the ratios of the ingredients in the composition, different production parameters (formulation ratios, mixing times) were evaluated, and formulation studies were carried out.

The method is as follows; The lipids were melted at 70-80°C, and surfactant was added on top of the molten lipid. This mixture was titrated with the water phase brought to the same temperature. This mixture was first mixed in a magnetic stirrer at 1000 rpm, then this pre-emulsion was passed through a sonicator (Bandelin, GmBh, Berlin, Germany) at 500 W and 20 kHz, and thus the formulations were prepared.

Different parameters were tried in preformulation studies.

1- Formulation of ingredient and ingredient quantity changes

- Lipid type
- lipid ratio
- Surfactant type
- Surfactant ratios

2- Changes in the method used in the production phase of the formulation

- Mixing time in a sonicator

The contents and preparation conditions of the formulations are shown in Table 1.

Table 1. The contents and preparation conditions of the formulations

Formulation	Oleic acid (mg)	Tween 80 (mg)	Water (ml)	Stirring speed (rpm)	Stirring time (minutes)	Sonicator conditions
F1	300	100	5	1000	15 (F1-1) 10 (F1-2) 5 (F1-3)	500 W ve 20 kHz
F2	200	200	5	1000	15 (F2-1) 10 (F2-2) 5 (F2-3)	500 W ve 20 kHz
F3	100	300	5	1000	15 (F3-1) 10 (F3-2) 5 (F3-3)	500 W ve 20 kHz
Formulation	**Oleic acid (mg)**	**Span 80 (mg)**	**Water (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F4	300	100	5	1000	15 (F4-1) 10 (F4-2) 5 (F4-3)	500 W ve 20 kHz
F5	200	200	5	1000	15 (F5-1) 10 (F5-2) 5 (F5-3)	500 W ve 20 kHz
F6	100	300	5	1000	15 (F6-1) 10 (F6-2) 5 (F6-3)	500 W ve 20 kHz
Formulation	**Capyrol 90 (mg)**	**Span 80 (mg)**	**Water (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F7	300	100	5	1000	15 (F7-1) 10 (F7-2) 5 (F7-3)	500 W ve 20 kHz
F8	200	200	5	1000	15 (F8-1) 10 (F8-2) 5 (F8-3)	500 W ve 20 kHz
F9	100	300	5	1000	15 (F9-1) 10 (F9-2) 5 (F9-3)	500 W ve 20 kHz

Table 1 (*continue*). The contents and preparation conditions of the formulations

Formulation	Capyrol 90 (mg) (mg)	Tween 80 (mg)	Water (ml)	Stirring speed (rpm)	Stirring time (minutes)	Sonication conditions
F10	300	100	5	1000	15 (F10-1) 10 (F10-2) 5 (F10-3)	500 W ve 20 kHz
F11	200	200	5	1000	15 (F11-1) 10 (F11-2) 5 (F11-3)	500 W ve 20 kHz
F12	100	300	5	1000	15 (F12-1) 10 (F12-2) 5 (F12-3)	500 W ve 20 kHz
Formulation	**Oleic acid (mg)**	**Lipoid S 75 (mg)**	**Water:Acetone: Ethanol (5:2.5:2.5 v/v) (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F13	300	100	5	1000	15 (F13-1) 10 (F13-2) 5 (F13-3)	500 W ve 20 kHz
F14	200	200	5	1000	15 (F14-1) 10 (F14-2) 5 (F14-3)	500 W ve 20 kHz
F15	100	300	5	1000	15 (F15-1) 10 (F15-2) 5 (F15-3)	500 W ve 20 kHz
Formulation	**Oleic acid (mg)**	**Lipoid S 100 (mg)**	**Water:Acetone:Ethanol (5:2.5:2.5 v/v) (ml)**	**Stirring speed (rpm)**	**Stirring time (minutes)**	**Sonication conditions**
F16	300	100	5	1000	15 (F16-1) 10 (F16-2) 5 (F16-3)	500 W ve 20 kHz
F17	200	200	5	1000	15 (F17-1) 10 (F17-2) 5 (F17-3)	500 W ve 20 kHz
F18	100	300	5	1000	15 (F18-1) 10 (F18-2) 5 (F18-3)	500 W ve 20 kHz

A total of 54 formulations were prepared. Among these formulations, F13, F14, F15, F16, F17, and F18 were canceled because aggregation was observed. Characterization studies of the remaining formulations were performed.

Characterization Studies of Prepared Formulations

Investigation of particle size properties

The prepared formulations were evaluated in terms of aggregate formation, particle size, and polydispersity index with Malvern Zetasizer (Malvern Nano ZS 90) in the particle size range of 3-1000 nm, at room temperature, with an angle of 173°. Samples were diluted with filtered, bidistilled water (pH=7) before evaluation. Dilutions were made at 1/400.

Zeta potential analysis

The formulations were evaluated with Malvern Zetasizer (Malvern Nano ZS 90) at 25°C, the dielectric constant of 78.5, the conductivity of 5 mS/cm, using DTS 1060C zeta cuvette, a field strength of 40 V/cm. Before measuring, samples were diluted with a certain amount of distilled water (pH=7). Dilutions were made at a ratio of 1/400.

Cytotoxicity

Cytotoxicity studies were conducted to determine the optimal dosage of Donepezil solution that is non-toxic to HT-22 mouse hippocampal cell lines. Various concentrations, ranging from 50 μg/ml to

5000 µg/ml, were evaluated. The primary objective of this study was to determine the optimal concentration of Donepezil that would provide maximum results while minimizing potential harm to cells. The outcome of this study would aid in establishing the recommended dosage of Donepezil for further research and clinical applications. The XTT method was used to evaluate cell viability, cytotoxicity, and proliferation. This method is a sensitive and user-friendly colorimetric assay that measures the conversion of a tetrazolium salt into a water-soluble orange formazan by mitochondrial dehydrogenases in metabolically active cells. The absorbance value at 570 nm in a microplate reader was used to measure viable cells. The cells were seeded in replicates of three at 100 µl at 1x104 cells/well in a 96-well plate and incubated for 24 hours in an incubator at 37°C and 5% CO_2. Different concentrations of Donepezil were then added to the cells. Donepezil was dissolved in a medium with dimethyl sulfoxide (DMSO) concentration of 0.1%. After a 24, 48, and 72-hour incubation period, a mixture prepared by adding activation solution to XTT reagent was added to each well and incubated for two hours. The absorbance values were measured in a microplate reader (Thermo Scientific Varioskan Flash Microplate Reader) at a wavelength of 570 nm. The CalcuSyn software was employed to determine the dosage that keeps cell viability above 70% compared to the control. Once the optimum dosage is established, formulations containing Donepezil at the appropriate dosage will be prepared for further research and clinical applications.

RESULT AND DISCUSSION

Results of Characterization Studies of Prepared Formulations

The results of the analyses measured on the Malvern zeta-sizer device are shown in Table 2. Formulations of each formulation with different mixing times (15, 10, and, 5 minutes) were prepared. Each formulation was studied as n=3, and Malvern Zeta-Sizer measurements were made.

Table 2. Malvern zeta sizer measurement results of formulations

Formulation	Particle size (nm)	PdI	Zeta Potential (mV)
F1-1	296.66 ± 5.41	0.39 ± 0.04	+12.85 ± 1.42
F1-2	135.80 ± 1.83	0.45 ± 0.04	+20.93 ± 3.30
F1-3	195.66 ± 7.41	0.52 ± 0.08	+25.41 ± 4.32
F2-1	227.80 ± 4.81	0.40 ± 0.01	-26.03 ± 7.18
F2-2	255.12 ± 3.25	0.45 ± 0.05	-25.04 ± 6.55
F2-3	229.60 ± 3.56	0.35 ± 0.03	-24.99 ± 5.18
F3-1	143.8 ± 6.24	0.53 ± 0.01	-25.60 ± 3.97
F3-2	59.89 ± 0.49	0.49 ± 0.01	-25.26 ± 1.65
F3-3	74.15 ± 2.55	0.51 ± 0.01	-25.89 ± 4.12
F4-1	114.33 ± 3.24	0.56 ± 0.03	+25.53 ± 6.31
F4-2	85.38 ± 2.16	0.52 ± 0.01	+29.01 ± 6.19
F4-3	81.02 ± 1.58	0.51 ± 0.02	+28.46 ± 2.61
F5-1	119.93 ± 2.66	0.51 ± 0.01	-20.63 ± 0.94
F5-2	91.94 ± 1.29	0.52 ± 0.01	-22.66 ± 2.25
F5-3	103.97 ± 1.17	0.53 ± 0.04	-25.91 ± 5.31
F6-1	99.29 ± 1.42	0.49 ± 0.01	+27.15 ± 2.87
F6-2	109.97 ± 3.95	0.49 ± 0.03	+23.01 ± 2.47
F6-3	103.47 ± 1.48	0.52 ± 0.02	+24.06 ± 3.79
F7-1	414.77 ± 50.79	0.59 ± 0.06	-23.99 ± 5.12
F7-2	288.90 ± 7.47	0.66 ± 0.12	-23.92 ± 2.44
F7-3	929.37 ± 39.58	0.75 ± 0.04	-24.07 ± 5.63

Table 2 (*continue*). Malvern zeta sizer measurement results of formulations

Formulation	Particle size (nm)	PdI	Zeta Potential (mV)
F8-1	764.933 ± 119.087	0.87 ±0.11	-24.77 ± 8.46
F8-2	1124.66 ± 52.54	0.93 ± 0.11	-28.53 ± 4.52
F8-3	906.80 ± 222.44	0.84 ± 0.13	-27.45 ± 3.52
F9-1	87.03 ± 1.88	0.27 ± 0.01	-26.57 ± 1.80
F9-2	72.25 ± 3.25	0.33 ± 0.02	-22.36 ± 2.45
F9-3	130.60 ± 2.74	0.43 ± 0.02	-21.17 ±0.55
F10-1	11.45 ± 0.06	0.23 ± 0.03	+25.19 ±4.76
F10-2	11.45 ± 0.06	0.14 ± 0.01	+21.66 ± 1.58
F10-3	10.31 ± 0.12	0.15 ± 0.01	+21.80 ± 1.88
F11-1	11.19 ± 0.01	0.17 ± 0.01	+24.84 ± 1.10
F11-2	14.93 ± 0.31	0.22 ± 0.01	+27.63 ± 3.21
F11-3	11.86 ± 0.09	0.18 ± 0.02	+28.56 ± 1.64
F12-1	9.72 ± 0.08	0.21 ± 0.03	-23.41 ± 2.91
F12-2	9.59 ± 0.04	0.15 ± 0.03	-26.76 ± 2.94
F12-3	10.22 ± 0.17	0.21 ± 0.01	-24.85 ± 3.95

After measurements, formulations with particle sizes in the range of 100-200 nm were selected. In the next studies, F1-2, F1-3, F3-1, F3-2, F4-1, F4-2, F4-3, F5-1, F5-2, F5-3, F6-1, F6-2, F6-3, F9-1, F9-3 formulations were continued. The graphs of the Malvern Zetasizer measurement results for the selected formulations are shown in Figure 1. Cytotoxicity studies were started.

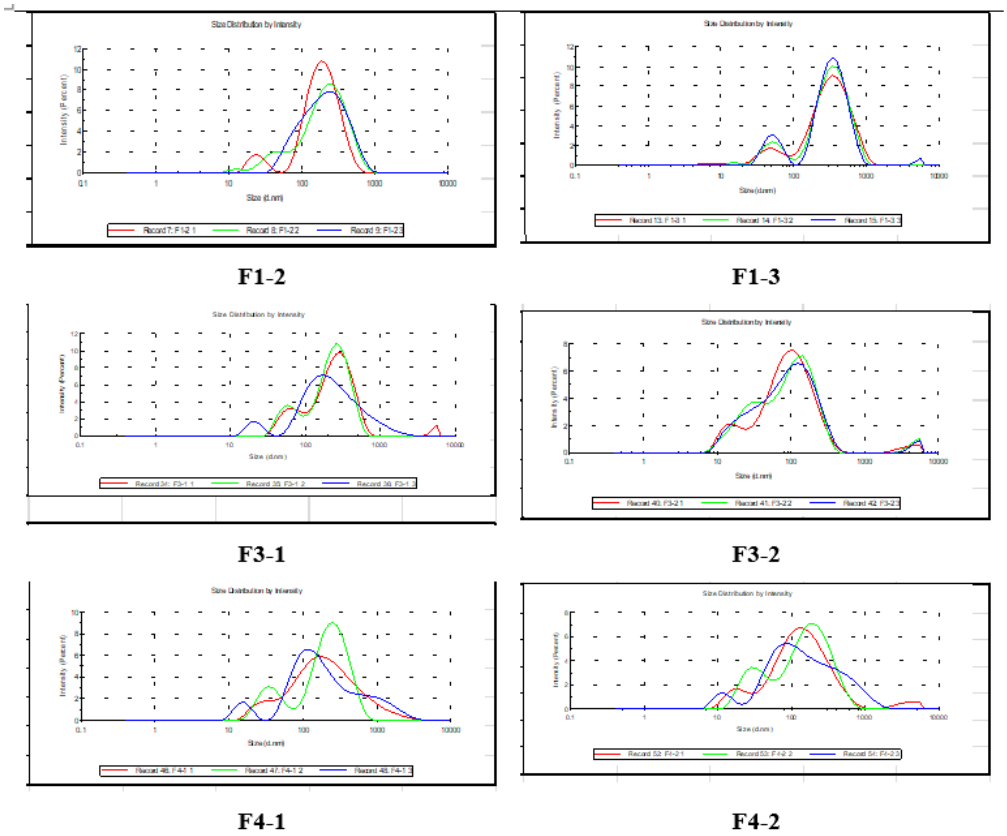

Figure 1. Graphs of Malvern Zetasizer measurement results for selected formulations

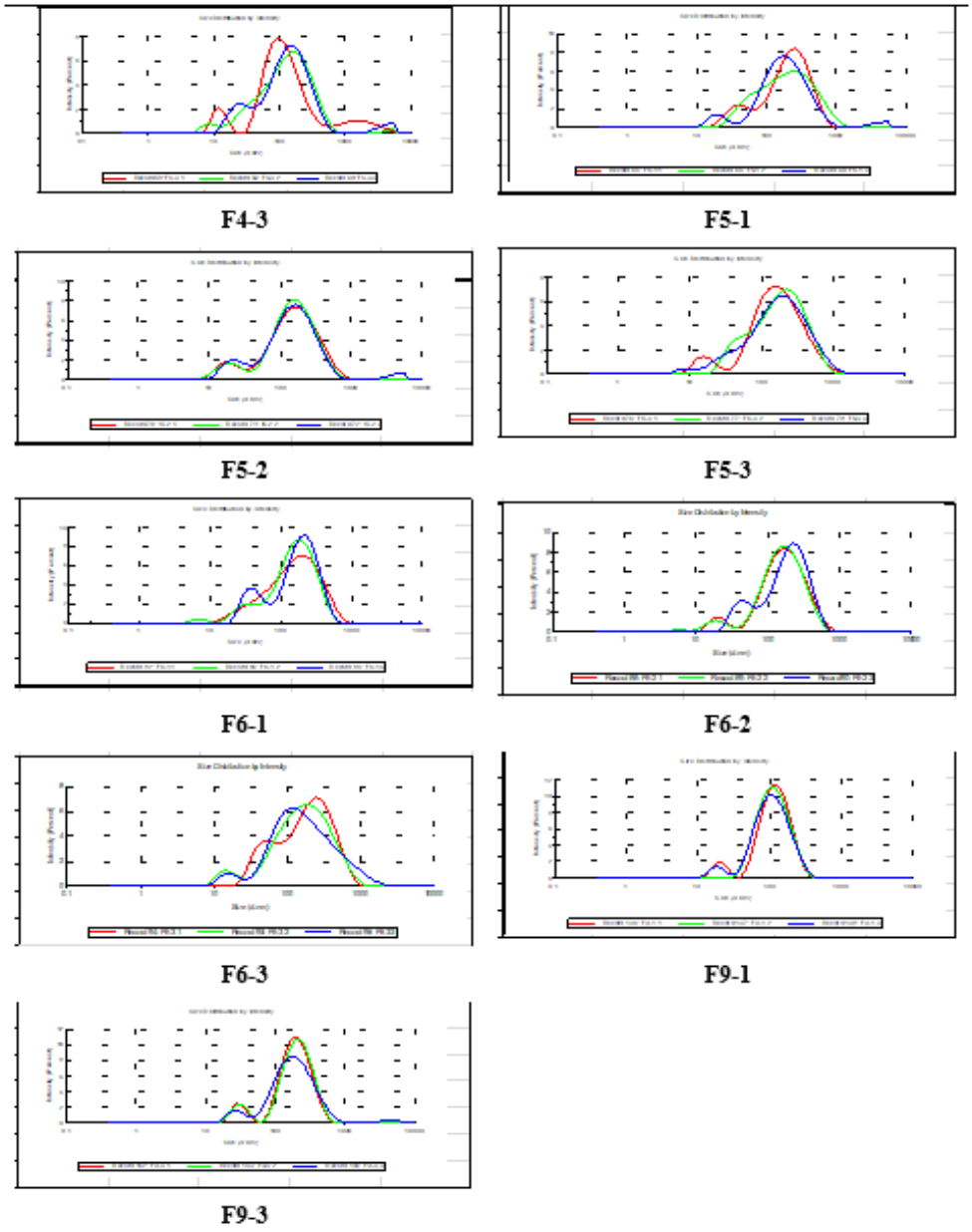

Figure 1 (*continue*). Graphs of Malvern Zetasizer measurement results for selected formulations

Results of Cytotoxicity Studies

The cytotoxicity results of different amounts of Donepezil solution are shown in Table 3 and Figure 2. Medium containing 0.1% DMSO was used for 100% viability.

Table 3. The cytotoxicity results from different amounts of Donepezil solution (n=3)

cell viability (%)	**Amount of Donepezil (ug/ml)**					
Time	**50**	**100**	**500**	**1000**	**2000**	**5000**
0	100	100	100	100	100	100
24	99.22±0.41	97.46±1.73	96.96±1.1	96.13±3.22	96.26±2.92	95.99±1.44
48	98.51±1.49	97.31±2.3	96.65±3.27	95.69±4.76	96.09±1.66	95.82±2.66
72	97.88±2.83	97.53±3.24	95.81±4.18	95.53±3.53	95.09±2.57	95.46±2.45

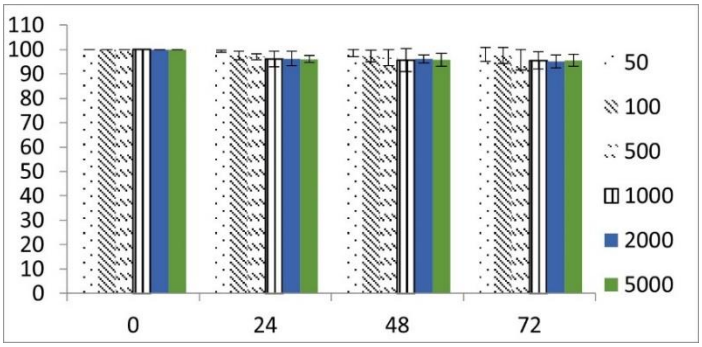

Figure 2. The cytotoxicity results from different amounts of Donepezil solution

Conclusion

The present study sought to develop nanoparticle carrier systems in lipid structure for the diagnosis of Alzheimer's disease, while also conducting cytotoxicity studies on the active substance Donepezil. Several formulations were prepared and evaluated, with a focus on selecting those that displayed optimal particle size, zeta potential, and PdI values capable of passing the blood-brain barrier. Formulations with particle sizes up to 200 nm were selected to pass the blood-brain barrier. Cytotoxicity studies have been conducted with these formulations. In addition, future studies will continue with formulations with positive zeta potential to pass the BBB and with pDI values below 0.5 in terms of homogeneity (F1-2, F6-1, and F6-2). In formulations with homogeneous distribution, this value is required to be 0.5 and below [16]. Moreover, the amount of Donepezil added to these chosen formulations was based on cytotoxicity results obtained from the active substance. Encouragingly, the study yielded promising results, suggesting that the developed formulations could prove instrumental in the diagnosis of Alzheimer's disease. Future research endeavors will continue to explore the potential of these formulations, using the selected formulations and determined amounts of Donepezil.

AUTHOR CONTRIBUTIONS

Concept: E.S.D., E.O., E.A.G.; Design: E.S.D., E.O., E.A.G.; Control: E.O., E.A.G.; Sources: E.S.D., E.O., E.A.G.; Materials: E.S.D., E.O., E.A.G.; Data Collection and/or Processing: E.S.D.; Analysis and/or Interpretation: E.S.D., E.O., E.A.G.; Literature Review: E.S.D.; Manuscript Writing: E.S.D., E.O., E.A.G.; Critical Review: E.S.D., E.O., E.A.G.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DETECTING DRUG-DRUG INTERACTIONS INDUCED BY ANTACIDS ENCOUNTERED IN A COMMUNITY PHARMACY: AN OBSERVATIONAL STUDY

BİR TOPLUM ECZANESİNDE KARŞILAŞILAN ANTİASİTLER KAYNAKLI İLAÇ ETKİLEŞİMLERİNİN TESPİTİ: GÖZLEMSEL BİR ÇALIŞMA

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ABSTRACT

Objective: *This study aimed to reveal drug-drug interactions (DDIs) due to antacids through programs used to detect DDIs.*
Material and Method: *Within the scope of this study, 207 prescriptions containing at least one antacid and a drug from a different pharmacological group were evaluated in terms of DDIs. Evaluations were made on the prescriptions received in a community pharmacy serving in Van, Türkiye. Three different DDI checking programs were used for this evaluation.*
Result and Discussion: *Antacid-induced DDIs were detected in 64 of the prescriptions. Interactions occurred between 52 active ingredient pairs, and it was revealed that DDIs were most common between calcium carbonate and famotidine. This interaction is minor and has been detected by only one database. Another common interaction was found between the calcium carbonate and cholecalciferol (Vitamin D) pair, and this interaction was reported as Level 2 and should be closely monitored in two different databases. As a result, DDIs induced by antiacids generally were found to be at moderate levels. However, it is seen that three DDI checking programs used in the study provide different results in detecting DDIs.*
Keywords: *Antacids, community pharmacy, drug-drug interactions, observational study*

ÖZ

Amaç: *Bu çalışma, ilaç-ilaç etkileşimlerini (DDIs) tespit etmek için kullanılan programlar aracılığıyla antiasitler nedeniyle oluşan DDI'leri tespit etmeyi amaçlamaktadır.*
Gereç ve Yöntem: *Bu çalışma kapsamında en az bir antiasit ve farklı farmakolojik gruptan bir ilaç içeren 207 reçete DDI açısından ele alınmıştır. Bu doğrultuda, Van'da hizmet veren bir toplum eczanesinde karşılanan reçeteler değerlendirilmiştir. Bu değerlendirmeler için üç farklı DDI kontrol programı kullanılmıştır.*
Sonuç ve Tartışma: *Reçetelerin 64'ünde antiasit kaynaklı DDI tespit edilmiştir. 52 aktif madde çifti arasında etkileşim meydana gelmiş olup, DDI'ların en yaygın olarak kalsiyum karbonat ve famotidin arasında olduğu ortaya konulmuştur. Bu etkileşimin minor düzeyde olup, yalnızca bir veritabanı tarafından tespit edilmiştir. Diğer bir sık karşılaşılan etkileşim ise kalsiyum karbonat ve kolekalsiferol (Vitamin D) çifti arasında bulunmuştur. Bu etkileşim Düzey 2 ve yakından takip*

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edilmesi gerekir olacak şekilde iki farklı program tarafından tespit edilmiştir. Sonuç olarak antiasitlerin neden olduğu DDI'ların genel olarak orta düzeyde ciddiyete sahip olduğu bulunmuştur. Ancak çalışmada kullanılan üç DDI kontrol programının DDI'ların tespitinde farklı sonuçlar verdiği görülmüştür.
Anahtar Kelimeler: *Antiasitler, gözlemsel çalışma, ilaç-ilaç etkileşimleri, toplum eczanesi*

INTRODUCTION

Antacids are alkaline substances generally used to neutralize excess acid in the stomach and alleviate dyspepsia symptoms [1,2]. They are widely used in the treatment of gastro-oesophageal reflux disease (GERD), duodenal and gastric ulcers, peptic ulcer, erosive esophagitis, Helicobacter pylori (HP) eradication, and dyspepsia [3,4].

In clinical practices, it is known that drug-drug interactions (DDIs) are very common, which may lead to synergistic or antagonistic medication responses in many cases. Since minor DDIs generally do not have a significant effect on clinical outcomes, no change in treatment is required in such cases. However, in moderate and serious interactions, many precautions must be taken, such as a dosage change, a change in the active ingredient, or closer patient monitoring [5]. Sadowski and Gugler and Allgayer put forth that DDIs caused by antacids do not cause serious health problems. Still, DDIs are common because patients generally do not consider antacids as a drug [6,7].

Unlu revealed that the most preferred drug for GERD in Turkey is PPIs, followed by antacids. Antacids can be included in the group of active substances that rarely may cause serious DDIs [8]. Ogava and Echizen revealed that antacid use is becoming increasingly common, and this increases the possibility of DDIs [9]. However, it is known that the ions of antacids containing calcium, magnesium, and aluminum are chelating agents, and they bind many drugs, such as digitoxin, tetracycline, indomethacin, aspirin, cimetidine, ranitidine, famotidine, theophylline, etc. Antacids also reduce the bioavailability of barbiturates, sulfonamides, and penicillin [2]. Maton and Burton stated that most antacids, except sodium bicarbonate, can reduce drug absorption through adsorption or chelation of other drugs [3]. Antacid-induced drug interactions can be prevented by rescheduling drug administration times. To avoid undesirable interactions, antacids are usually used two hours before or after taking any medication [4].

To the best of the author's knowledge, a limited number of studies have been conducted on detecting DDIs induced by antacids at the community pharmacy level. Therefore, this study aimed to detect the frequency of DDIs caused by prescribed antacids and their severity level with the help of three different national and international DDI checking programs.

MATERIAL AND METHOD

Prescriptions containing antacids received between 20 November 2022 and 20 April 2023 at a predetermined community pharmacy serving in the Van City center were examined by the researchers in terms of DDIs within the scope of this study. Firstly, the ICD-10 diagnostic code, the specialty area of the prescribing physician, the gender and age of the patient, and the number of items written on the prescription were obtained from prescriptions. Then, prescriptions were evaluated for possible DDIs. If a DDI was detected in the prescription, the degree of the DDI was also determined with the help of electronic DDI checker programs. The literature recommends evaluating data from at least three programs to get more reliable information in such studies [10,11].

For this reason, two of the programs to be used in the study were determined to be “Medscape” and “Drugs.com,” which are frequently preferred databases in the literature. In addition, “RxMediaPharma® Interactive Drug Information Resource,” commonly used in community pharmacies in Türkiye, has been included as the third program. A brief piece of information about these programs was given as follows.

1. RxMediaPharma: In RxMediaPharma, interaction levels are considered at three levels: Level 1 (high interaction), Level 2 (medium interaction), and Level 3 (low level of interaction).
2. Medscape: Medscape is a free online resource and divides drug-drug interactions into four groups: contraindicated, serious-use alternative, monitor closely, and minor.

3. Drugs.com: Drugs.com is a free online resource that evaluates drug interactions under four groups: major, moderate (recommended for use only in special cases), minor, and unknown.

Prescriptions containing at least one antacid and one different drug without antacids were included in the research. As a result of the evaluation with the concerned pharmacist, it was determined that approximately 60-70 prescriptions meeting the relevant criteria were met monthly at this pharmacy. Additionally, in studies with similar study designs, the number of evaluated prescriptions was determined according to affiliated pharmacies' filling prescription rates, and almost 100-200 prescriptions were investigated. In this regard, prescriptions that meet the study criteria were considered among all prescriptions filled at the relevant pharmacy during the research period.

The obtained data was subjected to descriptive statistical analysis via Microsoft Excel.

RESULT AND DISCUSSION

During the research period (20 November 2022 and 20 April 2023), 1128 prescriptions were filled at the corresponding community pharmacy. It was determined that approximately 18% of these prescriptions met the study's inclusion criteria. Hence, 207 prescriptions were evaluated in detail in terms of DDIs. 58% of these prescriptions were for women and 67% for patients over 40. The distribution of the prescribed physicians' specialty areas is given in Figure 1.

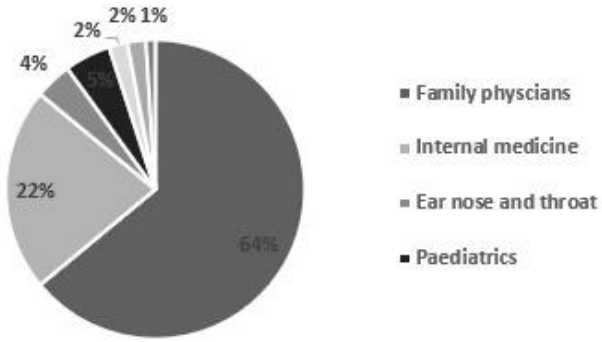

Figure 1. Prescribed physicians' specialty areas

According to Figure 1, family physicians mainly prescribe antacids, and internal medicine specialists follow them. Prescriptions were also evaluated in terms of diagnosis according to ICD-10 codes. It was determined that antacids were mainly prescribed for diagnosing K21, which refers to GERD (77%). When the antacids in the prescriptions were examined, it was seen that the most prescribed antacid was Calcium Carbonate. Figure 2 shows the distribution of them.

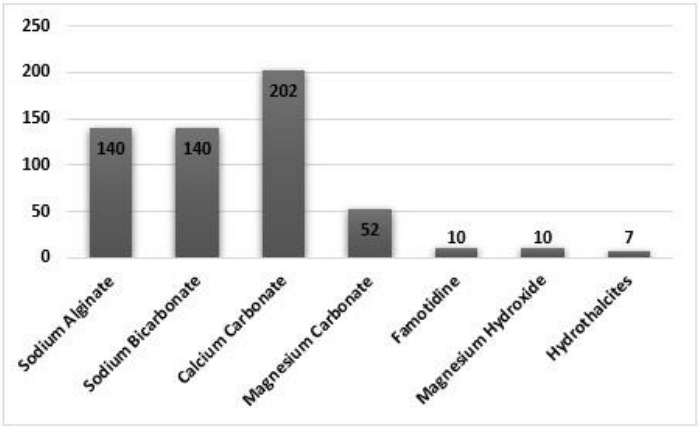

Figure 2. Distribution of prescribed antacids

While prescriptions were considered in terms of DDIs, an interaction was supposed to exist if a DDI was detected in any of the three databases. In this context, it was determined that 64 out of 207 prescriptions had DDIs. These 64 prescriptions were evaluated in more detail, and it was determined that sodium bicarbonate, calcium carbonate, magnesium carbonate, and famotidine caused 116 DDI cases. These cases were detected between 52 different active substance pairs. As stated in various literature, the findings obtained from three databases showed differences.

Table 1 presents the frequencies of DDIs encountered based on calcium carbonate due to the research conducted in three databases.

Table 1. Interactions with calcium carbonate

Pairs	Frequency	RxMediaPharma	Medscape	Drugs.com
Calcium carbonate-ferrous sulfate	2	Level 2	Minor	Moderate
Calcium carbonate-ramipril	4	No interactions	Monitor Closely	Minor
Calcium carbonate-metoprolol	3	No interactions	Monitor Closely	Moderate
Calcium carbonate-nitrofurantoin	4	No interactions	Monitor Closely	No interactions
Calcium carbonate-famotidin	10	No interactions	No interactions	Minor
Calcium carbonate-acetyl salicylic acid	5	No interactions	No interactions	Moderate
Calcium carbonate-amlodipin	2	No interactions	No interactions	Moderate
Calcium carbonate-ciprofloxacin	4	Level 2	Monitor Closely	Moderate
Calcium carbonate-lactulose	4	No interactions	Monitor Closely	Minor
Calcium carbonate-bisoprolol	1	No interactions	Monitor Closely	Moderate
Calcium carbonate-diltiazem	1	No interactions	Monitor Closely	Moderate
Calcium carbonate-levothyroxine	6	Level 2	Monitor Closely	Moderate
Calcium carbonate-sucralfat	3	No interactions	No interactions	Moderate
Calcium carbonate-cholecalciferol	8	Level 2	Monitor Closely	No interactions
Calcium carbonate-itraconazole	1	Level 2	Monitor Closely	Moderate
Calcium carbonate-azithromycin	2	No interactions	Monitor Closely	No interactions
Calcium carbonate-ferrous II glycine	1	No interactions	Monitor Closely	Moderate
Calcium carbonate-pancreatin	1	No interactions	No interactions	Moderate
Calcium carbonate-ibandronic acid	2	Level 2	Monitor Closely	Moderate
Calcium carbonate-allopurinol	1	No interactions	Monitor Closely	No interactions
Calcium carbonate-cefuroxime	3	Level 2	Monitor Closely	Moderate
Calcium carbonate-bisacodyl	4	Level 2	No interactions	Moderate
Calcium carbonate-ferrous II fumarate	2	Level 2	Monitor Closely	Moderate
Calcium carbonate-nebivolol	2	No interactions	Monitor Closely	Moderate
Calcium carbonate-hydrocortiazide	3	No interactions	No interactions	Moderate

It was determined that 61.3% of the interactions detected in the study were caused by calcium carbonate. In the light of Table 1, it was determined that the active ingredients ciprofloxacin, levothyroxine, itraconazole, ibandronic acid, cefuroxime, and ferrous II fumarate interact with calcium carbonate in all three databases.

The two active substances that have been found to interact most with calcium carbonate are famotidine and cholecalciferol. However, it is necessary to look at these interactions in more detail. Because there are products in the pharmaceutical market that contain these active substances in combination, Therefore, concerning cholecalciferol, it should be noted that the concurrent use of cholecalciferol with calcium salts is generally beneficial. Since vitamin D helps the absorption of calcium salts from the intestines, it is seen that these two active ingredients are used in combination in many preparations [12]. However, this combination may cause hypercalcemia in some patients. Concerning interaction with famotidine, although it has been detected by one of the databases, current studies mostly show that antiacids have no significant effects on famotidine's pharmacokinetics [13]. In

this context, it is thought that an interaction was detected between this active ingredient pair because the reference used in the database is not up-to-date.

The interaction between calcium carbonate and levothyroxine can be considered the most severe calcium carbonate-related interaction among the prescriptions evaluated within the scope of this study. In this interaction, which was detected in 6 different prescriptions, calcium carbonate reduces the gastrointestinal absorption of levothyroxine, negatively affecting levothyroxine's treatment success [14,15]. Thus, it is generally recommended to leave a two-hour break between the use of these two active substances to prevent interaction. Prescribing calcium carbonate and levothyroxine together is a common situation, and studies in the literature reveal that patients are generally unaware of the interaction between these two active substances and misuse them, which negatively affects treatment success [16,17]. In this context, pharmacists must inform patients about how to avoid this DDI.

Another vital interaction originating from calcium carbonate is with acetylsalicylic acid. In this study, this interaction was detected in all five prescriptions. This interaction must be considered, especially for hemodialysis patients [18].

Table 2 presents the frequencies of DDIs encountered based on sodium bicarbonate.

Table 2. Interactions with sodium bicarbonate

Pairs	Frequency	RxMediaPharma	Medscape	Drugs.com
Sodium bicarbonate-ferrous II sulfate	2	Level 2	Monitor Closely	Moderate
Sodium bicarbonate-ramipril	3	No interactions	Monitor Closely	Minor
Sodium bicarbonate-acetylsalicylicacid	1	No interactions	No interactions	Moderate
Sodium bicarbonate-famotidine	1	No interactions	No interactions	Minor
Sodium bicarbonate-pseudoephedrine	5	Level 1	Monitor Closely	Moderate
Sodium bicarbonate-lactulose	1	No interactions	Monitor Closely	Minor
Sodium bicarbonate-ciprofloxacin	3	Level 2	Monitor Closely	Moderate
Sodium bicarbonate-allopurinol	1	No interactions	Monitor Closely	No interactions
Sodium bicarbonate-cefuroxim	3	Level 2	Monitor Closely	Moderate
Sodium bicarbonate-bisacodyl	3	Level 2	Monitor Closely	Moderate
Sodium bicarbonate-nebivolol	2	No interactions	Monitor Closely	No interactions
Sodium bicarbonate-ferrous II fumarate	1	Level 2	Monitor Closely	Moderate
Sodium bicarbonate-nitrofurantoin	1	No interactions	Monitor Closely	No interactions
Sodium bicarbonate-metoprolol	1	No interactions	Monitor Closely	No interactions

Interactions with sodium bicarbonate 21.7% of the DDIs detected in the study were caused by sodium bicarbonate. It has been determined that the active ingredients ramipril, pseudoephedrine, ciprofloxacin, cefuroxime, and bisacodyl interact with sodium bicarbonate in three databases.

When the study findings are examined, it is possible to say that interactions caused by sodium bicarbonate are more serious. For example, dosage adjustments must be made if sodium bicarbonate and pseudoephedrine are used together. In this study, this interaction was detected in 5 prescriptions.

Table 3 presents the frequencies of drug-drug interactions encountered based on magnesium carbonate.

17% of the interactions detected in the study were due to magnesium carbonate. It has been determined that the active ingredient ciprofloxacin interacts with magnesium carbonate in three drug interaction databases. Similar to calcium carbonate, magnesium carbonate has interaction with famotidine and cholecalciferol. Different studies have reported that interactions between ciprofloxacin and antacids affect the absorption level, and the success of treatment is negatively affected [19,20].

As a result, it is clear that the selected three programs' results are different from each other in general. Three programs showed that out of 52 active substance pairs in which interactions were detected, 14 of them had interactions in common. RxMediaPharma detected 20 of them, Medscape detected 34 of them, and Drugs.com detected 42 of them. When looking at the interaction levels, it is seen that 5 of the interactions detected by RxMediaPharma are Level 1, and 15 are Level 2. It was determined that 33 of the interactions detected by Medscape were in the "Monitor closely" class, and 1

was in the "Minor" class. It was revealed that 32 of the interactions detected by Drugs.com were at the "Moderate" level, and 9 of them were at the "Minor" level.

Table 3. Interactions with magnesium carbonate

Pairs	Frequency	RxMediaPharma	Medscape	Drugs.com
Magnesium carbonate-sucralfate	1	No interactions	No interactions	Moderate
Magnesium carbonate-famotidine	3	No interactions	No interactions	Minor
Magnesium carbonate-acetyl salicylic acid	2	No interactions	No interactions	Moderate
Magnesium carbonate-amlodipine	1	Level 1	No interactions	No interactions
Magnesium carbonate-ciprofloxacin	1	Level 2	Monitor closely	Moderate
Magnesium carbonate-lactulose	2	No interactions	Monitor closely	Minor
Magnesium carbonate-bisoprolol	1	No interactions	Monitor closely	Minor
Magnesium carbonate- itraconazole	1	Level 2	No interactions	Moderate
Magnesium carbonate- azithromycin	1	No interactions	No interactions	Moderate
Magnesium carbonate- ferrous II glycine	3	Level 2	No interactions	Moderate
Magnesium carbonate- magnesiumoxide	1	No interactions	No interactions	Moderate
Magnesium carbonate-ibandronicacid	2	Level 2	No interactions	Moderate
Magnesium carbonate-cholecalciferol	3	No interactions	No interactions	Moderate

From the outcome of this observational study, it is possible to conclude that DDIs induced by antiacids generally were found moderate level. However, three DDI checking programs used in the study provide different results in detecting DDIs. This situation can lead to inconsistent results for pharmacists and other healthcare professionals and poses a risk to patient safety. In this context, in light of the findings of this study, to minimize this risk, it may be recommended that community pharmacists check the interactions from several programs instead of using a single program when detecting DDIs by combining their medical knowledge. Moreover, when the issue is considered in terms of the evaluation of health technologies, it is seen that there is a need to create a standard in such programs.

This observational study has possible limitations. The first one is evaluating only prescribed antacids. However, it should be noted that antacids also can be sold in community pharmacies without a prescription in Türkiye. Secondly, the data of this study only comes from one community pharmacy. Thus, to increase the generalizability of the results, more in-depth studies, where data is gathered from different pharmacists, will be needed in this area. Also, it should be noted that two international (free) and one national (not free) DDI checking programs were used in the study, so the generalizability of the results may also be increased by adding more programs.

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AUTHOR CONTRIBUTIONS

Concept: D.G., M.A.; Design: D.G., M.A.; Control: M.A.; Sources: D.G., M.A.; Materials: - ; Data Collection and/or Processing: D.G.; Analysis and/or Interpretation: D.G., M.A.; Literature Review: D.G., M.A.; Manuscript Writing: D.G., M.A.; Critical Review: M.A.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

This study was conducted after Van Yüzüncü Yıl University Non-interventional Research Ethics Committee approved the study ethically (Date:18/11/2022, Decision No: 2022/11-26).

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS IN AĞRI PROVINCE, TÜRKİYE: EXPLORING TRADITIONAL KNOWLEDGE AND THERAPEUTIC POTENTIAL

TÜRKİYE'NİN AĞRI İLİNDE TIBBİ BİTKİLERİN ETNOBOTANİK ÇALIŞMASI: GELENEKSEL BİLGİ VE TEDAVİ POTANSİYELİNİN KEŞFEDİLMESİ

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ABSTRACT

Objective: *This study was conducted to systematically document the use of plants, plant parts and preparation methods used by people in eight districts and sixty villages in Ağrı province.*
Material and Method: *The medicinal plant species employed by the indigenous population for therapeutic purposes were systematically gathered and identified. Comprehensive data on traditionally utilized information were compiled, and herbarium materials were prepared. These materials have been deposited at the Iğdır National Wild Life Museum (INWM), affiliated with Iğdır University.*
Result and Discussion: *This research identified a total of 58 taxa of medicinal plants belonging to 31 families. Among these, 50 species were found to grow naturally, while 8 species were cultivated. The predominant plant families included Asteraceae (8), Apiaceae (4), Lamiaceae (4), and Rosaceae (4). Infusion emerged as the most widely employed preparation method. The practice of traditional medicine remained prevalent among the population in Ağrı. Nevertheless, with the increasing availability of health services in the region, herbal medicine appeared to be more closely associated with healthcare and illness prevention than with curative purposes. The influx of new immigrants also contributed to the erosion of traditional knowledge. Notably, there is a discernible decline in traditional knowledge regarding the use of medicinal plants, both among younger generations and due to migration. Furthermore, this research serves as a foundational resource for prospective scientific inquiries aimed at the development of novel commercial drugs derived from plant sources.*
Keywords: *Ağrı, ethnobotany, medicinal plants, Türkiye*

ÖZ

Amaç: *Bu araştırma, Ağrı ilinin 8 ilçesi ve 60 köyünde yaşayan insanların yararlandıkları tıbbi bitkilerin kullanımı, bu bitkilerin kullanılan kısımlarını ve hazırlanma yöntemlerini kayıt altına almak amacıyla yapılmıştır.*
Gereç ve Yöntem: *Yöre halkının tedavi amacıyla kullandığı şifalı bitki türleri toplanarak, tanımlanmıştır. Geleneksel olarak kullanılan tüm bilgiler kayıt altına alınmış olup, herbaryum materyalleri hazırlanmış ve herbaryum örnekleri Iğdır Üniversitesi yaban hayatı müzesinde (INWM) depolanmıştır.*
Sonuç ve Tartışma: *Bu araştırmada 31 familyaya ait toplam 58 tıbbi bitki taksonu tanımlanmıştır.*

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Bunlardan 50 türün doğal, 8 türün ise kültür bitkisi olduğu tespit edilmiştir. En çok rastlanılan bitkilerin Asteraceae (8), Apiaceae (4), Lamiaceae (4), Rosaceae (4) familyalarına ait olduğu gözlenmiştir. En yaygın hazırlıklama şekli infüzyondur. Ağrı'da halk arasında geleneksel tıbbın kullanımı hâlâ yaygındır. Bununla birlikte, bölgedeki artan sağlık tesisleri sayesinde, bitkisel ilaçların daha çok sağlık bakımı ve hastalıkların önlenmesinde kullanıldığı tespit edilmiştir. Bölgede yeni göç ve genç nufüsun artuığı gözlenmiştir. Hem genç nesillerde hem de göç nedeniyle şifalı bitkilerin kullanımına ilişkin geleneksel bilgide kademeli bir kayıp olduğu gözlenmiştir. Ayrıca, bu araştırma, bitkisel kaynaklı yeni ticari ilaçlar geliştirmeyi amaçlayan gelecekteki bilimsel araştırmalar için temel bir kaynak görevi görecektir.
Anahtar Kelimeler: *Ağrı, etnobotani, tıbbi bitkiler, Türkiye*

INTRODUCTION

Throughout human history, plants have served as a resource, functioning both as a protective/therapeutic agent and as a tool [1]. In Türkiye, recognized as one of the world's significant biodiversity hotspots, more than 30% of the approximately 12.000 vascular plant taxa are endemic (about 4.000), surpassing the number of endemic species in European countries (1352) [2-4]. Türkiye encompasses diverse ecosystems owing to factors such as its geographical location, climate, geology, soil and water resources, and ecological advantages, including its position along bird migration routes [5-7]. The Anatolian population has accrued a wealth of knowledge in folk medicine over an extended period, attributable to the diversity of flora and fauna in the region, providing abundant sources of medicinal plants and animal remedies in both urban and rural areas. [8].

The flora of Eastern Anatolia varies depending on different ecological regions, geographical differences and different climates. The province of Ağrı, located in eastern Turkey, stands out for its rich biodiversity and cultural heritage, which provides a favorable environment for researching the traditional use of medicinal plants by indigenous communities [9]. With a land area of 11,376 km², Ağrı province encompasses around 1.4% of Anatolian and ranks as the 26th largest province in Türkiye in terms of surface area. [10].

The number of ethnobotanical studies conducted in Ağrı province is limited. Previous studyies have been confined to specific regions or mauontains rather than providing a comprehensive overview of Ağrı province [9,11-15]. This study aims to conduct a comprehensive ethnobotanical survey in Ağrı province, documenting and analyzing traditional knowledge and therapeutic practices, especially regarding medicinal plants. By collaborating closely with the inhabitants Diyadin (8 villages), Doubayazt (8 villages), Eleşkirt (7 villages), Hamur (7 villages), Patnos (8 villages), Taşlıçay (7 villages), Tutak (8 villages), and the center (7 villages) of Ağrı province, We aim to investigate the diversity of medicinal plants, identify plant species, document preparation methods, and understand the traditional uses of these plants for treating various ailments.

MATERIAL AND METHOD

The Study Area

Ağrı province, situated in the Eastern Anatolia Region of Türkiye, group into the B9 and B10 squares and is classified within the Iran-Turanian Plant Geography Region. As of 2022, it represents an Eastern Anatolian province with a population of 510.626 and covers a land area of 11.376 km². [9,16]. Iran to the east, Kars to the north, Erzurum to the northwest, Muş and Bitlis to the southwest, Van to the south, and Iğdır to the northeast surround Ağrı province (Figure 1).

Data collection

This study was supported within the scope of the 'Recording Traditional Knowledge Based on Biological Diversity in Ağrı Province Project' by the Ministry of Agriculture and Forestry. The villages where the study data were collected were determined by the ministry. During the visits to the villages, standardized and valid survey forms for each province were used in accordance with the technical specifications prepared by the ministry.

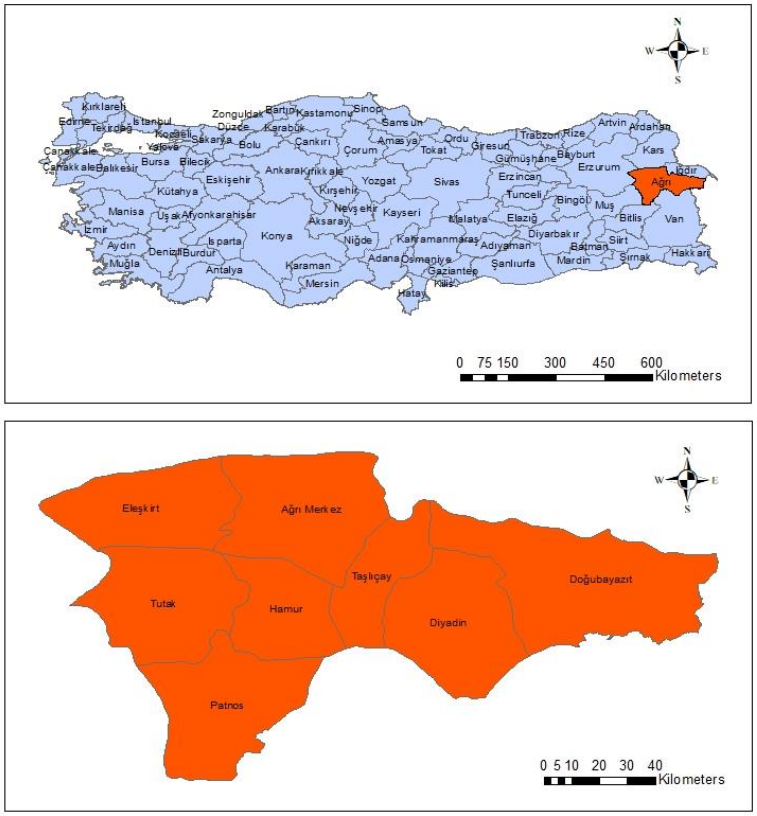

Figure 1. Geographical location of the investigation region

Ehnobotanical information was systematically collected through fieldwork, involving structured and semi-structured interviews with knowledgeable individuals from 60 villages, namely Sarca (1), Dumanlı (2), Soğancumaçay (3), Aşağıkent (4), Uçarkaya (5), Güneysu (6), Sarıharman (7), Armutlu (8), Güvercinli (9), Özdemir (10), Koçaklar (11), Kızkapan (12), Hasandolu (13), Akdilek (14), Yukarıgöçmez (15), Kılıçgediği (16), Mızrak (17), Uzunöz (18), Çukurkonak (19), Şekerbulak (20), Yenikent (21), Taşbudak (22), Otluca (23), Hayrangöl (24), Aşağıcihanbey (25), Tahir Beldesi (26), Gözaydın (27), Akyumak (28), Abdiköy (29), Söbetaş (30), Karlıca (31), Danakıran (32), Soğanlıtepe (33), Yuvacık (34), Sağlıksuyu (35), Karakazan (36), Gümüşkuşak (37), Uğurtaş (38), Gündoğdu (39), Yeltepe (40), Gözucu (41), Yukarıesen (42), Yukarıtoklu (43) Tanyolu (44), Kumluca (45), Dedebulak (46), Yanıkçukur (47), Karataş (48), Günbuldu (49), Toklucak (50), Batıbeyli (51), Pirali (52), Yukarıtütek (53), Bezirhane (54), Karaşeyh (55), Çalıköy (56), Kızılkaya (57), Çetenli (58), Üzengili (59), and Örtülü (60). A total of 158 individuals, encompassing midwives, shepherds, foresters, farmers, healers, beekeepers, housewives, teachers, village headmen, and plant collectors, were subjected to face-to-face interviews. Of the total informants, 38 were female (24.05%), while the remaining 120 were male (75.95%). A questionnaire was administered to gather information from the participants, and video photographs and audio recordings were obtained during the interviews, all conducted with the participants' consent. The interviews were conducted at random locations, including tea houses, mosque gardens, homes, fields, plateaus, etc. Throughout the study, Information regarding the local name of the plant, the therapeutic effects of the plant, the specific part(s) of the plant used, and the methods of preparation/application were documented based on the participants' responses.

Plant Materials

In 2021 and 2022, plant samples were systematically collected from various villages. The authors, Belkıs Muca YİĞİT, pressed and described the scientific names of the collected specimens, referencing authoritative botanical resources such as the Flora of Turkey and the East Aegean Islands, the Turkish Plants List (Vascular Plants), the Flora of the USSR, Flora Europaea, Flora Iranica, Flora of Iraq, and

Flora Palaestina [17-23]. The Plant List was used to determine the scientific names of plant species. [24]. Voucher specimens were meticulously preserved and archived at the Iğdır National Wildlife Museum (INWM).

Ethnobotanical Index

The Use Value (UV) index was calculated using the formula UV=ΣUi/N, where Ui represents the number of use reports indicating a taxon with a significant percentage, and N indicates the total number of information sources [25-28].

RESULT AND DISCUSSION

Interviews were conducted to determine the demographic characteristics of participants involved in the field research. A total of 158 individuals, representing diverse occupations including beekeepers, construction foremen, farmers, headmen, janitors, retirees, religious officers, engineers, teachers, housewives, managers, shepherds, as well as individuals engaged in herb and plant collection, were subjected to face-to-face interviews. Out of the total 158 participants contacted, 120 were male (75.95%), and the remaining 38 were female (24.05%). The age distribution of the participants is as follows: 3 informants under the age of 19, 21 informants between the ages of 19-35, 52 informants between the ages of 36-49, 52 informants between the ages of 50-70, and 30 informants above the age of 70. Furthermore, 15 participants reported having never attended a formal educational institution (Table 1).

Table 1. The demographic profile of the participants

Demographic characteristics	Number
Age Range	
Below 19	3
19-35	21
36-49	52
50-70	52
70 and above	30
Sex	
Women	38
Men	120
Educational levels	
Illiterate	15
Literate	17
Primary school	83
Secondary school	24
High school	13
University	6

All the interviewed people currently reside in the districts of Eleşkirt, Tutak, Patnos, Hamur, Taşlıçay, Diyadin, and Doğubayazıt in Ağrı, Türkiye. It was observed that many persons who benefit from medicinal plants are between the ages of 36 and 70 (104 people). Additionally, it has been observed that the predominant users of medicinal plants are males with primary school education or below (Table 1). Erzurum and Van are among the provinces where Ağrı has the most socio-cultural and economic interaction. Additionally, it has been revealed that people over the age of 50 benefit most from medicinal plants in Erzurum and Van [25,29]. A similar condition has been recorded in ethnobotanical research done on the surrounding provinces of Kars, Muş, and Iğdır [25,29-32]. In the Eastern Anatolia provinces (Erzurum, Van, Kars, Iğdır, Elazığ, Bingöl, Erzincan, and Tunceli) and the Eastern Black Sea Region (Bayburt, Gümüşhane, and Trabzon), the utilization of medicinal plants is predominantly observed among women, whereas in Ağrı, a higher prevalence is noted among men [25,29-37].

A comprehensive collection of 58 medicinal plant taxa covering 31 plant families was documented in the Ağrı province of Türkiye (Table 2). Asteraceae (8), Apiaceae (4), Lamiaceae (4) and Rosaceae (4) are the most common medicinal plant families. In total, 158 medicinal plant taxa were identified in Ağrı, distributed across 31 diffirent plant families, with 50 classified as wild species and 8 as cultivated plants. Table 2 provides an alphabetical listing of the 58 herbs identified in the region, organized by family and botanical name. Based on our findings, the most frequently utilized taxa include *Achillea arabica*, *Alcea calvertii*, *Beta vulgaris* var. *altissima*, *Malva neglecta*, *Plantago major*, *Rheum ribes*, and *Urtica dioica*. In the field of ethnobotany, various studies have been conducted in different regions to systematically document the wide diversity of plant species and their traditional uses. In the Van province, Mükemre et al. conducted a comprehensive ethnobotanical study, identifying a total of 73 taxa from 23 families [29]. Similarly, in the Bitlis province, Demir et al., undertook ethnobotanical research, documenting 71 taxa that represented 29 distinct families [38]. Furthermore, in Elazığ, another survey revealed the presence of 41 taxa spanning 17 different families [39]. Likewise, in the Erzurum province, an ethnobotanical study recorded 99 taxa belonging to 38 distinct families [25]. On the other hand, when studies in the Eastern Anatolia and Southeastern Anatolia regions are examined, it has been determined that *Helichrysum* sp., *Malva* sp., *Cephalaria* sp., *Rumex* sp., *Crataegus* sp., *Urtica dioca*, and *Rheum ribes* are mostly used in folk medicine [9-14,28-34].

Table 2. Traditional uses of plants in Ağrı (Türkiye)

Family	Plant species, and location	Local name	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Gymnospermae							
Pinaceae	*Pinus sylvestris* L.	Çam (INWM-0000059)	Res	Hea	Che	Peptic Ulcer	0.02
Angiospermae							
Amaranthaceae	*Beta vulgaris* var. *altissima* Döll.	Sılk, Sılkog, Hilog, Şekerpancarı (INWM-0000061)	Lea	Raw	Eat	Hypertension, Diabetes	0.06
Amaranthaceae	*Chenopodium album* L.	Selemask (INWM-0000080)	Lea	Inf	Ext	Wound	0.01
Amaryllidaceae	**Allium sativum* L.	Sarımsak (INWM-0000062)	Bul	Raw	Eat	Hypertension, Toothache	0.03
Amaryllidaceae	**Allium cepa* L.	Pivaz, Soğan (INWM-0000063)	Bul	Hea	Inh	Epilepsy	0.01
			Bul	Cru	Ext	Earache	0.03
Apiaceae	*Ammi visnaga* (L.) Lam.	Ziyan (INWM-0000064)	Fru	Raw	Che	Toothache	0.02
Apiaceae	*Ferula orientalis* L.	Helis, Heliz, Çakşır, Çaşır (INWM-0000065)	Roo	Raw	Eat	Urinary Tract İnfection	0.03
			Aer	Dec	Int	Nausea, Prostate, Asthma, Immunostimulant	0.01
Apiaceae	*Heracleum platytaenium* Boiss.	So, Sö, Söh (INWM-0000066)	Aer	Raw	Eat	Diabetes, Anti-İnflammatory, Analgesic	0.01
Apiaceae	*Prangos ferulacea* (L.) Lindl.	Deliçaşır (INWM-0000067)	Aer	Gar	Ext	Toothache	0.03
Asphodelaceae	*Eremurus spectabilis* M. Bieb.	Gurik, Gulig, Gülik (INWM-00000116)	Lea	Cru	Int	Peptic Ulcer	0.01
			Aer	Inf	Int	Immunostimulant, Constipation,	0.04
Asteraceae	*Artemisia absinthium* L.	Havşan, Gziyahavşan, Havaju (INWM-0000068)	Aer	Inf	Int	Diabetes, Urinary Tract İnfection, Diarrhea	0.04
			Flo	Raw	Int	Immunostimulant, Sedative	0.01
Asteraceae	*Achillea arabica* Kotschy	Gılikakiçik, Sarı çiçek, Civanperçemi (INWM-0000069)	Aer	Inf	Int	Peptic Ulcer, Hagi-nitis, Hemorrhoids, Antihelmintic, Wounds	0.07

Table 2 (*continue*). Traditional uses of plants in Ağrı (Türkiye)

Family	Plant species, and location	Local name	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Asteraceae	*Achillea millefolium* L.	Civanperçemi, Dermanimid, Gulilk, Dermediva (INWM-0000070)	Aer	Cru	Ext	Wounds	0.04
			Aer	Inf	Int	Anti-İnflammatory, Amenore, Diarrhea, Peptic Ulcer	0.03
Asteraceae	*Anthemis cretica* L.	Patpat, Papatya (INWM-0000071)	Aer	Inf	Int	Immunostimulant, Digestive, Peptic Ulcer, Urinary Tract İnfection	0.04
Asteraceae	*Cichorium intybus* L.	Çakçak, kajık, Kermeşo, Çekçek (INWM-0000072)	Aer	Inf	Int	Myalgia, Asthma, Allergy, Hemorrhoids, Analgesic	0.03
Asteraceae	*Gundelia tournefortii* L.	Dağ Sakızı, Kereng, Benişteganog, Kanog, Sakız otu, Geleng, Ganog, Gelengkusi, Benîştê, Kengel, Gereng, Benîştê (INWM-0000073)	Aer	Raw	Eat	Immunostimulant,	0.01
			Lat	Raw	Che	Diabetes, Toothache	0.03
			Lat	Mixed with butter	Ext	Ambustion, Wound,	0.02
			Roo	Dec	Int	Asthma	0.01
Asteraceae	*Tragopogon buphthalmoides* (DC.) Boiss.	Sping, Spitag (INWM-000074)	Lea	Raw	Eat	Diabetes	0.03
Asteraceae	*Tussilago farfara* L.	Kersim, Karşim, Sarmalık, Sarma (INWM-000075)	Lea	Inf	Int	Anti-İnflammatory	0.01
Boraginaceae	*Alkanna orientalis* (L.) Boiss.	Hewajo, Havajo, Havaju, Kök boya (INWM-000076)	Roo	Boi	Int	Anti-İnflammatory	0.02
			Roo	Boi	Ext	Wounds, Conjunctivitis, Ambustion, Scabies, Earache	0.05
			Aer	Inf	Int	Diabetes, Hypercholesteremia, Analgesic, Hemorrhoids,	0.03
Boraginaceae	*Anchusa azurea* Mill.	Tilki Otu, Mıjmıjık (INWM-0000077)	Lea	Inf	Int	Hernia	0.04
Caprifoliaceae	*Cephalaria procera* Fisch.& Lall	Pelemir, Gulinga (INWM-0000078)	Lat	Raw	Ext	Hemostatic	0.02
Cannabaceae	*Cannabis sativa* L.	Kenevir (INWM-0000079)	See	Hea	Int	Sedative-Hypnotic	0.01
Euphorbiaceae	*Euphorbia seguieriana* Neck.	Sütleğen (INWM-0000081)	Lat	Raw	Int	Constipation	0.01
Fabaceae	*Astragalus brachycalyx* Fisch. ex Boiss.	Guni, Gunig, Geven (INWM-000082)	Aer	Dec	Ext	Alopecia Areata, Wounds	0.03
			Ole	Raw	Int	Diabetes	0.01
Fabaceae	*Glycyrrhiza glabra* L.	Mayam, Sus (INWM-000083)	Roo	Dec	Int	Asthma	0.01
Fabaceae	*Onobrychis carduchorum* C.C. Tensen	Gete (INWM-000084)	Aer	Mixed with butter	Ext	Wounds	0.01
Iridaceae	*Gladiolus kotschyanus* Boiss.	Kılıç otu (INWM-000085)	Aer	Inf	Int	Peptic Ulcer	0.01
Juglandaceae	**Juglans regia* L.	Ceviz, El Cevizi (INWM-000086)	See	Cru	Int	Peptic Ulcer	0.01

Table 2 (*continue*). Traditional uses of plants in Ağrı (Türkiye)

Family	Plant species, and location	Local name	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Lamiaceae	*Mentha longifolia* (L.) L.	Nane, Punk (INWM-000087)	Lea	Inf	Int	Catarrh, Asthma, Peptic Ulcer, Constipation	0.03
			Lea	Cru	Ext	Toothache, Scorpion Sting, Snakebite	0.02
Lamiaceae	*Teucrium polium* L.	Mervend, Acı Tal, Merven, Mevran, Mervent (INWM-000088)	Lea	Inf	Int	Immunostimulant, İnfertility, Diarrhea, Anti-İnflammatory, Asthma	0.04
			Lea	Cru	Ext	Wounds, Vaginitis	0.03
			Lea	Raw	Int	Peptic Ulcer, Stomachache	0.03
Lamiaceae	*Teucrium chamaedrys* L.	Kısamahmut (INWM-000089)	Aer	Inf	Int	Renal Calculi, Digestive	0.01
Lamiaceae	*Thymus fallax* Fisch. & C.A Mey.	Catri, Catiri, Dağ Kekiği (INWM-000090)	Aer	Inf	Int	Peptic Ulcer, Diabetes, Antipyretic, Asthma	0.03
			Lea	Cru	Ext	Toothache, Wound	0.02
Malvaceae	*Alcea calvertii* (Boiss.) Boiss.	Hiro, Hırabeng (INWM-000091)	Aer	Mixed with milk	Ext	Scorpion Sting, Snakebite, Wounds	0.01
			Aer	Inf	Int	Peptic Ulcer, Diabetes, Hemorrhoids, Vaginitis	0.07
			Aer	Inf	Ext	Earache, Conjunc-tivitis, Stomachache, Dermatological diseases	0.02
Malvaceae	*Malva neglecta* Wallr.	Dolık, Dolıg Tolıg, Tolık (INWM-000092)	Aer	Dec	Ext	Vaginitis	0.01
			Aer	Dec	Int	Peptic Ulcer, Anti-İnflammatory, İmmunostimulant	0.11
			Aer	Raw	Che	Toothache	0.02
			Lea	Dec	Ext	Wounds	0.03
			Lea	Coo	Int	Constipation, Derma-tological diseases	0.03
Moraceae	*Ficus carica* L.	Babesir (INWM-000093)	Fru	Raw	Eat	Hemorrhoids	0.01
Nitrariaceae	*Peganum harmala* L.	Üzerlik (INWM-000094)	Aer	Hea	Inh	Asthma, Sedative	0.01
Oleaceae	**Olea europaea* L.	Zeytin (INWM-000095)	Ole	Raw	Ext	Wounds, Anti-İnflammatory	0.01
Papaveraceae	*Papaver fugax* Poir.	Amıg, Amık (INWM-000096)	Roo	Raw	Eat	Immunostimulant, Peptic Ulcer	0.01
Papaveraceae	*Papaver somniferum* L.	Yabani haşhaş (INWM-000097)	Lat	Raw	Ext	Toothache	0.01
Plantaginaceae	*Plantago major* L.	Damar otu, Belhevis (INWM-000098)	Lea	Raw	Ext	Furoncle, Mastitis, Wounds, Anti-İnflammatory, Vaginitis, Conjunctivitis	0.09
			Lea	Inf	Int	Peptic Ulcer, Renal Calculi, Immunostimulant, Stomachache, Toothache	0.04

Table 2 (*continue*). Traditional uses of plants in Ağrı (Türkiye)

Family	Plant species, and location	Local name	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Poaceae	**Triticum aestivum* L.	Saman (INWM-000099)	Lea	Cru	Ext	Conjunctivitis	0.01
Polygonaceae	*Polygonum cognatum* Meissn.	Kuş epeleği, Nanicivigi, Gulikakiçik (INWM-00000100)	Aer	Inf	Int	Peptic Ulcer, Diabetes	0.02
Polygonaceae	*Rheum ribes* L.	Ribis, Işgın, Rabis, Reviz, Rıbiz (INWM-00000101)	Aer	Raw	Int	Diabetes, Peptic Ulcer	0.02
			Roo	Dec	Int	Diabetes, Renal Calculi	0.06
			Who	Raw	Int	Constipation	0.03
Polygonaceae	*Rumex crispus* L.	Tırşo Tırşoaga (INWM-00000102)	Lea	Cru	Ext	Toothache	0.01
			Lea	Dec	Ext	Hemorrhoids	0.02
			Lea	Dec	Int	Anti-İnflammatory	0.01
			Roo	Dec	Int	Diabetes, Acute Tonsilitis,	0.01
Ranunculaceae	*Thalictrum minus* var. *minus* L.	Katranotu, Karakatranotu (INWM-00000103)	Aer	Inf	Int	Diabetes, Anti-İnflammatory, Digestive	0.03
Rosaceae	*Armeniaca vulgaris* Lam.	Kayısı (INWM-00000104)	Fru	Raw	Eat	Hepatitis, Acute Tonsilitis, Hoarseness	0.01
Rosaceae	*Crataegus orientalis* Pall. ex M.Bieb.	Gıvij, Talik, Alıç (INWM-00000105)	Fru	Dec	Int	Cardiac Diseases, Diabetes, Asthma, Rheumatic Pain	0.02
Rosaceae	*Pyrus elaeagnifolia* Pall.	Dağ Armudu, Karçin (INWM-00000106)	Fru	Raw	Eat	Diarrhea	0.02
Rosaceae	*Sorbus persica* Hedl.	Biog (INWM-00000107)	Fru	Raw	Eat	Immunostimulant	0.01
Rutaceae	**Citrus limon* (L.) Burm.f.	Limon (INWM-00000108)	Per	Cru	Ext	Hernia	0.01
Salicaceae	*Salix alba* L.	Darabi, Söğüt (INWM-00000109)	Bar	Hea	Ext	Wounds, Hernia	0.02
Scrophulariaceae	*Verbascum oreodoxum* Hub.-Mor.	Mavjork, Majerk, Mamujark, Cavreşk, Majork (INWM-00000110)	Aer	Dec	Ext	Furoncle, Wounds	0.01
			Aer	Inf	Int	Diabetes, Hemorrhoids, Rheumatic Pain	0.03
			Lea	Inf	Int	Immunostimulant, Abortifacient	0.02
Solanaceae	*Hyoscyamus niger* L.	Delipıtpıt, Delipatpat (INWM-00000111)	Aer	Hea	Inh	Toothache, Asthma	0.01
Solanaceae	**Lycopersicon esculentum* Mill.	Domates (INWM-00000112)	Fru	Coo	Ext	Ambustion	0.01
Solanaceae	**Solanum tuberosum* L.	Patates (INWM-00000113)	Tub	Cru	Ext	Wounds, Ambustion	0.03
Urticaceae	*Urtica dioica* L.	Gezeng, Gezgezk, Kevgesk, Gezgezik, Isırgan otu, Gezgez, (INWM-00000114)	Lea	Inf	Int	Catarrh, Hemorrhoids, Asthma, Peptic Ulcer, İmmunostimulant, Antipyretic, Hypertension	0.09
			Aer	Inf	Ext	Rheumatic Pain, Vaginitis, Anti-İnflammatory, Dermatologic disorders	0.13

Table 2 (*continue*). Traditional uses of plants in Ağrı (Türkiye)

Family	Plant species, and location	Local name	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Viburnaceae	*Viburnum lantana* L.	Dendereşk, Ayı meyvesi (INWM-0000060)	Fru	Inf	Int	Hypertension, Diabetes, Anti-İnflammatory, Digestive, Immunostimulant	0.05
Zygophyllaceae	*Zygophyllum fabago* L.	Kotibun (INWM-00000115)	Roo	Cru	Ext	Rheumatic Pain	0.01
			Lea	Inf	Int	Immunostimulant	0.01

[a] Plant part(s) used: Aer: Aerial parts; Bar: Bark; Bul: Bulbus; Flo: Flowers; Fru: Fruits; Lat: Latex; Lea: Leaves; Ole: Oleum; Res: Resin; Roo: Roots; See: Seeds; Per: Pericarp; Tub: Tuber; Who: Whole plant

[b] Prep: Preparations; Boi: Boiled; Cooked: Coo; Cru: Crushed; Dec: Decoction; Hea: Heated; Inf: Infusion; Che: Chewable; Mix; Mixed

[c] Adm.: Int: Internal use; Ext: External use; Eat: Eaten as meal; Gar: Gargle; Inh: Inhalation

*Cultivated plants

The plant organs most commonly used to prepare remedies included the aerial parts (31), leaves (22), roots (9), fruits (8), latex (5), bulbus (3), seeds (2), and oleum (2), with occasional utilization of tuber, bark, pericarp, flowers, and resina in some remedies. Additionally, local inhabitants occasionally incorporated other components, such as butter and milk, in remedy preparations. Major methods for preparing drugs included infusion (26), raw application (24), decoction (13), crushing (12), and heating (11) (Table 2). Remedies were predominantly administered internally (48%). Remarkably, the dosage of medicinal preparations often lacked precision; Terms such as "pinch" or "spoonful" were commonly used.

We systematically documented the local names of plants as indicated by the informants. In certain instances, a single vernacular name was attributed to more than one plant species, potentially leading to confusion and a potential reduction in the safe use of plants. Conversely, some plants were associated with more than one vernacular name (e.g., *Salix alba*: Darabi, söğüt; *Rheum ribes*: Ribis, Işgın Rabis, Reviz, Rıbiz). While most plant names have Turkish origins, we also identified Kurdish names. [25-28].

The authors compared their findings to those of previous comprehensive ethnobotanical research carried out in the region of Ağrı [9-15]. The most frequently utilized medicinal plant species in Ağrı were identified as *Urtica dioica*, *Malva neglecta*, *Mentha longifolia*, *Rheum ribes*, *Rumex acetosella*, *Plantago major*, *Plantogo arabica*, *Achillea arabica* and *Alcea calvarii*, and these were documented in these literatures [9-15]. In addition to this information, all of the previous studies were carried out only in certain regions of Ağrı [9-15].

Alcea calvarii (0.07), *Achillea arabica* (0.07), *Beta vulgaris* var. *altissima* (0.06), *Malva neglecta* (0.11), *Plantago major* (0.09), *Rheum ribes* (0.06), *Urtica dioica* (0.13), and *Viburnum lantana* (0.05) had the highest UVs (Table 2). The informants utilized medical plants mainly for the treatment of peptic ulcer, diabetes, immunostimulation, wound healing, and asthma. It has been determined that the number of plants used for cardiovascular problems, epilepsy, prostate issues, myalgia, hypercholesterolemia, abortifacient purposes, hemostatic, and infertility are the lowest. Other studies in Eastern Anatolia have observed that medicinal plants are mostly used in cancer, diabetes, respiratory tract disorders, cardiovascular system disorder, urinary tract system disorders and gastro-intestinal disorders [9-15].

It has been reported the *Alcea calvarii* was used as ınternally as antidiabetic agent in Ağrı. In the *in vitro* study conducted in this direction, it was determined that *Alcea* sp. showed antidiabetic and antiulcer activity [45-46]. Its use as an antiulcer and antidiabetic has also been recorded in other ethnobotanical studies [13,14,29]. Again, it has also been determined that *Achillea arabica* is used at a high rate in folk medicine in Ağrı province. *In vivo* studies have also showed that *Achillea* sp. have antiulcer, antidiabetic, and wound healing activities [47-49]. Furthermore, its use as an antiulcer, antidiabetic, and wound healing agent has also been recorded in other ethnobotanical studies [13,14,25,26,28]. *Urtica dioica* is traditionally used in Ağrı in the treatment of catarrh, hemorrhoids, asthma, peptic ulcer, antipyretic, hypertension and as an immunostimulant. Local people living in Ağrı stated that they used the infusion of this plant. Again, local people reported that this plant is also used

externally for rheumatic disorders, dermatologic disorders, and vaginitis. In previous studies, it was determined that the *Urtica dioica* plant has antiasthmatic, antihemorrhoidal, antiulcer, antihypertensive, and immunostimulatory activities activity [40-44]. It has also been reported that the *Urtica dioica* species is used in folk medicine in Erzincan, Van, and Erzurum [25,27,28]. *Malva neglecta*, *Plantago major*, *Rheum ribes*, and *Viburnum lantana* are plants which are used in Türkiye and across the World [25,28-32,50].

Our study was carried out on the whole of Ağrı and compared to the previous ethbotanical studies on the province of Ağrı. *Pinus sylvestris*, *Beta vulgaris* var. *altissima*, *Ammi visnaga*, *Heracleum platytaenium*, *Achillea arabica*, *Tragopogon buphthalmoides*, *Alkanna orientalis*, *Cephalaria procera*, *Euphorbia seguieriana*, *Glycyrrhiza glabra*, *Gladiolus kotschyanus*, *Papaver fugax*, *Papaver somniferum*, *Polygonum cognatum*, *Thalictrum minus* var. *minus*, *Verbascum oreodoxum*, *Hyoscyamus niger*, and *Zygophyllum fabago* were recorded for the first time in the province of Ağrı [9-15], however it is known that these plant are used as folk medicine in other regions of Anatolia [25-39].

It has been observed that those who do not trust modern medicine give more importance to this traditional knowledge. Furthermore, due to terrorist incidents, some villages in the study area have been evacuated, leading to continued migration from other regions. New settlers may not be familiar with or may not utilize this existing knowledge. Given these challenges, there is a heightened risk of losing traditional knowledge. In a region characterized by challenging geographical conditions and local issues, this study aims to mitigate the loss of ethnobotanical knowledge, serving as an important and meaningful resource for Ağrı.

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AUTHOR CONTRIBUTIONS

Concept: B.M.Y., S.G.; Design: B.M.Y., S.G.; Control: B.M.Y., S.G.; Sources: B.M.Y., S.G.; Materials: B.M.Y., S.G.; Data Collection and/or Processing: B.M.Y., S.G.; Analysis and/or Interpretation: B.M.Y., S.G.; Literature Review: B.M.Y., S.G.; Manuscript Writing: B.M.Y., S.G.; Critical Review: B.M.Y., S.G.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ÖZGÜN MAKALE / ORIGINAL ARTICLE

ANTİ-APOPTOTİK PROTEİN İÇİN YENİ İNHİBİTÖRLERİN *İN-SİLİKO* YÖNTEMLERLE ARAŞTIRILMASI

INVESTIGATION OF NEW INHIBITORS FOR ANTI-APOPTOTIC PROTEIN BY IN-SILICO METHODS

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ÖZ

Amaç: *Bu araştırma makalesinin amacı, terapötik müdahale için yeni yollar keşfetmek üzere in-siliko yöntemler kullanarak anti-apoptotik protein inhibitörlerini incelemektir. Bununla birlikte ilaç keşif süreçlerini hızlandırmada hesaplamalı yaklaşımların potansiyelini vurgulamakta ve daha fazla deneysel doğrulama için umut verici adayları belirlemek için uygun maliyetli ve zaman açısından verimli bir strateji sunmayı hedeflemektedir.*
Gereç ve Yöntem: *Bu çalışmada, önemli anti-apoptotik proteinleri hedef alan potansiyel inhibitörleri belirlemek için gelişmiş hesaplama teknikleri kullanılmıştır. Moleküler yerleştirmeden yararlanarak, hedef proteinlerle bağlanma ilgileri ve etkileşim modellerini tespit etmek için çeşitli kimyasal kütüphaneleri sistematik olarak taranmıştır Çalışma, potansiyel ilaç adaylarının tasarımını optimize etmek için bu etkileşimlerin yapısal inceliklerini aydınlatmaya odaklanmıştır.*
Sonuç ve Tartışma: *Bulgularımız, yüksek bağlanma afiniteleri ve uygun farmakokinetik özelliklere sahip umut verici öncü bileşikleri ortaya çıkarmakta ve bunları daha fazla deneysel doğrulama için aday olarak sunmaktadır. Bu çalışmada elde edilen hesaplamalı içgörüler, anti-apoptotik protein inhibitörleri hakkındaki artan bilgi birikimine katkıda bulunmakla kalmayıp, aynı zamanda anormal apoptotik düzenleme ile karakterize edilen hastalıklarla mücadelede yeni terapötiklerin rasyonel tasarımı için bir temel sağlamaktadır.*
Anahtar Kelimeler: *Anti-apoptotik protein, inhibitör, in-siliko*

ABSTRACT

Objective: *The aim of this research article is to study anti-apoptotic protein inhibitors using in-silico methods to discover new avenues for therapeutic intervention. It also highlights the potential of computational approaches in accelerating drug discovery processes and aims to provide a cost-effective and time-efficient strategy to identify promising candidates for further experimental validation.*
Material and Method: *In this study, advanced computational techniques were used to identify potential inhibitors targeting important anti-apoptotic proteins. Taking advantage of molecular docking, various chemical libraries were systematically screened to detect binding interests and interaction patterns with target proteins. The study focused on elucidating the structural subtleties of these interactions to optimize the design of potential drug candidates.*
Result and Discussion: *Our findings reveal promising lead compounds with high binding affinities and favorable pharmacokinetic properties and present them as candidates for further experimental*

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validation. The computational insights obtained in this study not only contribute to the growing body of knowledge on anti-apoptotic protein inhibitors, but also provide a basis for the rational design of novel therapeutics to combat diseases characterized by abnormal apoptotic regulation.
Keywords: *Anti-apoptotic protein, inhibitor, in-silico*

GİRİŞ

Anti-apoptotik protein BCL-2, programlanmış hücre ölümünün düzenlenmesinde çok önemli bir rol oynar. BCL-2'nin Bax ve Bak gibi pro-apoptotik proteinlerin aktivasyonunu kısıtlayarak apoptozu engellediği bulunmuştur [1]. Ayrıca, anti-apoptotik BCL-2 ve pro-apoptotik Bax arasındaki denge apoptoz indüksiyonunun düzenlenmesi için gereklidir [2]. Çalışmalar ayrıca BCL-2'nin endoplazmik retikulumda kalsiyum homeostazının korunmasında rol oynadığını ve düşük hücre dışı Ca^{2+}'nın antiapoptotik etkisini azalttığını göstermiştir, bu da sitozolik Ca2+ yükselmesinin optimal ER havuzu dolumu ve BCL-2 tarafından apoptoz inhibisyonu için gerekli olabileceğini düşündürmektedir [3]. Ayrıca, Bim/Bcl-2 dengesinin naif ve hafızalı T hücresi homeostazını korumak için kritik olduğu tespit edilmiş ve BCL-2'nin bağışıklık hücresi düzenlemesindeki önemi vurgulanmıştır [4]. Dahası, BCL-2'nin mikroRNA'lar tarafından düzenlenmesi çeşitli patolojik durumlarla ilişkilendirilmiştir. Örneğin, yukarı regüle miR-1183'ün BCL-2 ailesindeki apoptoz proteinlerinin dengesini bozduğu, romatizmal kalp hastalığı bağlamında apoptoz kaskad reaksiyonlarının artmasına ve anti-apoptoz etkilerinin azalmasına yol açtığı gösterilmiştir [5]. Ayrıca, BCL-XL ve MCL-1 anti-apoptotik proteinlerinin ikili inhibisyonunun kanser hücrelerinin sitotoksisitesini artırdığını gösteren çalışmalarla BCL-2'yi hedeflemenin terapötik potansiyeli araştırılmıştır [6]. Ayrıca, BCL-2'nin diğer proteinlerle etkileşimi de araştırılmıştır. Örneğin, BCL-2'nin BCL-2 benzeri bir şekilde işlev gören bir dizi sadece BH3 proteinini ve aktif Bax'ı bağladığı ve nötralize ettiği bulunmuştur [7]. Ayrıca, ülseratif kolitin iltihaplı kolon mukozasında Bax'ın aşağı regülasyonu, BCL-2'nin antiapoptotik etkisine karşı koyma ile ilişkilendirilmiştir [8]. Sonuç olarak, BCL-2 apoptozun önemli bir düzenleyicisidir ve diğer proteinler ve mikroRNA'lar ile karmaşık etkileşimleri, bağışıklık hücresi homeostazı, kanser ve kalp hastalığı dahil olmak üzere çeşitli fizyolojik ve patolojik süreçlerde önemli roller oynamaktadır.

GEREÇ VE YÖNTEM

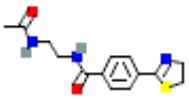
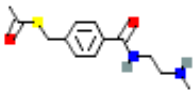
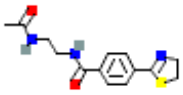
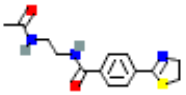
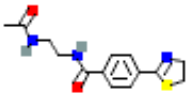
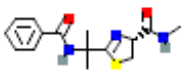
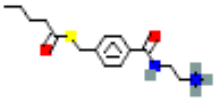
Anti-apoptotik proteinin yapısı (PDB ID: 7YB7) PDB veritabanından indirilmiştir. Molekül yapılarının benzerliği PubCHEM veritabanından araştırılmış benzerlik oranı %93 olacak şekilde ayarlanmıştır. (Tablo 1). Protein yapısının indirilmesi ve temizlenmesi için Biovia Discovery Studio program [9] kullanılmıştır. Docking analizi için Autodock [10] yazılımı ile vina [11] ve vinardo [12] kuvvet alanları, DFT hesabında ise Gaussian [13] programı kullanılmıştır (Versiyon G09), DFT hesaplarının görselleştirmesinde Gausview5 [14] programı kullanılmıştır. Ligandların 3 boyutlu yapıları pubchem sitesinden indirilmiştir.

SONUÇ VE TARTIŞMA

Tablo 2'de görüldüğü gibi, docking sonuçlarına göre bağlanma enerjisi en düşük olan molekül (4R)-2-(2-benzamidopropan-2-il)-*N*-metil-4,5-dihidro-1,3-tiyazol-4-karboksamid (PubChemID: 11141281)'dir. Tablo incelendiğinde, Anti-apoptotik protein ait PDB ID: 7YB7 yapısında bulunan inhibitorün (PubChem ID: 168654843) bağlanma enerjisi -6.5 kcal/mol iken benzerlik taramasında göre bulunan (4R)-2-(2-benzamidopropan-2-il)-*N*-metil-4,5-dihidro-1,3-tiyazol-4-karboksamid (PubChem ID: 11141281) adlı molekülün bağlanma enerjisi -6.8 kcal/mol olarak bulunmuştur. Docking sonuçlarının analizinde sadece bağlanma enerjisini dikkate almak yanıltıcı sonuçlar doğurabilir. Sadece docking skoruna göre yapılan analizler protein-ligand etkileşimi hakkında sınırlı bilgi erişimi sağlar. Bu sınırlılıktan kaçınmak için çalışmaya konu olan protein ile 168654843 ve 11141281 ID'li moleküllere ait ayrıntılı etkileşim analizleri yapılmıştır. Şekil 1'e göre, 168654843 ID'li molekül TYR130 rezidüsü ile hidrojen ve Pi bağı, TYR77 rezidüsü ve ALA81 rezidüsü ile de Pi-Alkyl bağı yapmaktadır (Şekil 2A ve 2B). SAS analiziyle bakıldığında (Şekil 2C) bağ kurulan bu bölgelerin su ile etkileştiği, bağ kuran bölgelerin sui le etkileşme ihtimallerinin yüksek olduğu görülmektedir. Bu durum 80, 84, 85, 88, 89,

92, 126 ve 129 no'lu rezidülerle olan van der Waals etkleşimlerinin etkisini azaltmaktadır. 11141281 ID'li bileşik ile olan etkileşim incelendiğinde LEU87 rezdüsü ile hidrojen ve alkil etkileşimleri olduğu görülmektedir. MET65 rezidüsü ile kurulan pi-Sulfur etkileşimin bağlanma enerjisine büyük katkısı olduğu görülmektedir. ALA99, ARG96, VAL83 rezidüleriyle Pi-Alkyl etkileşimleri bulunmaktadır. TYR58, GLU86 ve GLU64 rezidüleri van der Waals etkileşimleri içerisindedir. SAS analizi yapıldığında, bağların yoğun olduğu aromatik halka'nın proteinin SAS değeri düşük kısmıyla etkileşitği görülmektedir. Bağ yapılan bölge su ile daha az temas ettiği için kurulan etkileşme nispeten daha güçlü olmaktadır.

Tablo 1. Çalışmada kullanılan moleküllerin yapıları, molekül formülleri, moleküler ağırlıkları ve 2D gösterimleri

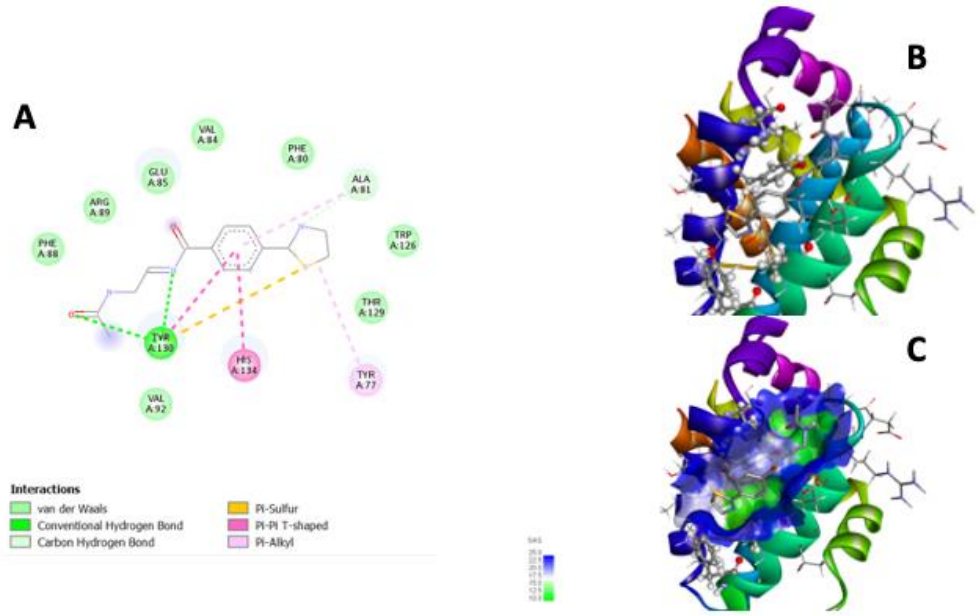
Molekül	MF*	MA**	2D Yapısı	PubChem ID
***N*-(2-asetamidoetil)-4-(4,5-dihidro-1,3-tiyazol-2-il)benzamid**	$C_{14}H_{17}N_3O_2S$	291.37 g/mol		168654843
***S*-[[4-[2-(metilamino)etilkarbamoil]-fenil]metil] etanetiyoat ***	$C_{13}H_{18}N_2O_2S$	266.36 g/mol		156074004
***S*-[[4-(2-aminoetilkarbamoil)-fenil]metil] etanetiyoat**	$C_{12}H_{16}N_2O_2S$	252.33 g/mol		156038047
***S*-[[4-(2-aminoetilkarbamoil)-fenil]metil] pentanetiyoat**	$C_{15}H_{22}N_2O_2S$	294.4 g/mol		146404377
***N*-(2-asetamido-etil)-4-etilsulfanil-metil)benzamid**	$C_{14}H_{20}N_2O_2S$	280.39 g/mol		119041195
(4*R*)-2-(2-benzamidopropan-2-il)-N-metil-4,5-dihidro-1,3-tiyazol-4-karboksamit	$C_{15}H_{19}N_3O_2S$	305.4 g/mol		11141281
2-[[4-(Pentanoilsülfanil-metil)benzoil]amino]etilazanium	$C_{15}H_{23}N_2O_2S^+$	295.4 g/mol		167587006

* Molekül Formülü ** Molekül Ağırlığı

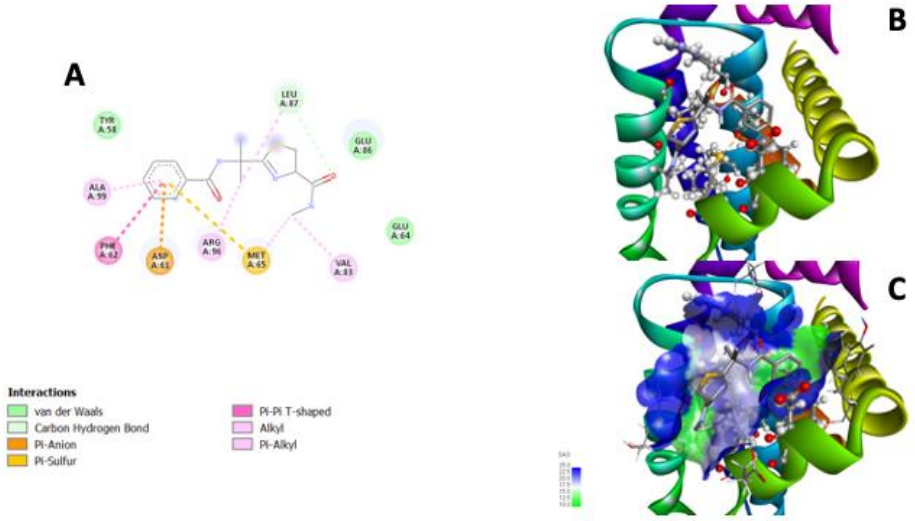
Docking Sonuçlarının Analizi

Tablo 2. Çalışmada kullanılan moleküllerin docking skorları

Ligand (PubChem ID)	Bağlanma Affinitesi kcal/mol (Vina)	Bağlanma Affinitesi kcal/mol (Vinardo)
11141281	-6.882	-5.786
168654843	-6.502	-5.043
167587006	-6.001	-4.615
119041195	-5.83	-4.812
156074004	-5.712	-4.952
156038047	-5.588	-4.766
146404377	-5.52	-4.269



Şekil 1. 168654843 ID'li molekülün docking sonuçlarının 2 boyutlu (A), 3 boyutlu (B) ve SAS gösterimi (C)

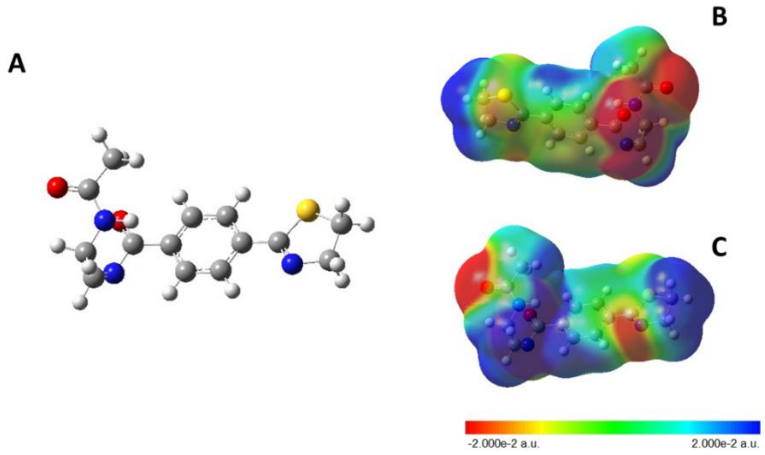


Şekil 2. 11141281 ID'li molekülün docking sonuçlarının 2 boyutlu (A), 3 boyutlu (B) ve SAS gösterimi (C)

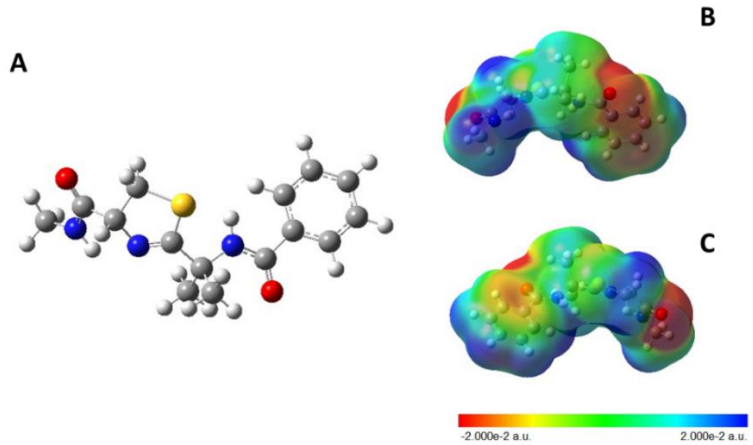
168654843 ve 11141281 ID'li Moleküllerin Fiziksel ve Kimyasal Özelliklerinin Analizi

Yapılan DFT hesapları ile her iki yapı da ayrıntılı olarak incelenmiştir. 168654843 ID'li molekülün polar olduğu tespit edilmiş ve dipol momenti 6.441854 Debye ve optimizasyon enerjisi -1255.417971 Hartree olarak hesaplanmıştır. Şekil 3A'da 168654843 ID'li molekülün optimizasyon hesabı sonucu elde edilmiş kararlı yapısı görülmektedir. Moleküle ait elektrostatik yük haritası önden Şekil 2B ve arkadan Şekil 3C'de hesaplanmıştır. Oksijenin bulunduğu bölgede elektron donörü olarak davranma eğiliminin yüksek olduğu görülmektedir. 2 boyutlu docking etkileşiminde de görüldüğü gibi bu bölge protein ile hidrojen bağı kurmaktadır. Molekülün mavi kısımlarının donör olarak davranma potansiyelinin; özellikle nitrojen atomlarının bulunduğu bölgede yüksek olduğu görülmektedir. 11141281 ID'li molekülün DFT hesapları sonucunda (Şekil 4) bu molekülün de diğeri gibi polar olduğu

tespit edilmiştir. Dipol momenti 1.058436 Debye ve optimizasyon enerjisi-1295.963944 Hartree olarak hesaplanmıştır. Optimizasyon enerjisi değerine göre 11141281 ID'li molekülün diğerine göre daha kararlı olduğunu söyleyebiliriz. Elektrostatik yük haritası değerlendirmesine göre 11141281 ID'li molekül de hem donör hem de akseptör olarak davranma özelliğine sahiptir. Ancak, bu molekülün negatif yüzeyi nispeten küçük olduğu için akseptör olarak davranma kabiliyeti diğer moleküle göre daha azdır (Şekil 4B ve 4C).

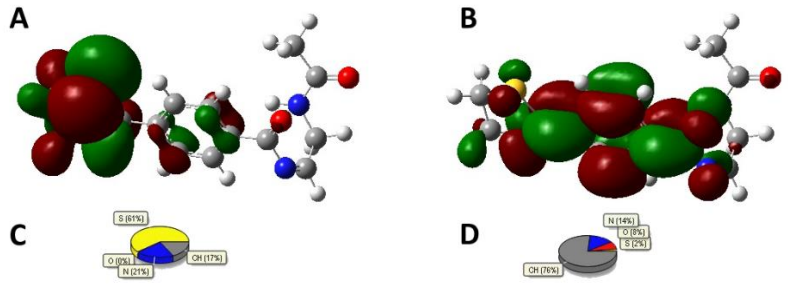


Şekil 3. 168654843 ID'li moleküle ait optimize yapı (A), Elektronik yük yoğunluğu haritasının önden (B) ve arkadan (C) görüntüsü

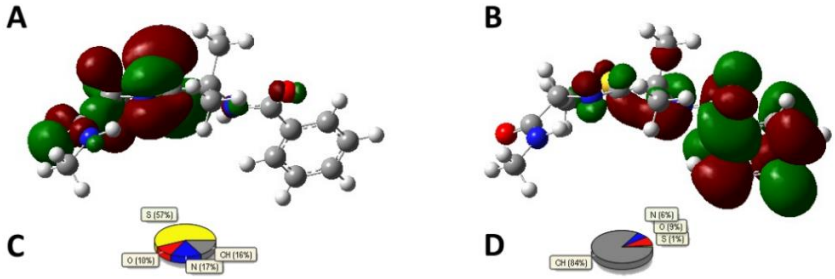


Şekil 4. 11141281 ID'li moleküle ait optimize yapı (A), Elektronik yük yoğunluğu haritasının önden (B) ve arkadan (C) görüntüsü

Moleküler düzeyde yapılan HOMO-LUMO etkileşimlerinin incelenmesinde ise 168654843 ID'li molekülde yükün, molekülün orta yerinde lokalize olduğu görülmektedir (Şekil 5A ve 5B). Bu durum etkileşimin etkinliğini azaltmaktadır. Bu molekülün HOMO orbitallerinde aktif olan fragmentler Kükürt, Azot ve Karbon elementleridir (Şekil 5C). 11141281 ID'li molekülde, molekül için yük transferi olduğu görülmektedir (Şekil 6A ve 6B). Başta bir deyişle molekül içi yük hareketliliği bu molekülde daha fazladır. Burada valans bandından iletkenlik bandına daha hızlı elektron aktarılmakta ve molekül polarizasyonunu korumaktadır. Bu durum etkileşme anında, etkileşmenin etkinliğini arttırmaktadır ki bu da docking etkileşme enerjilerinin verildiği Tablo1 kapsamında gösterilmiştir. Bu molekülün HOMO orbitallerinde aktif fragmentler Kükürt, Azot, Karbon ve diğer molekülden farklı olarak Oksijen'dir (Şekil 6C). Bu da molekülün Oksijen atomlarının da akseptör olarak davranmasına neden olmaktadır. Bu davranış afinite değerini, diğer moleküle göre arttırmakta ve Tablo 1'deki değerin nedenini açıklamaktadır.



Şekil 5. 168654843 ID'li moleküle ait HOMO (A), LUMO (B) ve HOMO Fragment (C) ve LUMO (D) Fragment gösterimi



Şekil 6. 11141281 ID'li moleküle ait HOMO (A), LUMO (B) ve HOMO Fragment (C) ve LUMO Fragment gösterimi

Sonuç olarak, anti-apoptotik protein inhibitörlerine yönelik *in-siliko* araştırmamız, düzensiz apoptotik süreçlerle kendini gösteren hastalıklarda terapötik müdahale için potansiyel yollara ilişkin değerli bilgiler sağlamıştır. Bu çalışmada kullanılan hesaplama yöntemleri ile önemli anti-apoptotik proteinlere karşı umut verici inhibitör özelliklere sahip yeni bileşiklerin tanımlanması ve karakterizasyonu gösterilmiştir. Moleküler etkileşimlerin, moleküler yerleştirme ve dinamik simülasyonları yoluyla sistematik analizi, inhibitörler ve hedef proteinler arasındaki etkileşimi yöneten karmaşık bağlanma modellerin ve yapısal farkların ayırt edilmesi sağlanmıştır. Bu yaklaşım, gelişmiş farmakokinetik profillere sahip güçlü ve seçici inhibitörlerin rasyonel tasarımı için kritik önem taşımaktadır. Yüksek bağlanma afinitesine sahip öncü bileşiklerin tanımlanması, deneysel doğrulama için öncül bilgiler sunarak, hesaplama tahminlerinin somut farmasötik çözümlere dönüştürülmesinin önünü açmaktadır. Hesaplama yöntemlerinin çıktıları deneysel çalışmalar ile tamamlandığında, daha verimli ve kolaylaştırılmış bir ilaç keşif sürecine olanak sağlamaktadır. Hesaplamalı biyologlar, tıbbi kimyagerler ve deneysel biyologlar arasındaki işbirliği, *in-siliko* bulguların klinik olarak ilgili terapötiklere başarılı bir şekilde dönüştürülmesinin anahtarıdır. Ayrıca, biyolojik sistemlerin dinamik yapısı, öngörüsel doğruluklarını artırmak için hesaplama modellerinin ve yöntemlerinin sürekli olarak iyileştirilmesini gerektirmektedir. Bu araştırma, sadece anti-apoptotik protein inhibisyonunun anlaşılmasına katkıda bulunmakla kalmayıp, aynı zamanda ilaç keşfini hızlandırmada *in-siliko* metodolojilerinin daha geniş potansiyele sahip olduğunun da altını çizmektedir.

YAZAR KATKILARI

Kavram: M.A.U.; Tasarım: M.A.U.; Denetim: M.A.U.; Kaynaklar: M.A.U.; Malzemeler: M.A.U.; Veri Toplama ve/veya İşleme: M.A.U.; Analiz ve/veya Yorumlama: M.A.U.; Literatür Taraması: M.A.U.; Makalenin Yazılması: M.A.U.; Kritik İnceleme: M.A.U.; Diğer: -

ÇIKAR ÇATIŞMASI BEYANI

Yazar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan eder.

ETİK KURUL ONAYI

Yazar bu çalışma için etik kurul onayının zorunlu olmadığını beyan etmektedir.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

EVALUATION OF THE IMPACT OF DIFFERENT SUPERDISINTEGRANTS ON THE *IN VITRO* CHARACTERIZATION PARAMETERS OF ORALLY DISINTEGRATING TABLETS CONTAINING KETOPROFEN

FARKLI SÜPER DAĞITICILARIN KETOPROFEN İÇEREN AĞIZDA DAĞILAN TABLETLERİN İN VİTRO KARAKTERİZASYON PARAMETRELERİ ÜZERİNDEKİ ETKİSİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *Orally Disintegrating Tablets (ODTs) have revolutionized pharmaceutical drug delivery, offering a patient-friendly alternative for those struggling with conventional tablet swallowing. This study delves into the impact of superdisintegrants (crospovidone, sodium starch glycolate, and croscarmellose sodium) on the in vitro characterization of Ketoprofen-containing ODTs. ODTs are designed to rapidly disintegrate in the oral cavity without water, enhancing patient compliance, ensuring faster therapeutic onset, and providing convenience.*

Material and Method: *The micromeritic properties of pre-compression Ketoprofen ODT blends were assessed for bulk density, tapped density, Hausner ratio, and compressibility index. ODTs were formulated using a direct compression method to maintain component uniformity. Comprehensive characterization included weight variation, tablet hardness, friability, wetting time, and in vitro disintegration time assessments. The drug content was determined through UV spectrophotometry of dissolved ODTs, and dissolution studies were conducted in pH 6.8 phosphate buffer using USP apparatus XXIV.*

Result and Discussion: *Results showed uniform tablet mass and favorable powder mixture flowability, ensuring ODT physical properties. Tablets exhibited excellent mechanical resistance with consistent hardness and low friability loss. All formulations demonstrated high and uniform drug content. Different superdisintegrants influenced wetting, disintegration, and dissolution times. Crospovidone exhibited the fastest wetting time but longer disintegration times, attributed to increased tablet hardness. Dissolution studies revealed that crospovidone-containing ODTs had faster drug release compared to croscarmellose sodium and sodium starch glycolate, aligning with literature findings. The study emphasized the importance of considering both wetting and disintegration times for a comprehensive evaluation of ODT performance. Croscarmellose sodium and sodium starch glycolate hindered drug release, forming gel-like masses impeding dissolution, while crospovidone enhanced drug release in formulated ODTs. In conclusion, the study provides valuable insights for pharmaceutical development and patient-centric drug delivery solutions, showcasing the influence of superdisintegrants on ODT performance and emphasizing the importance of considering various parameters for comprehensive evaluation.*

Keywords: *Croscarmellose sodium, crospovidone, ketoprofen, orally disintegrating tablet (ODTs),*

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sodium starch glycolate

ÖZ

Amaç: *Ağızda Dağılan Tabletler (ADT'ler), geleneksel tabletleri yutmakta zorluk çeken hastalar için önemli bir alternatif sağlayarak farmasötik ilaç dağıtımında önemli bir ilerleme kat etmiştir. Bu çalışma, Ketoprofen içeren ADT'lerin in vitro karakterizasyonu üzerinde krospovidon, sodyum nişasta glikolat ve kroskarmeloz sodyum gibi süper dağıtıcıların etkisine odaklanmaktadır. ODT'ler, hızlı parçalanma, gelişmiş hasta uyumu ve terapötik başlangıç hızı sağlamak üzere ağız içinde su kullanmadan tasarlanmıştır.*

Gereç ve Yöntem: *Basım öncesi ketoprofen içeren ADT toz karışımları mikromeritik özellikleri, hacim yoğunluğu, sıkıştırılmış yoğunluk, Hausner oranı ve sıkıştırılabilirlik indeksi değerlendirilmiştir. ADT'ler, doğrudan basım yöntemi kullanılarak hazırlanmıştır. Karakterizasyon çalışmaları kapsamında, ağırlık sapması tayini, tablet sertliği, kırılma dayanıklılığı, ıslanma süresi ve in vitro çözünme hızı testleri yapılmıştır. Etken madde miktarı, UV spektrofotometrik yöntem ile belirlenmiştir. Çözünme hızı çalışmaları, USP XXIV'e göre pH 6.8 fosfat tamponunda belirli zaman aralıklarında etken madde miktarının ölçülmesi ile değerlendirilmiştir.*

Sonuç ve Tartışma: *ADT'lerin fiziksel özellikleri, özellikle içerdikleri ketoprofen bakımından incelenerek toz karışımlarının homojenliğini ve istenen akıcılığı sağlama hedeflenmiştir. Tabletlerin mekanik direnci uygun, sertlik değerleri istenen aralıkta ve kırılganlıklarının yeterli düzeyde olduğu tespit edilmiştir. Ayrıca, tüm formülasyonlarda yüksek ve homojen etken madde içeriği gözlemlenmiştir. Farklı süper dağıtıcılarla hazırlanan ADT'lerin ıslanma, çözünme ve dağılma süreleri üzerindeki etkileri araştırılmış, krospovidonun diğerlerine göre daha hızlı ıslanma sağladığı belirlenmiştir. Ancak, krospovidon içeren tabletlerin in vitro çözünme sürelerindeki gecikmenin muhtemelen daha yüksek tablet sertliğinden kaynaklandığı gözlemlenmiştir. Çözünme hızı çalışmaları, krospovidon içeren ADT'lerin kroskarmeloz sodyum ve sodyum nişasta glikolat içerenlere göre daha hızlı etken madde salımı sağladığını göstermiş ve bu sonuç, literatürdeki bulgularla uyumlu bulunmuştur. Çalışma, ADT'lerin performansının kapsamlı bir değerlendirmesi için hem ıslanma hem de dağılma sürelerinin önemine vurgu yapmıştır. Kroskarmeloz sodyum ve sodyum nişasta glikolatın çözünmeyi geciktiren jel benzeri kütleler oluşturduğu, krospovidon içeren ADT'lerde ise etken madde salımının daha hızlı olduğu gözlemlenmiştir.*

Anahtar Kelimeler: *Ağızda hızlı dağılan tablet (ADTs), ketoprofen, kroskarmeloz sodyum, krospovidon, sodyum nişasta glikolat*

INTRODUCTION

Orally Disintegrating Tablets (ODTs) represent a significant advancement in pharmaceutical drug delivery, offering a patient-friendly alternative to traditional oral dosage forms. These tablets, designed to rapidly disintegrate in the oral cavity without the need for water, provide a convenient option for individuals facing challenges in swallowing conventional tablets or capsules. Formulated using techniques such as direct compression, freeze-drying, or sublimation, ODTs rely on carefully chosen excipients, including superdisintegrants to facilitate quick tablet breakdown. Their advantages include improved patient compliance, particularly in pediatric and geriatric populations, as well as a faster onset of therapeutic effects and enhanced convenience for on-the-go use. ODTs find applications across various therapeutic areas, from analgesics to antipsychotics, showcasing their versatility in accommodating different drug classes. Regulatory agencies, such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), provide guidelines to ensure the safety, efficacy, and quality of ODTs. Despite their benefits, challenges like taste-masking and stability maintenance persist, prompting ongoing research into innovative technologies like 3D printing and nanotechnology. In essence, ODTs have become a cornerstone in modern pharmaceutical development, reflecting a commitment to patient-centric drug delivery solutions [1].

The efficacy of ODTs depends critically on the selection and synergistic interaction of excipients. While crospovidone, sodium starch glycolate andcroscarmellose sodium are prominent superdisintegrants in orally disintegrating tablets (ODTs), their efficacy is fundamentally dependent on the synergistic interaction with other excipients. Superdisintegrants, are those substances, which facilitate the faster disintegration with smaller quantity in contrast to disintegrants. Widely

employed superdisintegrants, including croscarmellose sodium, crospovidone and sodium starch glycolate, demonstrate high efficiency at low concentration levels (2-5 w/w%) in tablet formulations, effectively enhancing the speed and completeness of tablet disintegration [2].

Croscarmellose sodium is derived from the sodium salt of cross-linked, partially O-carboxymethylated cellulose, whereas sodium starch glycolate originates from the sodium salt of a carboxymethyl ether of starch or a cross-linked carboxymethyl ether of starch. Both substances are anionic sodium salts, and their polymer backbones primarily consist of repeating glucose units [3].

On the contrary, crospovidone constitutes an insoluble, cross-linked homopolymer of *N*-vinyl-2-pyrrolidone and is characterized as nonionic. Chemically, the repetitive structure of crospovidone closely resembles that of N-methylpyrrolidone (NMP), a water-miscible, polar aprotic solvent renowned for its high interfacial activity and employed as a solubilizer in various applications. Crospovidone experiences substantial hydration and swelling, generating disruptive pressure within the tablet matrix. In contrast, croscarmellose sodium leverages its elastic properties; it readily undergoes deformation under compression, snapping back to its original shape and leading to tablet fragmentation [4].

Sodium starch glycolate is the sodium salt of carboxymethyl ether. Sodium starch glycoate is a white to off-white, bland, odorless, moderately free streaming powder. It absorbs water quickly, bringing about swelling which prompts fast breaking down of tablets and granules [5].

Bulking agents like microcrystalline cellulose provide the foundational framework for the tablet, while binders like polyvinylpyrrolidone ensure ingredient cohesion before the superdisintegrants exert their action. Lubricants like magnesium stearate facilitate smooth tableting and prevent unwanted sticking. Furthermore, flavorants and sweeteners play a crucial role in masking the often unpleasant taste of the active pharmaceutical ingredient (API) and its excipient partners. This delicately balanced composition of excipients, precisely harmonized, underpins the rapid disintegration that defines ODTs [1].

In this study, Ketoprofen was chosen as the model active ingredient, and the aim was to investigate the impact of superdisintegrants with different disintegration mechanisms namely; croscarmellose sodium, crospovidone and sodium starch glycolate on the *in vitro* characterization parameters of ODT formulations.

To comprehend the impacts of superdisintegrants in the prepared ODT formulations and compare superdisintegrants from different groups, several essential *in vitro* controls must be undertaken. Examining the micromeritic properties of a powder mass is essential for refining manufacturing processes, maintaining uniform dosage forms, predicting compression behavior, and improving the overall performance and stability of pharmaceutical formulations during storage. Special attention is given to evaluating disintegration time, which determines how quickly the tablet breaks down in the mouth. Ensuring uniformity in dosage units is achieved through tests like content uniformity. Friability testing assesses the tablet's resistance to abrasion during handling and transportation, ensuring its structural integrity. Additionally, hardness testing gauges the tablet's mechanical strength. *In vitro* dissolution studies are crucial for understanding the drug release profile from the ODT, offering valuable insights into its bioavailability. Evaluating wetting and *in vitro* dispersion time are also essential for ensuring the stability and shelf life of ODTs [6]. Collectively, a comprehensive range of *in vitro* tests validates the quality, functionality, and performance of ODTs, ensuring optimal outcomes for patients.

MATERIAL AND METHOD

Materials

Ketoprofen (Dolder, Germany), Aerosil (Colloidal silicon dioxide; Evonik Rohm GmbH, Germany), Polyvinylpyrrolidone K-30 (Crospovidone; Fluka, Germany), Microcrystalline cellulose (Avicel PH 101; FMC Biopolymer, Philadelphia), Aspartame (Deva Holding, Turkey), ++++Sodium starch glycolate (Explotab; JRS Pharma, Germany), Magnesium stearate (Riedel de Haen, Germany), Pregelatinized starch (Lyclatab, Roquette, France) and Menthol (OKimya, Turkey).

Methods

Micromeritic Properties of Ketoprofen ODTs

Before compression, the micromeritic properties of ketoprofen ODT blends were assessed for various formulations. The mixture blends for all formulations underwent pre-compression parameter evaluations, including tapped density, bulk density, compressibility index and Hausner ratio. These parameters were analyzed to compare the initial powder volume with the final (tapped) volume, providing insights into the flowability of the ODT powder blends. Bulk density was determined following the USP method I, while tapped density was determined using a tapped density tester (Aymes, Turkey) derved from the USP method II. The Hausner ratio and compressibility index were calculated using Equations (1) and (2) respectively [7]. These evaluations contribute to the comprehension of powder blend characteristics prior to compression, assisting in forecasting the flowability of ketoprofen ODT formulations.

$$\text{Hausner ratio} = \text{tapped density}/\text{bulk density} \quad \text{(Equation 1)}$$

$$\text{Compressibility index \%} = (\text{tapped density} - \text{bulk density}) \times 100 / \text{tapped density} \quad \text{(Equation 2)}$$

Tables 1 and 2 provide information on the composition of ketoprofen orally disintegrating tablets (ODTs) and the micromeritic properties of the ODT powder blends, respectively.

Table 1. Formulation details for Orally Disintegrating Tablets (ODTs) containing ketoprofen

Component	Amount (mg)	Function	F1	F2	F3
Ketoprofen	100	Active Pharmaceutical Ingredient (API)	✓	✓	✓
Microcrystalline cellulose	150	Bulking agent	✓	✓	✓
Pregelatinized starch	50	Disintegrant	✓	✓	✓
Polyvinylpyrrolidone	30	Binder	✓	✓	✓
Colloidal silicon dioxide	10	Glidant	✓	✓	✓
Magnesium stearate	5	Lubricant	✓	✓	✓
Aspartame	5	Sweetener	✓	✓	✓
Menthol	2	Flavorant	✓	✓	✓
***Superdisintegrant**					
*Croscarmellose sodium	5	Swelling and mechanical disruption	✓		
* Crospovidone	5	Wicking and swelling		✓	
* Sodium starch glycolate	5	Wicking and gel formation			✓

Table 2. Micromeritic properties of ketoprofen Orally Disintegrating Tablets (ODTs) (n=3±SD)

ODT Formulations	F1	F2	F3
Bulk density (g/ml)	0.51±0.33	0.45±0.22	0.33±0.37
Tapped density (g/ml)	0.58±0.42	0.528±0.37	0.31±0.29
Hausner ratio	1.16±0.52	1.16±0.63	1.04±0.41
Compressibility index (%)	14.06±0.41	14.24±0.51	19.67±0.31

Preparation of Ketoprofen ODTs

The preparation of the ODTs containing Ketoprofen involves a systematic direct compression method to ensure optimal therapeutic efficacy and patient compliance. Commencing with the accurate weighing of each component, including the active pharmaceutical ingredient (API) Ketoprofen, bulking agent microcrystalline cellulose, disintegrant (with each ODT formulation containing the same amount

but featuring a different type), binder polyvinylpyrrolidone, glidant colloidal silicon dioxide, lubricant magnesium stearate, sweetener aspartame, flavorant menthol, and the critical superdisintegrant croscarmellose sodium, the formulation is assembled. For the preparation of all formulations (F1, F2, and F3), precise weighing of all components, excluding the lubricant and glidant, was followed by thorough mixing in a cubic mixer (Erweka, Germany) for 15 minutes. Subsequently, the resulting blend underwent lubrication with magnesium stearate for an additional 5 minutes, after which the mixture was directly compressed into tablets. The quantities of all tablet components, except superdisintegrants, were consistently maintained. Using an eccentric single-punch tabletting machine (Korsch, Germany), flat-faced tablets weighing 357 mg and measuring 12 mm in diameter were manufactured. The tablet thickness and hardness were consistently controlled at 3.0 ± 0.1 mm and 3.5 ± 0.5 kg, respectively, across all formulations. Detailed information on the physical properties of the ODT formulations is presented in Table 3.

Table 3. Physical properties of ketoprofen Orally Disintegrating Tablets (ODTs) (n=3±SD)

	F1	**F2**	**F3**
Hardness (kg)	3.50±0.24	3.92±0.28	3.32±0.32
Friability (%)	0.68±0.15	0.59±0.29	0.48±0.21
Content uniformity (%)	99.85±0.36	100.04±0.29	99.97±0.33
Wetting time (sec)	44.33±2.48	34.67±3.10	62.00±2.31
***In vitro* disintegration time (sec)**	39.27±3.25	47.67±1.15	40.20±4.16

Characterization of ODTs

Weight Variation

To evaluate weight variation, twenty randomly chosen Orally Disintegrating Tablets (ODTs) from each formulation were individually weighed using a Sartorius BL 210S scale in Göttingen, Germany. The individual weights were subsequently compared with the average weight to ascertain the extent of weight variation. Additionally, the diameter and thickness of ten ODTs from each formulation were measured using a micrometer [8].

Tablet Hardness

To evaluate tablet hardness, which signifies the force required to break a tablet through radial compression, we utilized a tablet hardness tester, specifically the Monsanto tablet hardness tester. This measurement was performed to assess the tablets' crushing tolerance [8].

Measurement of Friability

To measure friability, ten tablets were weighed and introduced into the friabilator (Roche Friabilitor, Ludwigshafen, Germany). The tablets underwent rotation at 25 rpm, and subsequently, the friability percentage was computed [9].

Measurement of Wetting Time and *In Vitro* Disintegration Time

A folded piece of paper tissue (10.75 mm x 12 mm) was arranged in a culture dish with a diameter of 6.5 cm, filled with 6 ml of water. Subsequently, a tablet was positioned on the paper, and the duration for full wetting was documented. After achieving complete wetting, the tablet was then weighed. The *in vitro* disintegration time test assessed the tablets' capacity to break down into small fragments in fluid. The time taken for the tablet to completely disintegrate in the fluid was recorded. Three repetitions for each formulation were conducted [7].

Determination of Drug Amount

In the initial stage, ten Orally Disintegrating Tablets (ODTs) were weighed and finely powdered using a mortar from Ildam Kimya, Turkey. The average weight of a tablet was then calculated. An amount equivalent to the average weight of the tablet's content was accurately measured from the

powdered tablets. A small quantity of ethyl alcohol was added to dissolve the active material, and the solution was adjusted to a volume of 100 ml in a volumetric flask from Ildam Kimya, Turkey. The mixture underwent sonication for 10 minutes and was subsequently filtered. Next, 1 ml of this solution was transferred to another volumetric flask and diluted to 25 ml with pH 6.8 phosphate buffer. The absorbance value at 261 nm in this solution was determined using UV spectrophotometry, and the drug amount in the sample was calculated using the calibration equation [7].

Dissolution Studies

In vitro drug release was evaluated employing USP apparatus XXIV (paddle assembly) with a rotation speed of 50 rpm, while maintaining the temperature at 37 ± 5°C in 900 ml of pH 6.8 phosphate buffer as the dissolution medium. The percentage of drug release was calculated by withdrawing a 5 ml aliquot at different time intervals, filtering it through Whatman filter paper, and assaying it at 261 nm. To maintain the original volume, an equal volume of fresh dissolution medium was replenished. The dissolution studies were carried out in triplicate [7].

RESULT AND DISCUSSION

Physical Properties of the Tablet Blend

To ensure the uniformity of tablet mass, an analysis of the powder mixture's flow properties was conducted prior to compression. The powder mixture exhibited favorable flowability, as indicated by low Hausner's ratio (ranging from 1.04 ± 0.41 to 1.16 ± 0.63) and compressibility index values (ranging from 14.06 ± 0.41 to 19.67 ± 0.31). The free-flowing nature of the tablet powder mixture resulted in tablets with uniform weight and acceptable weight variation (3.61 %) due to consistent die fill. The tablets demonstrated good mechanical resistance, reflected in hardness values (ranging from 3.32±0.32 to 3.92±0.28 kg/cm²) and low friability loss (ranging from 0.48±0.21 to 0.68±0.15 %). Additionally, all ketoprofen ODT formulations exhibited high and uniform drug content, ranging from 99.85±0.36 to 100.04±0.29 (Table 3).

The Impact of Superdisintegrant Selection on the Wetting, Disintegration, and Dissolution Times of Ketoprofen ODTs

In the development of ODTs, optimizing the wetting time is crucial, as wetting serves as the initial stage of both disintegration and dissolution. In this study, the wetting time for ketoprofen ODTs ranged from 34.67±3.10 to 62.00±2.31 seconds, meeting the official requirements (not exceeding <3 minutes) for orodispersible tablets [10]. All the ODTs incorporating various disintegrants exhibited water absorption, leading to slight swelling and subsequent disintegration. This behavior aligns with the findings from literature research, indicating that sodium starch glycolate, crospovidone, and croscarmellose sodium disintegrate through analogous wicking and swelling processes. Due to their porous particle morphology, these disintegrants draw water into the tablet via capillary action, inducing secondary swelling, breakage of interparticle bonds, and ultimately facilitating tablet disintegration [11]. The wetting time of orally disintegrating tablets (ODTs) containing different superdisintegrants followed the order; crospovidone < sodium starch glycolate < croscarmellose sodium. Crospovidone demonstrated faster wetting of the tablets compared to croscarmellose sodium and sodium starch glycolate. ODTs with crospovidone (F2 formulation) outperformed those with other superdisintegrants, exhibiting a shorter wetting time 34.67±3.10 seconds (Table 3). The rapid swelling and dispersion of crospovidone in water, attributed to its superior hydration capacity, were evident. The high degree of swelling of crospovidone, was associated with a wicking mechanism that drew water into the tablet through capillary action.

In contrast, F3 formulation that contains sodium starch glycolate, known for its high water absorption rate and a swelling capacity of 6%, exhibited swelled tablets with wetting time 62.00 ±2.31seconds.

ODTs formulated with croscarmellose sodium (F1 formulation) displayed moderate swelling and wetting time 44.33±2.48 seconds (Table 3). Despite croscarmellose sodium's limited water solubility, it exhibited a higher degree of swelling, up to 4-8 times its initial volume.

The *in vitro* disintegration time of all ODTs was also assessed, and Table 3**.** presents the average disintegration time for all ODT samples, ranging from 39.27±3.25 to 47.67±1.15seconds. In the case of sodium starch glycolate and croscarmellose sodium, the *in vitro* disintegration times consistently proved shorter than the wetting times for all ODTs in this study. However, for ODTs containing crospovidone, a different trend emerged compared to the wetting test, with *in vitro* disintegration times longer than the wetting times. This difference is likely attributed to the greater hardness of the ODTs containing crospovidone.

In the realm of physiological circumstances, the breakdown of ODTs in the mouth involves two phases. The first phase encompasses saliva absorption, initiated upon placing the tablet on the tongue, followed by the second phase where the tablet breaks down into minute particles. This second phase is associated with the pressure between the tongue and the upper hard palate. Neglecting this pressure aspect, relying solely on wetting time calculations may prove insufficient for defining ODTs and could introduce biases in result evaluation.

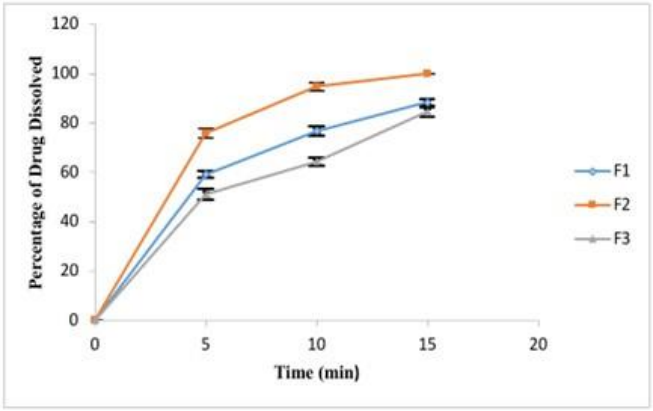

Figure 1. *In vitro* dissolution parameters in pH 6.8 phosphate buffer (n=3 ± SD)

Table 4. *In vitro* dissolution parameters in pH 6.8 phosphate buffer (n=3)

Formulation Code	D_5(%)[a]	D_{10} (%)[a]	D_{15} (%)[a]	$t_{\%50}$[b]	$t_{\%90}$[b]
F1	59.12±0.66	76.77±0.91	88.42±0.69	>30	>30
F2	75.86±0.98	94.73±0.84	≥100	>30	>30
F3	51.08±1.16	64.33±0.89	84.62±0.93	>30	>30

a D5, D10, D15: percent of drug dissolved in 5, 10 and 15 min.
b $t_{50\%,}t_{90\%}$: time to dissolve 50% and 90% of drug from tablet

The influence of superdisintegrants on the dissolution of ketoprofen from ODTs is depicted in Figure 1 and summarized in Table 4. *In vitro* dissolution studies were conducted to assess the impact of Croscarmellose Sodium (F1), Crospovidone (F2) and Sodium Starch Glycolate (F3) on the release profile of ketoprofen in the formulated ODTs. ODTs incorporated F1 and F3, the drug release improved to 64.33±0.89 to 76.77±0.91 % in 10 minutes respectively, though deemed insufficient for an ODT formulation. This behavior is attributed to the swelling ofCroscarmellose Sodium and Sodium starch glycolate, forming a gel-like mass that entraps some of the drug, hindering its release. Consistent with the literature, drug release from ketoprofen orally disintegrating tablets (ODTs) containing Crospovidone has been observed to be faster in dissolution rate studies [12].

AUTHOR CONTRIBUTIONS

Concept: T.C.; Design: T.C.; Control: T.C.; Sources: T.C.; Materials: T.C.; Data Collection and/or Processing: T.C.; Analysis and/or Interpretation: T.C.; Literature Review: T.C.; Manuscript Writing: T.C.; Critical Review: T.C.; Other: -

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

IN SILICO APPROACHES ON PHENYLALANINE HYDROXYLASE INHIBITOR-RELATED COMPOUNDS USED IN PARKINSON'S DISEASE TREATMENT

PARKİNSON HASTALIĞI TEDAVİSİNDE KULLANILAN FENİLALANİN HİDROKSİLAZ İNHİBİTÖRÜ İLE İLİŞKİLİ BİLEŞİKLERE İLİŞKİN İN SİLİKO YAKLAŞIMLAR

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ABSTRACT

Objective: *In Parkinson's disease, Levodopa with Carbidopa addresses dopamine deficiency. Phenylalanine hydroxylase catalyzes phenylalanine to tyrosine conversion crucial for dopamine synthesis. Inhibiting phenylalanine hydroxylase may enhance Carbidopa's effects, preventing peripheral dopamine synthesis. The study used virtual scanning, molecular docking, and dynamics simulation to explore phenylalanine hydroxylase interactions with Carbidopa and similar ligands. ADME/T assessments and drug similarity tests were conducted to evaluate therapeutic potential in biological systems.*
Material and Method: *A molecular docking study was performed on the structures obtained from the PubChem database and human PAH (PDB ID: 6PAH) using Autodock Vina within Chimera 1.16. Furthermore, the ligands underwent ADME/T assays, which are crucial aspects in drug development.*
Result and Discussion: *The study suggests that 2-(2-Aminohydrazinyl)-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid shows promise as a phenylalanine hydroxylase inhibitor for Parkinson's disease treatment, but further research is needed to assess its safety, efficacy, and specificity, particularly in extracerebral regions, while also exploring its potential to improve the effectiveness of Levadopa/Carbidopa combination therapy.*
Keywords: *Drug development, inhibitor, in silico, Parkinson's disease, phenylalanine hydroxylase*

ÖZ

Amaç: *Parkinson hastalığında, Levodopa ile Carbidopa kullanılarak dopamin eksikliği ele alınır. Fenilalanin hidroksilaz, dopamin sentezi için önemli olan fenilalanin'in tirozin'e dönüşümünü katalizler. Fenilalanin hidroksilaz'ın inhibe edilmesi, periferik dopamin sentezini engelleyerek Carbidopa'nın etkilerini artırabilir. Çalışmada, fenilalanin hidroksilazın Carbidopa ve benzeri ligandlarla etkileşimlerini araştırmak için sanal tarama, moleküler bağlanma ve dinamik simulasyon kullanıldı. ADME/T değerlendirmeleri ve ilaç benzerlik testleri biyolojik sistemlerdeki terapötik potansiyeli değerlendirmek amacıyla yapıldı.*
Gereç ve Yöntem: *PubChem veritabanından elde edilen yapılar ve insan fenialanin hidroksilaz (PDB Kimlik Numarası: 6PAH) üzerinde bir moleküler bağlanma çalışması, Autodock Vina'nın Chimera 1.16 içinde kullanılarak gerçekleştirildi. Ayrıca, ligandlar ilaç geliştirme sürecinde önemli bir aşama olan ADME/T analizlerine tabi tutuldu.*

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Sonuç ve Tartışma: *Çalışma, 2-(2-Aminohydrazinyl)-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid'in Parkinson hastalığının tedavisinde fenilalanin hidroksilaz inhibitörü olarak umut vadettiğini öne sürüyor. Ancak, özellikle ekstraserebral bölgelerde güvenlik, etkinlik ve özgünlük değerlendirmek için daha fazla araştırmaya ihtiyaç olduğunu ve aynı zamanda Levadopa/Carbidopa kombinasyon tedavisinin etkinliğini artırma potansiyelini keşfetmenin önemli olduğunu belirtiyor.*
Anahtar Kelimeler: *Fenilalanin hidroksilaz, ilaç geliştirme, inhibitör, in siliko, Parkinson hastalığı*

INTRODUCTION

Alzheimer's disease (AD) stands as the most prevalent neurodegenerative disease globally [1], with Parkinson's disease (PD) following closely behind [2]. The disease is characterized by the degeneration of dopamine neurons, leading to dopamine deficiency, which is the major deficit underlying PD. The reduction in dopamine levels eventually produces motor symptoms, such as bradykinesia and akinesia [3]. Although various pharmacological treatments, such as the use of dopamine agonists and monoamine oxidase inhibitors, have been effective in alleviating the disease symptoms, treatment with 3,4-dihydroxyphenylalanine (levodopa, L-DOPA, or DAH), the precursor of dopamine, is currently the safest and most effective strategy [4,5]. However, the efficacy of this treatment is limited by the conversion of L-DOPA to dopamine in the extracerebral regions, reducing the bioavailability of L-DOPA in the brain. Thus, L-DOPA is commonly used in combination with Carbidopa, an inhibitor of DOPA decarboxylase (DDC), which inhibits the conversion of L-DOPA to dopamine in the periphery owing to its inability to cross the blood-brain barrier (BBB) [6,7]. This increases the amount of L-DOPA reaching the brain, where it can be converted to dopamine to relieve symptoms associated with PD.

The diagnosis and treatment of Parkinson's disease emphasize the importance of carbidopa-levodopa formulations, often used alongside other medications [8]. Advanced treatments such as deep brain stimulation and levodopa-carbidopa enteral suspension help manage medication-resistant symptoms [9]. Despite the initially promising effects of dual L-DOPA/Carbidopa therapy, in recent years, frequent fluctuations in dopamine levels have become an issue [10]. While the underlying reasons remain to be elucidated, several measures have been taken to compensate for these fluctuations [11]. including repeated L-DOPA intake or switching to gel-based from oral applications. However, despite their effectiveness in managing symptoms, levodopa-carbidopa therapy has several shortcomings and limitations. One major limitation is the development of motor fluctuations, where patients experience alternating periods of increased mobility (on periods) and worsened symptoms (off periods), significantly affecting their quality of life [12,13]. Long-term use can lead to the development of levodopa-induced dyskinesias (LIDs) [14], involuntary movements that worsen over time. Additionally, while levodopa-carbidopa effectively addresses motor symptoms, it may have limited efficacy in managing non-motor symptoms such as cognitive impairment, psychiatric symptoms, and sleep disturbances. Furthermore, levodopa-carbidopa primarily provides symptomatic relief and does not alter the underlying disease progression in Parkinson's disease. Therefore, the recommended L-DOPA dose of 400 mg/day should not be exceeded [8,15].

Coupling the existing treatments with the targeting of other steps in the dopamine synthesis pathway might be an effective strategy to reduce peripheral L-DOPA levels during PD therapy.

The enzyme phenylalanine hydroxylase (PAH) (EC 1.14.16.1) converts L-phenylalanine into L-tyrosine, which is then hydroxylated to form L-DOPA by the enzyme Tyrosine hydroxylase (TH) [16,17]. These tetrameric enzymes consist of regulatory N-terminal domains, catalytic domains with structural similarity, and C-terminal domains responsible for oligomerization. This study initially explored Carbidopa's affinity to PAH's active site to understand its potential interference with L-DOPA synthesis. Next, chemicals structurally similar to Carbidopa were screened as ligands using molecular docking, a technique integrating computational approaches in drug design. Molecular dynamics simulation was then employed to further elucidate the binding mechanism and conformational behavior of the target protein and potential inhibitors [18,19,20,21], Additionally, the ligands underwent

ADME/T assays to assess their pharmacokinetics and safety profile, crucial in drug development [22,23].

MATERIAL AND METHOD

Virtual Screening and Selection of Target Protein and Ligands

L-DOPA is a catechol that can bind the Fe(III) at the active site of PAH, causing a reversible shift to the inactive form of the enzyme. The crystallographic structure of L-DOPA-bound 6PAH was downloaded from the Protein Data Bank. Carbidopa (A) and structurally similar 3-(3,4-Dihydroxyphenyl)-2-hydrazinylpropanoic acid (B), 3-(3,4-Dihydroxyphenyl)-2-hydrazinyl-2-methyl propanoate (C), 6-[3-(3,4-Dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoyl]oxyhexanoic acid (D), Ethyl 3-(3,4-dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoate (Carbidopa Ethyl Ester) (E), 2-(2-aminohydrazinyl)-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid (F), and 2-[(3,4-dihydroxy phenyl)methyl]-2-hydrazinylbutanoic acid (G) were selected as ligands.

Preparation of Protein Structure and Ligands

The target protein PDB file was obtained [24-26], ligands (small molecules) and water molecules attached to the protein structure were deleted from the 3D crystallographic structure, and the final protein structure was saved as a Mol2 file by adding polar hydrogen atoms and charges. SMILES notations were copied from the PubChem database. Energy minimization was achieved using the Build Structure tool embedded in Chimera. Ligands were saved in Mol2 file for docking.

Final Docking with Autodock Vina Embedded in Chimera (1.16)

The ligand molecule was imported in PDBQT format into the output file. AutoDock Vina command prompt was run by entering the previously determined active site coordinates, and the results were analyzed [18].

Grid Box Preparation

The active site of DAH bound to 6PAH in its crystallographic structure was identified by taking the arithmetic average of the x, y, and z coordinates of DAH (Table 1). The search area for the grid box was taken as 30 × 30 × 30 Å [26].

Table 1. Coordinate calculation for the 6PAH-bound DAH ligand

Residue Name (RES)	Chain Identifier (C)	Sequence Number Insertion Code (SSEQI)	x	y	z	Resolution	B-factor	Atom
DAH	A	600	-7.111	27.750	4.352	1.00	56.41	N
DAH	A	600	-7.194	26.678	3.307	1.00	56.32	C
DAH	A	600	-8.666	26.319	2.931	1.00	56.53	C
DAH	A	600	-8.977	25.836	1.814	1.00	56.42	O
DAH	A	600	-6.300	25.430	3.605	1.00	55.47	C
DAH	A	600	-5.066	25.551	4.533	1.00	54.66	C
DAH	A	600	-5.196	26.087	5.871	1.00	54.32	C
DAH	A	600	-3.783	25.126	4.068	1.00	53.88	C
DAH	A	600	-4.066	26.186	6.716	1.00	53.73	C
DAH	A	600	-2.645	25.223	4.912	1.00	53.38	C
DAH	A	600	-2.787	25.760	6.259	1.00	53.40	C
DAH	A	600	-1.435	24.795	4.426	1.00	52.27	O
DAH	A	600	-1.708	25.875	7.121	1.00	52.85	O
DAH	A	600	-9.580	26.579	3.957	1.00	57.03	O

The arithmetic mean of the x, y, and z coordinates is respectively -5.322, 25.943, and 4.562.

Computer-Based Analysis of Pharmacokinetics and Drug-likeness

Drug similarity as well as oral bioavailability (Lipinski's rule of 5) [27] and safety profile of the compounds selected as drug candidates were evaluated with ADME/T (http://www.swissadme.ch/, http://lmmd.ecust.edu.cn/admetsar2) which are used especially to estimate pharmacokinetic properties.

Software Used

Windows 10 Microsoft operating system was installed. The UCSF Chimera (1.16) program (https://www.cgl.ucsf.edu/chimera/download.html) was run for docking with AutoDock Vina [18], hosted by The Scripps Research Institute (USA), frequently used for molecular docking simulations [28,29]. Protein Data Bank (https://www.rcsb.org/) and PubChem were searched for protein and chemical (ligand) structures, respectively. IGemDOCK V2.1 was used for the elimination of ligands by pre-scoring. Plip-tool (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) [30] and the ProteinsPlus web server (https://proteins.plus/) were used to analyze the bonds and distances between the target protein and selected ligands. The iMod server package (https://imods.iqfr.csic.es/) was used for modeling, image processing, and 2D and 3D data analyses [31]. The ligands were subjected to ADME/T assays to evaluate their pharmacokinetics and safety profile, which are vital aspects in drug development. (http://www.swissadme.ch/, http://lmmd.ecust.edu.cn/admetsar2)

RESULT AND DISCUSSION

This *in silico* structure-based study conducted to predict ligand-target interactions [32] revealed proteins that are structurally similar to DAH, which might interact with PAH (Figure 1).

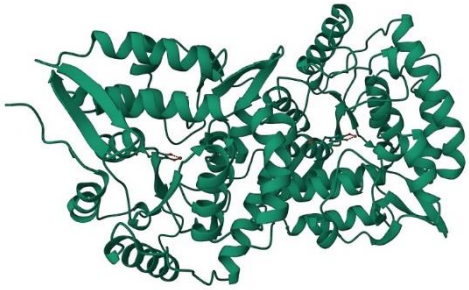


Figure 1. The crystal structure of human 6PAH

The surface of a protein presents a distribution of charge and hydrophobicity that enables interactions with suitable surfaces [33,34] and influences the conformational stability of the protein structure. Surface properties also affect interactions with other biomolecules, which can contribute to the modification of a protein's behavior and stability, affecting its bioactivity [35]. In this respect, hydrogen bonding (at positions G131, L133, Y209, and E214) and hydrophobic interactions (at V129, F138, and P165) play important roles in the binding of the donepezil ligand to human PAH (Figure 2).

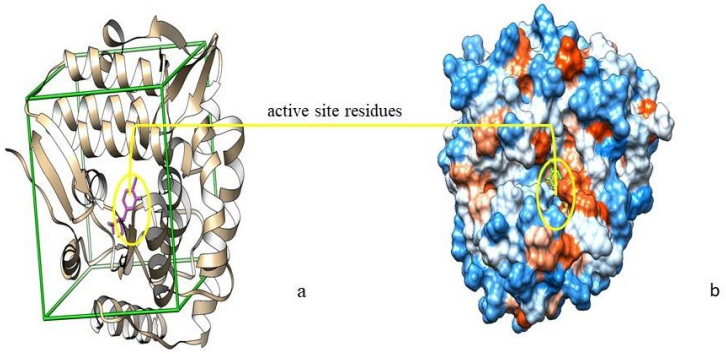
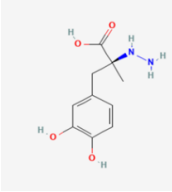
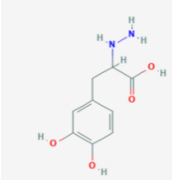
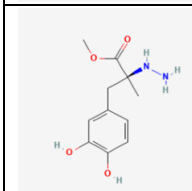
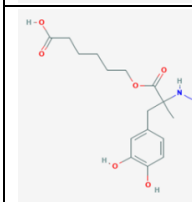
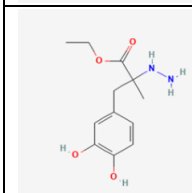
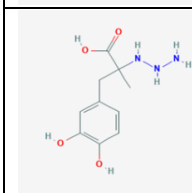
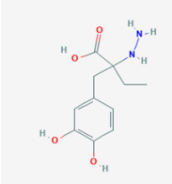


Figure 2. Visualization of a) the protein structure and b) surface hydrophobicity with active site residues in the crystal structure of 6PAH

The predicted binding affinities are given in kcal/mol. Root mean square deviation (RMSD) values are computed using the optimal mode as the basis. The difference between the rmsd/lb (RMSD lower bound) and rmsd/ub (RMSD upper bound) metrics is determined by how atoms are matched in the distance calculation. The ligands (Table 2) were pre-screened using the Igemdock software. The binding energies of ligands are shown in Table 3. L-DOPA (DAH) is found as a complex in the crystal structure of recombinant human PAH.

Table 2. Structures of the ligands obtained from PubChem

Structure	Ligand	PubChem ID
	L-alpha-methyldopa hydrazine (Carbidopa) (A)	CID: 34359
	3-(3,4-Dihydroxyphenyl)-2-hydrazinylpropanoic acid (B)	CID: 13687898
	3-(3,4-Dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoate (C)	CID: 21964612
	6-[3-(3,4-Dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoyl]oxyhexanoic acid (D)	CID: 21964631
	Ethyl 3-(3,4-dihydroxyphenyl)-2-hydrazinyl-2-methyl-propanoate (Carbidopa Ethyl Ester) (E)	CID: 21964632
	2-(2-Aminohydrazinyl)-3-(3,4-dihydroxyphenyl)-2-methyl propanoic acid (F)	CID: 22234765
	2-[(3,4-Dihydroxyphenyl)methyl]-2-hydrazinylbutanoic acid (G)	CID: 53679931

Carbidopa (A), utilized alongside L-DOPA in PD therapy [7], and six structurally similar ligands (B), (C), (D), (E), (F), and (G) were positioned within the drug binding pocket of the human PAH enzyme (Table 2 and Figure 3, 4). The binding energies of the protein-ligand complexes obtained from molecular docking were compared with the RMSD values of the corresponding compounds (Table 3). Carbidopa (A) was chosen as the positive control due to its structural resemblance to DAH, and its comparison with the other six ligands confirmed their potential binding activity against PAH. The docking scores of the structures-B (-6.7 kcal/mol), C (-6.9 kcal/mol), D (-6.8 kcal/mol), E (-6.6 kcal/mol), F (-7.1 kcal/mol), and G (-6.6 kcal/mol)-were consistent. The binding energy was calculated as -92.03 kcal/mol for (B), -88.92 kcal/mol for (C), -99.58 kcal/mol for (D), -88.02 kcal/mol for (E), -100.45 kcal/mol for (F), and -89.96 kcal/mol for (G) (Table 3). These findings suggested that 2-(2-Aminohydrazinyl)-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid (F) is more effective in binding to PAH than all currently known and tested ligands. Detailed examination of the protein-ligand bonds revealed the presence of hydrogen bonds and hydrophobic interactions between all ligands and PAH, similar to the 6PAH-DAH complex. Additionally, π-stacking was observed in (B), and both π-stacking and salt bridges were formed in (E) and (F). Ligands were found to interact with 5 to 7 amino acid residues at the active site of 6PAH.

Table 3. Docking scores and binding energies of the ligands for PAH and RMSD values

Ligand molecules	Binding affinity (kcal/mol)	RMSD lower bound	RMSD upper bound	Binding energy (kcal/mol)
(A)	-6.7	1.974	2.325	-92.36
(B)	-6.7	3.962	5.859	-92.03
(C)	-6.9	2.540	3.819	-88.92
(D)	-6.8	9.163	11.938	-99.58
(E)	-6.6	1.656	2.351	-88.02
(F)	-7.1	9.191	10.781	-100.45
(G)	-6.6	8.941	11.111	-89.96

RMSD, root mean square deviation

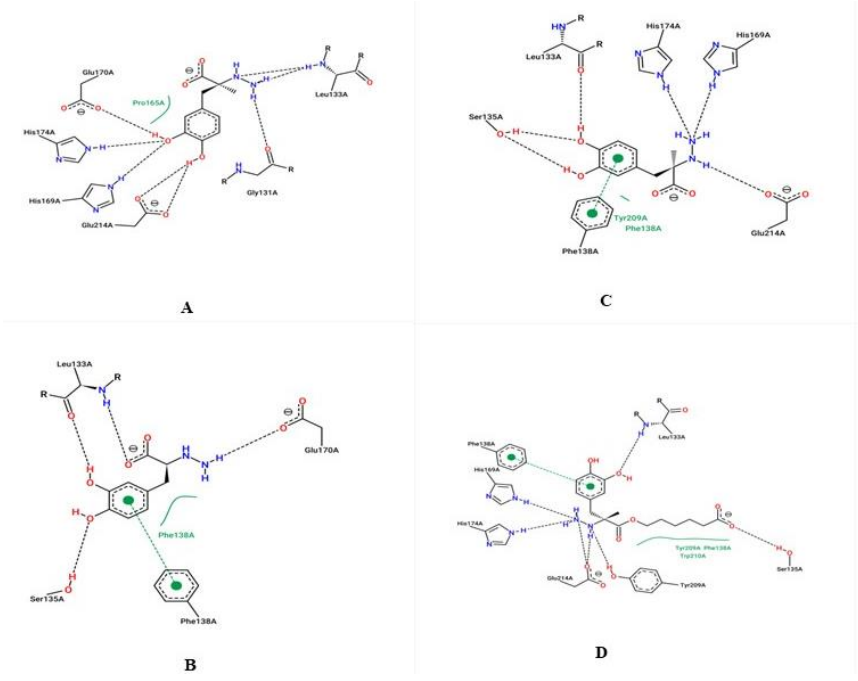


Figure 3. Interactions of target protein and the selected ligands (A, B, C, D). Protein-ligand interaction profiles are named by the letter of the respective ligand

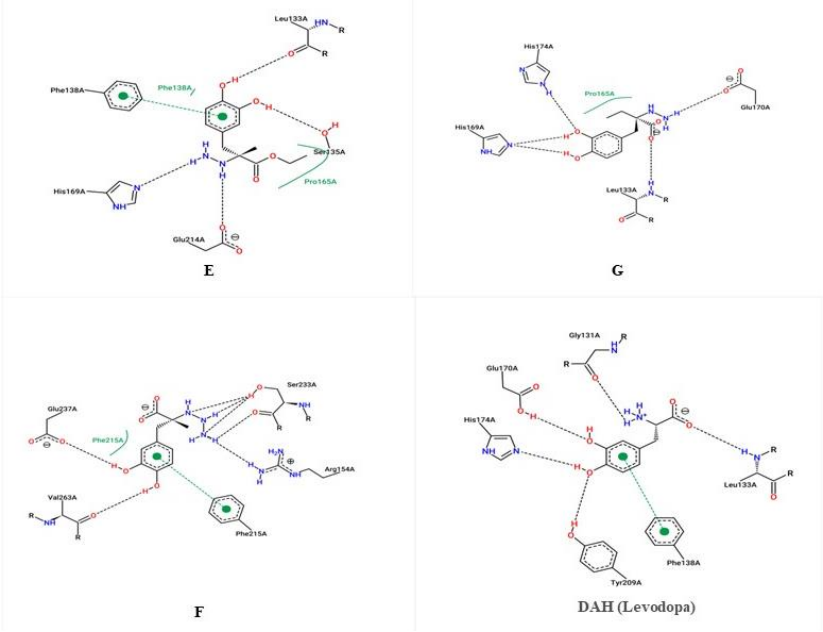


Figure 4. Interactions of target protein and the selected ligands (E, F, G). Protein-ligand interaction profiles are named by the letter of the respective ligand

L132, F138, and P165 hydrophobic interactions and G131, L133, and H169 hydrogen bonds were observed when Carbidopa (A) was bound to the PAH enzyme. L133, S135, and H148 hydrogen bonds, L132 and F138 hydrophobic interactions, and F138 π-stacking interactions were observed for (B). S135, H174, Y209, and G214 hydrogen bonds and L132, F138, and Q214 hydrophobic forces were found to contribute to the interaction between (C) and 6PAH. G131, L133, and Y209 hydrogen bonds and F138, P165, A206, T209, and W210 hydrophobic interactions were noted in our modeling for (D)-6PAH. S135 and Q214 hydrogen bonds, V129, L132, F138, and Y209 hydrophobic interactions, F138 π-stacking interactions, and H169 and H174 salt bridges were observed for (E)-6PAH. A154, S233, and G265 hydrogen bonds, F215 π-stacking interactions, and H169 salt bridges were noted in (F)-6PAH modeling. L133, H148, Y150, and H169 hydrogen bonds and L132, F138, and P165 hydrophobic forces played a role in the interaction between (G) and 6PAH. When the ligands were examined, the lowest hydrogen bond (H-A) distance was reported as 2.09 Å in positive control Carbidopa (A). Meanwhile, the lowest H-A values in the (F) ligand were found as 2.17 Å, 2.80 Å, 2.35 Å, and 2.78 Å. These results indicated that the binding between PAH and (F) may be stronger in comparison with the other ligands.

Protein's three-dimensional configuration is intricately shaped by a delicate interplay of weak forces, notably hydrogen bonds, salt bridges, and the hydrophobic effect, crucial for proper folding and structural integrity. Additionally, the cation-π interaction, an increasingly acknowledged non-covalent binding force in structural biology, has become more prominent. Both theoretical and experimental studies have demonstrated that cation-π interactions can be quite strong, both in the gas phase and in aqueous environments [36]. Therefore, the hydrogen bonds (with the shortest H-A distance), salt bridges, and π-stacking interactions present in the structure of 2-(2-Aminohydrazinyl)-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid ligand are crucial for determining the three-dimensional structure of proteins and are among the reasons why this ligand is preferred.

Flexibility is a crucial factor for protein-protein interactions or the interactions of biological macromolecules with their substrates [37]. The iMOD server was used to calculate the flexibility of the protein, as well as molecular motion [38]. The NMA study of the complex showed mobility and flexibility (Figure 5A). Deformability is associated with flexibility, and the B factor is associated with protein mobility [39]. The B factor graphs in Figure 5B represent the comparison between the NMA and PDB fields of the complex. The highly deformable regions of the (F)-6PAH complex are indicated by the highest peaks. Eigenvalue and variance plots (Figure 5C) of the (F)-6PAH complex showed an

inverse relationship with the normal mode. 4.709380e-0.4 is the eigenvalue of the complex calculated with the iMod server. The correlation between the residues of the (F)-6PAH complex is displayed by the covariance map. Blue, red, and white colors between residues in the matrix indicate anticorrelation, correlation, and uncorrelated motion, respectively (Figure 5-D). Interatomic relationships are shown in Figure 5E. Dark gray shows the stiffer parts; the covariance matrices and elastic maps gave reasonable values.

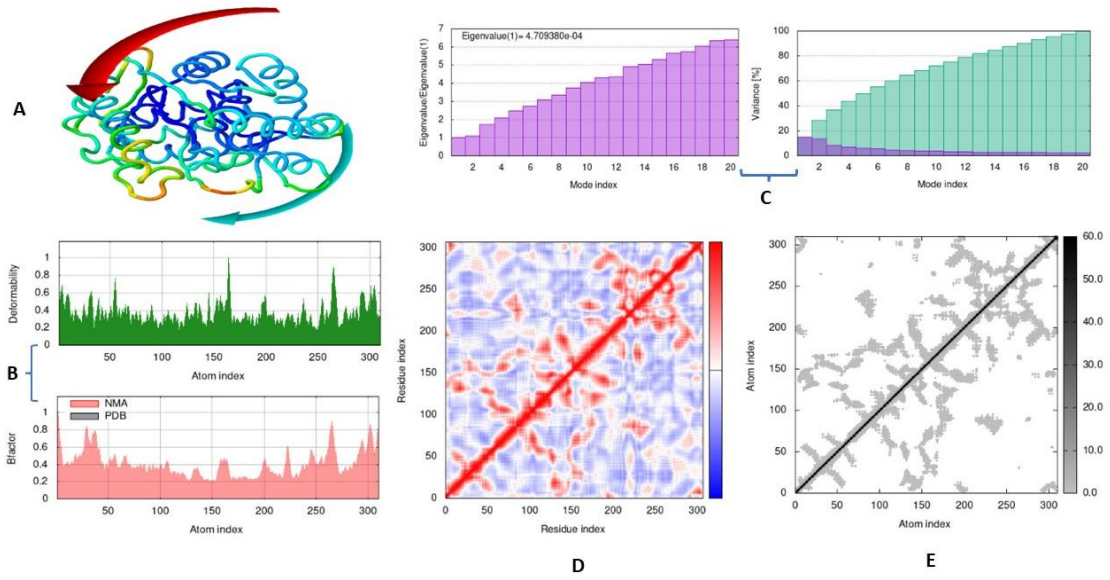


Figure 5. The results from molecular dynamics simulations in iMods pertain to the (F)-6PAH complex. A. Molecular mobility assessed by normal mode analysis (NMA) of the docked proteins. The colored affine arrows show the mobility. B. Deformability and the B-factor plot. C. Eigenvalue (left) and variance plots (right). The calculated eigenvalue is 4.709380e-0.4. In the variance plot D, cumulative variance is illustrated by the green-shaded bars, while individual variance is indicated by the purple-shaded bars. Covariance map. Blue, anticorrelation; red, correlation; white, uncorrelated motion. E. Elastic network model. Dark gray shows the stiffer parts

The exploration of the ADME properties of drugs using computer-based models and calculations is essential in the processes of drug development. Simultaneously, Lipinski's Rule of Five for Drug Molecules specifies that a potential drug candidate must adhere to four specific physical and chemical properties within acceptable limits to attain high oral bioavailability. [40]. According to Lipinski's rule of five, an orally active drug can have no more than one violation of these conditions [41]. When evaluating ligands that meet Lipinski criteria, the assessment of Carbidopa and ligands with similar structures as potential drug candidates was conducted using the SwissADME platform. According to the results, it was found that the log P values of all compounds were below 5, and the molecular weight of each compound was within the acceptable range (MW<500). The number of H-bond acceptors (≤10) and donors (≤5) also fell within acceptable limits. Only the F ligand showed 6 H-bond donors (Table 4). However, other pharmacokinetic and pharmacodynamic factors should also be considered. Seven compounds were identified within the topological polar surface area range (TPSA; <140). The number of rotatable bonds for all compounds was within the acceptable range (≤10) as shown in Table 4. When all ligands were evaluated in terms of lipophilicity (LIPO; -0.7<XlogP3<5), size (150<MW<500), polarity (20<TPSA<30), solubility (INSOLU, -6<log S<0), saturation (INSATU; 0.25<carbon fraction in sp3 hybridization<1), and flexibility (FLEX, 0<rotatable bonds<9), deviation was observed in saturation for ligand B (0.22) and in flexibility for ligand D (9). Other ligands are within the optimal range. The analysis results demonstrated that the five properties fell within the favorable range, categorizing the compound as a drug-like molecule. Except for compound D, the other ligands, especially ligands F and G, can be considered suitable for injectable administration as they are within

the appropriate flexibility range [42]. Additionally, when pharmacokinetic properties were evaluated, it was predicted that other ligands, like Carbidopa, did not cross the BBB. According to ADMET analysis, the acute oral toxicity of other ligands, like Carbidopa, is classified as Category III (slightly toxic). (Category III includes compounds with LD_{50} values greater than 500mg/kg but less than 5000mg/kg (http://lmmd.ecust.edu.cn/admetsar2) [43].

Table 4. List of pharmacokinetic properties of ligands

Properties	**Physicochemical properties**								**Lipophili-city**	**Water Solubility**	**Pharmaco-kinetics**	**Drug-likeness**	**Medicinal chemistry**
Parameters	Molecular weight (g/mol)	Number of heavy atoms	Number of aromatic heavy atoms	Number of rotatable bonds	Number of H-bond acceptors	Number of H-bond donors	Molar Refrac-tivity	TPSA (Å²)	Log Po/w	LogS (ESOL)	GI absorbtion/ BBB permeant	Lipinski/ violation	Synthetic accecibility
A	226.23	16	6	4	6	5	57.19	115.81	-2.17	0.11	High/No	Yes; 0 violation	2.25
B	212.20	15	6	4	6	5	52.35	115.81	-2.98	0.69	High/No	Yes; 0 violation	2.15
C	225.22	16	6	4	6	4	55.25	118.64	-2.17	0.12	High/No	Yes; 0 violation	2.21
D	340.37	24	6	11	8	5	87.32	142.11	-1.27	-0.61	Low/No	Yes; 0 violation	3.21
E	254.28	18	6	6	6	4	66.32	104.81	0.81	-1.78	High/No	Yes; 0 violation	2.66
F	241.24	17	6	5	6	6	60.00	127.84	-2.40	0.25	High/No	Yes; 1 violation: NHorOH>5	2.47
G	240.26	17	6	5	6	5	62.00	115.81	-1.64	-0.23	High/No	Yes; 0 violation	2.36

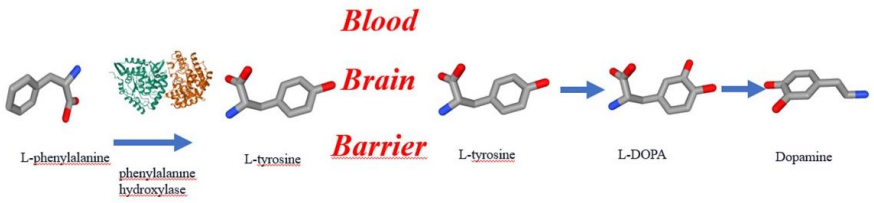


Figure 6. Phenylalanine tyrosine metabolic pathway

L-DOPA is widely used in Parkinson's's disease (PD) treatment, yet concerns persist about its fluctuating levels during prolonged therapy [44], with underlying reasons not fully understood [10]. These fluctuations may result from negative feedback due to L-DOPA binding with PAH. In physiological conditions, L-DOPA, derived from tyrosine conversion by TH activity (Figure 6), binds to the iron on PAH, temporarily shifting the enzyme to its inactive state and inhibiting L-DOPA synthesis [45]. During L-DOPA treatment, acute peripheral level increases may induce this negative feedback loop. Given the potential interference of normal pathway activity with appropriate dosage levels and intervals, targeting this pathway during PD therapy could be beneficial. Carbidopa, used with L-DOPA to decrease its conversion to dopamine in peripheral regions, shares structural similarities with L-DOPA. Our study found that carbidopa binds to PAH with a binding affinity of -6.87 kcal/mol and energy of -92.36 kcal/mol, potentially regulating L-DOPA levels. However, combination therapy stability in this aspect remains uncertain. Additionally, we identified 2-(2-Aminohydrazinyl)-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid as a promising PAH inhibitor, exhibiting improved activity with a binding affinity of -7.1 kcal/mol, energy of -100.45 kcal/mol, and favorable molecular dynamics. Most ligands were suitable for injectable administration, with carbidopa-like ligands predicted to have limited BBB penetration. Nonetheless, caution is necessary in interpreting these findings due to potential risks of excessive PAH inhibition, especially in phenylketonuria [46].

Further studies should investigate the safety and efficacy of the proposed compound in various dosage settings over time and explore the specificity of the inhibitory effects in extracerebral regions. Despite these limitations, the study suggests the potential of the compound to enhance the efficacy of L-DOPA/Carbidopa combination therapy in Parkinson's's disease treatment.

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AUTHOR CONTRIBUTIONS

Concept: H.A., E.S.; Design: H.A., E.S.; Control: H.A., E.S.; Sources: H.A., E.S.; Materials: H.A., E.S.; Data Collection and/or Processing: H.A., E.S.; Analysis and/or Interpretation: H.A., E.S.; Literature Review: H.A., E.S.; Manuscript Writing: H.A., E.S.; Critical Review: H.A., E.S.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

LIQUID AND SOLID SELF-EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS) CONTAINING VALSARTAN: STABILITY ASSESSMENT AND PERMEABILITY STUDIES

VALSARTAN İÇEREN KATI VE SIVI KENDİLİĞİNDEN EMÜLSİFİYE OLAN SİSTEMLER (SEDDS): STABİLİTE DEĞERLENDİRMESİ VE PERMEABİLİTE ÇALIŞMALARI

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ABSTRACT

Objective: *Valsartan (VST) is a Biopharmaceutical classification system (BSC) class II active ingredient with a bioavailability of approximately 25% and is utilized to treat high blood pressure (hypertension). This study aimed was to showcase the stability and increase the permeability of VST by developing self-emulsifying drug delivery systems (SEDDS) and solidified SEDDS (S-SEDDS) formulations.*

Material and Method: *The ratios of the components were determined by the pseudo-ternary phase diagram, and the characterization studies were conducted in the previous study. Stability was performed in long-term (25±2˚C, 60±5% relative humidity) and accelerated (40±2˚C, 75±5% relative humidity) conditions. The intestinal permeability of SEDDS formulations was evaluated by Caco-2 cells.*

Result and Discussion: *Formulations for 12 month, droplet sizes were found to be 67.52 ± 5.26 nm and 176.93 ± 17.34 nm for SEDDS of VST (VST-SEDDS) and S-SEDDS of VST (VST-S-SEDDS), respectively. During this period, polydispersity indexes were: VST-SEDDS, 0.56±0.1; VST-S-SEDDS, 0.58±0.05. Both formulations increased VST permeability across Caco-2 cells: VST-SEDDS by 2.32x (powder) and 2.18x (commercial); VST-S-SEDDS by 1.38x (powder) and 1.30x (commercial). The formulation components did not have cytotoxic effects. These results demonstrated that newly developed VST-SEDDS and VST-S-SEDDS formulations with high permeability may be a desirable approach for antihypertensive therapy.*

Keywords: *Caco-2 cell line, permeability, self-emulsifying drug delivery system, solidified self-emulsifying drug delivery system, valsartan*

ÖZ

Amaç: *Valsartan (VST), biyoyararlanımı yaklaşık %25 olan Biyofarmasötik Sınıflandırma Sistemi (BSS) sınıf II aktif maddedir ve yüksek kan basıncını (hipertansiyon) tedavi etmek için kullanılır. Bu çalışmanın amacı, kendi kendine emülsifiye olan ilaç dağıtım sistemleri (SEDDS) ve katılaştırılmış*

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SEDDS (S-SEDDS) formülasyonları geliştirerek VST'nin stabilitesini göstermek ve geçirgenliğini artırmaktır.

Gereç ve Yöntem: *Bir önceki çalışmada bileşenlerin oranları üçgen faz diyagramı ile belirlenmiş olup, karakterizasyon çalışmaları yapılmıştır. Bu çalışmada, uzun süreli (25±2˚C, 60±5% bağıl nem) ve hızlandırılmış (40±2˚C, 75±5% bağıl nem) koşullarda stabilite çalışmaları gerçekleştirildi. SEDDS formülasyonlarının bağırsak geçirgenliği Caco-2 hücreleri tarafından değerlendirildi.*

Sonuç ve Tartışma: *Formülasyonların 12 ay boyunca partikül boyutları VST içeren SEDDS (VST-SEDDS) formülasyuonunda 67.52 ± 5.26 nm, VST içeren S-SEDDS (VST-S-SEDDS) formülasyonunda 176.93 ± 17.34 nm olarak bulundu. Bu süre boyunca VST-SEDDS ve VST-S-SEDDS formülasyonunun polidispersite indeksleri sırasıyla 0.56±0.1, 0.58±0.05 olarak bulundu. Her iki formülasyon da Caco-2 hücreleri boyunca VST geçirgenliğini artırdı: VST-SEDDS 2,32 kat (toz) ve 2,18 kat (ticari); VST-S-SEDDS 1,38x (toz) ve 1,30x (ticari). Formülasyonların bileşenlerinde sitotoksik etki görülmedi. Bu sonuçlar, yüksek geçirgenliğe sahip yeni geliştirilen VST-SEDDS ve VST-S-SEDDS formülasyonlarının antihipertansif tedavi için arzu edilen bir yaklaşım olabileceğini gösterdi.*

Anahtar Kelimeler: *Caco-2 hücre hattı, kendi kendine emülsifiye olan ilaç dağıtım sistemleri, katılaştırılmış kendi kendine emülsifiye olan ilaç dağıtım sistemleri, permeabilite, valsartan*

INTRODUCTION

Hypertension, a primary risk factor for cardiovascular diseases, impacts over 46% of the global adult population, totaling approximately 1.28 billion individuals worldwide, as The World Health Organization (WHO) reported in its 2023 report [1]. Valsartan (VST) is employed in managing high blood pressure and is an approved angiotensin II receptor blocker for treating hypertension in adults. It has been marketed in adults in Europe at doses of 80-160 mg since 1996; since 2006, it has been as the highest dose of 320 mg [2]. VST has an acidic structure, and its solubility is very low (<0.1 mg/ml), depending on pH, at the aqueous phase. VST belongs to the low solubility, high permeability (class II) category of the biopharmaceutical drug classification system (BCS) [3]. It has a bioavailability of approximately 25% because of low water solubility. Increasing the solubility, or reducing the first pass effect through the liver may be approached to increase bioavailability.

Self-emulsifying drug delivery systems (SEDDS) formulation approach used for years to increase solubility and bioavailability. SEDDS formulation contains oil as a solid or liquid form, surfactant, and cosurfactant. After the SEDDSs are taken orally, the drugs trapped in the triglycerides in their compositions are released from the enterocytes through the villus in the small intestine to the lymphatic pathway through exocytosis by chylomicrons and pass into the blood from the small intestine. Thus, the active substance enters the lymphatic system and passes into the blood circulation from there, and the drug is protected from the first-pass effect of the liver [4]. Developing self-emulsifying drug delivery systems (SEDDS) is crucial, particularly for drugs with low bioavailability. However, liquid SEDDS formulations present various drawbacks, including drug leakage, reduced stability, and limited drug loading capacity.

Different solidification techniques (such as adsorption on solid carriers, nanoparticulate systems, spray drying, melt extrusion, or melt granulation) are used for liquid SEDDS formulations that can be converted into solid SEDDS (S-SEDDS) formulations. Long-term storage and high stability can be achieved with S-SEDDS. The developed S-SEDDS can take various forms, such as dry emulsions, self-emulsifying capsules, micro/nano-particles, pellets/tablets, or suppositories [5,6].

In the previous study, SEDDS formulation was developed using VST active ingredient (VST-SEDDS), and characterization studies were carried out. In this study it was used isopropyl myristate as oil phase, Capyrol 90 and Tween 20 as surfactants and Transcutol HP as co-surfactant. After performing phase diagrams studies, 1:1 surfactant/co-surfactant ratio was determineted extensive microemulsion area. For the VST-SEDDS formulation, VST was dissolved in SEDDS formulation composition at 80 mg/0.5 ml ratio. The VST-S-SEDDS formulation from the VST-SEDDS formulation was carried out using a wet granulation for solid carrier adsorption technique using Avicel pH 101 and HPMC in previous study. The characterization results of the formulations obtained by using HPMC and Avicel pH 101 as solid carriers were compared, and studies were continued with Avicel 101 due to its higher flow properties.

[7]. The solid carrier adsorption technique was used in this study because it has many advantages, such as being simple and fast, and being able to be carried out without using organic solvents. This study aims is to complete the stability studies and show that the bioavailability of VST, which has low solubility and bioavailability from liquid and solid SEDDS formulations, increases by passing through Caco-2 cells. For this purpose, permeability studies on Caco-2 cell lines were divided into experimental groups: powder VST, VST-SEDDS, VST-S-SEDDS, and commercial product. Dispersions containing 40 µg/ml VST were applied to the well plates in all experimental groups, from apical to basolateral direction and from basolateral to apical direction. In addition, cytotoxicity studies were conducted using Caco-2 cell lines. According to the results, SEDDS formulations had increased permeability compared to the commercial product, and therefore, it would be predicted to increase bioavailability. It was also determined that the VST-S-SEDDS formulation had higher permeability values than the liquid VST-SEDDS formulation.

MATERIAL AND METHOD

Materials

VST was generously provided by Bilim Pharmaceuticals (Beyoglu, Istanbul). Capyrol® 90 (Propylene glycol monocaprylate) and Transcutol® HP (Diethylene glycol monoethyl ether) were graciously supplied by Gattefossé (Saint-Priest, France). Isopropyl myristate, Tween® 20 (Polyoxyethylene sorbitan monolaurate), and Avicel pH 101 were acquired from Sigma Aldrich (Darmstadt, Germany). HBSS was also obtained from Sigma Aldrich (Darmstadt, Germany). All the solvents employed in the analytical studies were of high-performance liquid chromatography (HPLC) grade.

Methods

Preparation of VST-SEDDS and VST-S-SEDDS

A Pseudo-ternary phase diagram with oil, surfactant, and co-surfactant determined as a result of solubility studies was drawn using the water titration method. The emulsion area on the pseudo-ternary phase diagram was utilized to identify suitable phases and determine the proportion of each component [8]. Thermodynamic stability studies were carried out with the formulation giving the highest area in the ternary phase diagram. The thermodynamic stability of SEDDS formulations was evaluated by carrying out freeze-thaw, heating-cooling cycles, and centrifugation tests [9].
VST-SEDDS formulation was adsorbed onto Avicel pH 101, a type of inert carrier for preparing VST-S-SEDDS. VST-S-SEDDS formulation was prepared using a wet granulation technique. An oven at 45˚C for approximately 1 hour was used to dry the wet granulation. After that, characterization studies were performed for both formulations.

HPLC Studies

HPLC method was developed and validated for VST quantification in VST-SEDDS and VST-S-SEDDS formulations using HPLC with an Agilent (HP 1100, USA) Series. UV-DAD detector and Zorbax SB C18 (150 mm × 4.6 mm, 3.5 µm) column were used for VST analysis. As the mobile phase, a mixture of Acetonitrile: 0.1M phosphate buffer (55:45, v/v) was adjusted to pH 2.7 with trifluoroacetic acid. The injection volume was 10 µl, and the flow rate was set at 1 ml/min, with a wavelength of 250 nm [10]. The mobile phase was used as the solvent to determine the amounts of the developed VST-SEDDS and VST-S-SEDDS formulations of stability studies. The buffer solution (HBSS) was used as a dilution solution to evaluate permeability studies and observe the buffer solution effect [11]. Linearity, working range, limit of detection (LOD), and limit of quantification (LOQ) parameters were examined.

Chemical and Physical Stability

Chemical and physical stability were assessed over a 12-month storage period under two temperature/relative humidity (RH) conditions: 25 ± 2 °C/60 ± 5 RH% and 40 ± 2 °C/75 ± 5 RH%. Stability studies were carried out by filling the VTS-SEDDS into a vial and VST-S-SEDDS into the

bottle. Physical appearance, electrical conductivity, pH, density, refractive index, polydispersity index, droplet size, viscosity, and active ingredient quantification were monitored throughout stability.

In vitro Permeability Studies

Caco-2 human colon epithelial cancer cell lines are used to forecast the intestinal permeability of drugs because they mimic the small intestinal epithelium [12]. For permeability studies, Caco 2 cells were incubated (37°C/ 90% humidity and 5% CO_2) in flasks until the appropriate count and size. When the appropriate count was reached, permeability studies were conducted using Caco-2 cells prepared by seeding 5×10^5 cells in each of six wells with transwell polycarbonate membrane (pore size 0.4 µm, filtration area 4.67 cm^2, Corning, USA) and they were utilized 21 days after seeding [13]. Transepithelial electrical resistance (TEER) value was measured from the apical direction to the basolateral direction (A-B) and from the basolateral direction to the apical direction (B-A) to show that cell integrity was achieved at the beginning and end of the experiment. For *in vitro* permeability studies, 80 mg of VST, VST-SEDDS equivalent to 80 mg of VST, and commercial product were employed. The solutions obtained by diluting 80 mg VST, VST-SEDDS equivalent to 80 mg VST, and the commercial product in HBSS were applied (Valsartan concentration 40 µg/ml). A volume of 1.5 ml of solution was applied to A-B and, a volume of 2.6 ml of solution was applied to B-A direction for each well. Samples of 200 µl were collected at 0, 30, 60, 90, and 120 minutes (n=3). The apparent permeability coefficient (P_{app}, cm s^{-1}) was calculated from the slope (dQ/t) of the linear portion of the plots depicting the cumulative amounts of permeated VST (Q) over time (t), as per Equation 1 [14]. The quantity of drug remaining on the surface and inside of the polycarbonate membrane was also determined to assess the method's validity. The Efflux ratio was utilized to evaluate the P-gp inhibitory effect of the substances, and Equation 2 shows how the efflux value is calculated [15].

Equation 1.

$$\text{Papp(Permeability)} = \frac{\text{dQ}}{\text{dt}} \text{x} \frac{1}{\text{Co x A x 60}}$$

In Equation 1, P_{app} is the apparent permeability (cm/s), dQ/dt is the steady state flux, A is the diffusion area of the monolayer (in cm^2), Co is the initial concentration of the drug in the donor compartment (µM), and 60 is a conversion factor for time.

Equation 2.

$$\text{The efflux ratio} = \frac{\text{Papp(B} - \text{A)}}{\text{Papp(A} - \text{B)}}$$

In Equation 2, P_{app} (A-B): Apparent permeability of apical direction to basolateral direction (cm/s), P_{app} (B-A): Apparent permeability of basolateral direction to apical direction (cm/s).

Cytotoxicity Test

A cytotoxicity study was conducted to observe the potential cytotoxic effect of formulation components on Caco-2 cells [16]. Caco-2 cells were seeded in a 96-well plate (pore size 0.4 µm, Corning, USA), each well at a density of 1×10^{-4} cells and incubated for 24 h at 37 ± 0.5 °C in a CO_2 incubator. To examine cytotoxicity powder VST, VST-SEDDS formulation, VST-S-SEDDS formulation, and commercial tablet product, were dissolved in Hanks' Balanced Salt Solution (HBSS) and incubated with Caco-2 cells for 24, 48, and 72 hours. At the end of these periods, 100 µl of (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) (5 mg/ml of stock MTT / phosphate buffer saline (PBS) solution) was added to each of the plate and incubated for 4 hours. The solutions were removed, and 100 µl dimethyl sulfoxide (DMSO) was added to each 96-well. To test cell viability, absorbance values at 540 nm were used an ELISA microplate reader UV spectrophotometer (Thermo vario scan-FHA multi-plate reader) [17].

Statistical Evaluation

Statistical significance was assessed using the Student t-test and one-way ANOVA test. Differences were deemed significant at the 95% confidence level ($p < 0.05$). The results were presented as the mean of the obtained values, and the corresponding standard deviation (±).

RESULT AND DISCUSSION

Preparation of VST-SEDDS and VST-S-SEDDS

Details of the method development, ternary phase diagram and characterization studies of the optimum formulation were described in a previous [7]. As per the study, isopropyl myristate was employed as the oil phase, Capyrol 90 and Tween 20 as surfactants, and Transcutol HP as a co-surfactant. 0.5 ml of the developed SEDDS formulation contains 80 mg VST. Thermodynamic stability studies evaluate the ability of the SEDDS formulation to withstand stress conditions [18]. In the optimal SEDDS formulation, the heating-cooling, freeze-thaw cycles, and centrifugation test were carried out within the scope of the thermodynamic study. At the end of these studies, no phase separation was observed in the VST-SEDDS formulation, and it was shown that the developed formulation was not affected by stress conditions. The droplet size of the VST-SEDDS formulation was obtained at 72.63 ± 0.45 nm, and the zeta potential value was found to be 0.069 ±0.005 mV. In addition, the VST-SEDDS formulation polydispersity index (PDI) was found to be 0.449. Self-emulsifying systems, in particular, utilize a classification system known as the Lipid Formulation Classification System (LFCS). This system, introduced by Pouton, facilitates a systematic and rational approach to formulation [19]. According to LFCS, SEDDS formulations with a 50-100 nm droplet size are in the Type IIIB class. Due to the droplet size of the developed VST-SEDDS formulation, it was observed to have a Type IIIB classification system that shows greater dispersion rates then other types [20]. When the SEDDS formulation was diluted 1:1000 with water, the droplet size (<100nm) did not change over 24 hours.

VST-S-SEDDS formulation was developed based on VST-SEDDS formulation using the wet granulation technique with Avicel pH 101. 500 mg of the developed VST-S-SEDDS formulation contains 80 mg VST. After diluting with the amount of water that creates the optimum formulation, droplet size and PDI analyzes were performed and these results were found to be 186±5.23 nm and 0.51±0.30, respectively. Due to the droplet size of the developed VST-S-SEDDS formulation, it was observed to have a Type IIIA classification system. According to the Lipid Formulation Classification System (LFCS) proposed by Pouton, while Type III A systems need to be digested more than Type IIIB systems for absorption in the in vivo environment. Since the solvent capacity of the Type IIIB systems is lower, the possibility of precipitation in the in vivo environment may be higher than the Type IIIA systems [19]. While VST-SEDDS is in the Type IIIB class according to droplet size, the VST-S-SEDDS formulation developed by the solid phase absorption method is in the Type IIIA class according to the droplet size. The reason for this change can be interpreted as the change in dispersibility behavior and droplet size after the absorption of VST-S-SEDDS into Avicel. In solidification drug delivery systems, predicting the flow characteristics of powders for manufacture is especially important. Powder characterization evaluations of VST-S-SEDDS formulation were carried out using European Pharmacopoeia guidance [21]. Therefore, the Hausner ratio and Carr index, derived from bulk and tapped density were found to predict powder flowability. Bulk density, tapped density, Hausner ratio and Carr index results calculated for the developed VST-S-SEDDS formulation were 0.288, 0.4301, 1.492, and 32.98%, respectively. Granules with a Hausner ratio below 1.25 and 16-20% compressibility percentage are known to have good fluidity [21]. For this reason, it may be recommended to add glidants and/or lubricants as formulation components to increase the flow properties of the developed formulation [22]. However, since using a lubricant to increase flowability would cause problems fitting into capsule No. 00, the dosage form was planned to be a sachet. Accordingly, in the characterization studies carried out, the dimensional analysis of VST-S-SEDDS was found to be 98.4% in the 2000-1400 µm mesh size range. The emulsification time of the solidified self-emulsions and the droplet size after redispersibility were measured and found to be 30 seconds and 186.3±1.362 nm, respectively. It has been observed that this formulation belongs to Type IIIA from the LFCS with its particle size. Its rapid

emulsification in water compared to the emulsification time supported the idea of using it in the sachet dosage form.

This formulation has greater dispersion rates and requirements of enzymatic digestion are not necessary [19].

HPLC Studies

HPLC method was developed and validated to determine drug content [7]. The calibration curve was drawn for HBSS medium, and LOD and LOQ values were calculated (Table 1).

Table 1. Linearity, LOD and LOQ results of permeability medium

Media	Concentration range (µg/ml)	Equation	R^2	LOD (µg/ml)	LOQ (µg/ml)
HBSS	0.1-50	y= 17.516x+3.3711	0.9992	0.700	0.210

Chemical and Physical Stability

It was observed that there were no significant physical and chemical changes in the stability results of the developed formulation carried out at 25±2˚C, 60±5% relative humidity (long term) and 40±2˚C, 75±5% relative humidity (accelerated) for 12 months (Table 2 and Table 3).

Table 2. The stability results of VST-SEDDS formulation at 25 ± 2˚C/60 ± 5 RH% and at 40 ± 2˚C/75 ± 5 RH% (n=3)

Conditions* / Parameters	0. Month		1. Month		3. Month		6. Month		9. Month		12. Month	
	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.
pH*	3.74	3.74	3.75	3.76	3.75	3.75	3.76	3.75	3.75	3.75	3.74	3.76
Refractive index **	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46
Electrical conductivity (µs) **	86.0	86.0	86.0	87.0	86.0	87.0	87.0	87.0	86.0	87.0	86.0	86.0
Density (g/ml)	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Droplet size (nm)**	72.6 ±1.56	72.6 ±1.56	70.2 ±0.96	73.7 ±1.94	73.3 ±1.56	79.0 ±2.95	62.1 ±1.25	63.8 ±0.96	65.6 ±1.4	67.5 ±1.76	61.3 ±1.62	63.9 ±1.85
Polydispersity index	0.45 ±0.21	0.45 ±0.35	0.77 ±0.25	0.42 ±0.21	0.48 ±0.30	0.67 ±0.31	0.64 ±0.29	0.58 ±0.32	0.49 ±0.34	0.59 ±0.33	0.64 ±0.30	0.56 ±0.28
Viscosity (cP)	47.0	47.0	47.1	47.0	47.1	47.0	47.0	47.1	47.0	47.0	47.1	47.1
Assay (%)	95.2 ±0.50	95.2 ±0.50	95.8 ±0.60	95.0 ±0.50	95.5 ±0.30	96.3 ±0.60	95.5 ±2.00	96.8 ±0.50	95.5 ±0.91	96.8 ±1.01	96.5 ±1.56	97.3 ±1.49
Physical appearance	Homogeneous, Transparent, No Phase Separation											

* 1. condition: 25 ± 2 ◦C/60 ± 5 RH%; 2. Condition: 40 ± 2 ◦C/75 ± 5 RH%; ** Diluted with the amount of water that creates the optimum formulation.

Droplet sizes for VST-SEDDS and VST-S-SEDDS during the long-term stability condition were 67.52 ± 6.07 nm and 176.93 ± 17.34 nm, respectively. The polydispersity index (PDI) gives a measure of particle size distribution. A PDI <0.5 indicates a homogeneous system and a narrow particle size distribution [23]. VST-SEDDS formulation polydispersity index (PDI) was found to be 0.449. After diluting with the amount of water that creates the optimum VST-S-SEDDS formulation, PDI analyzes were performed and it was found to be 0.51±0.30. During stability period, Polydispersity indexes (PDI) of VST-SEDDS and VST-S-SEDDS formulation were found to be 0.56±0.1, 0.58±0.05, respectively. The PDI values of the formulations did not change for 12 months and were approximately 0.5, indicating

that the formulations maintained their monodisperse structure throughout this period. The accelerated stability test, it was found to be 70.11 ± 6.07 nm for VST-SEDDS and 192.53 ± 22.16 nm for SEDDS. During all stability studies, when VST-SEDDS and VST-S-SEDDS are evaluated on their own, there were no statistically significant changes in droplet size and active substance assay ($p<0.05$). Solidified SEDDS increases the solubility of lipophilic drugs, reduces their biodegradation, and provides a better stability profile.

Table 3. The stability results of VST-S-SEDDS formulation (n=3)

Conditions* / Parameters	0. Month		1. Month		3. Month		6. Month		9. Month		12. Month	
	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.	1.	2.
Droplet size (nm)**	186 ±5.23	186 ±5.23	184 ±4.96	179 ±4.78	142 ±3.56	169 ±4.26	181 ±9.47	233 ±6.73	178 ±4.82	195 ±3.42	189 ±8.62	190 ±5.41
Polydispersity index**	0.51 ±0.30	0.53 ±0.28	0.49 ±0.22	0.58 ±0.40	0.51 ±0.33	0.61 ±0.28	0.58 ±0.18	0.63 ±0.26	0.60 ±0.25	0.62 ±0.35	0.61 ±0.28	0.63 ±0.32
Assay (%)	100.0 ±0.51	100.0 ±0.51	95.7 ± 1.45	104.3 ±0.96	103.0 ± 1.03	104.0 ± 1.53	102.0 ± 1.02	104.0 ± 0.95	106.1 ± 0.62	103.0 ± 1.52	104.0 ± 1.32	104.0 ± 0.85
Physical appearance**	Homogeneous, Transparent, No Phase Separation											

* 1. condition: 25 ± 2 ◦C/60 ± 5 RH%; 2. Condition: 40 ± 2 ◦C/75 ± 5 RH%; ** Dilute with the amount of water that creates the optimum formulation.

In vitro Permeability Studies

Caco-2 cells resemble intestinal epithelium due to their tight junctions and microvilli-like structure [24]. For this purpose, permeability studies of powder VST, VST-SEDDS, VST-S-SEDDS, and commercial product were performed with Caco-2 cell lines. They calculated P_{app} for apical to basolateral (A-B) and basolateral to apical (B-A) direction (Table 4). If $P_{app} > 10^{-6}$ cm/s, it can be said to have high permeability [25]. When the efflux value is >2, there is a possibility that the formulation will be exposed to efflux flow, which is a factor that reduces permeability (Table 4).

Table 4. Apparent permeability coefficient and Efflux value (n=3)

	Powder Valsartan	Commercial product	VST-SEDDS	VST-S-SEDDS
$P_{app\ A\text{-}B}$±SD (cm/s)	$37.3x10^{-5}$ ±0.01	$39.7x10^{-5}$ ±0.006	$86.5x10^{-5}$ ±0.007	$51.5x10^{-5}$± 0.002
$P_{app\ B\text{-}A}$±SD (cm/s)	$72.9.x10^{-5}$±0.01	$63.4x10^{-5}$± 0.008	$75.6x10^{-5}$ ± 0.016	$52.1x10^{-5}$± 0.009
Efflux	1.95±0.698	1.59±0.69	0.87±0.047	1.01±1.66

According to the results, it was observed that VST-SEDDS and VST-S-SEDDS formulations increased the permeability of VST by 2.32 and 1.38 times, respectively, compared to powder VST and by 2.18 and 1.30 times, compared to the commercial product. It was observed that the permeability was increased similarly with the S-SEDDS formulation developed in the study of Timur et al. [26]. The surfactants and co-surfactants used in SEDDS formulations increase permeability by opening tight junctions and reducing efflux membrane transport activity [27]. In addition, when the amount of VST drug remaining on the surface and inside of the polycarbonate membrane was analyzed, no amount of VST was found in the samples. This result indicates that VST is not retained in the membrane and that all added represents a permeability study.

Transepithelial electrical resistance (TEER) is measured across the cellular monolayer and is a convenient and relatively sensitive measure of the integrity and permeability of the monolayer on cell lines. The TEER value range of cells with cell integrity s hould be 500-1200 $\Omega.cm^2$ [28]. Throughout

this study, TEER values in the Caco-2 cell line for A-B and B-A were in the range of 1100-1200 Ω .cm^2 in all pre- and post-experiment measurements and were found to comply with the acceptance criteria.

In vitro permeability studies showed that SEDDS formulations increased the permeability of Caco-2 cell lines. Finally, developing valsartan-containing VST-SEDDS and VST-S-SEDDS may be a desirable for improved bioavailable antihypertensive therapy.

Cytotoxicity Test

As Caco-2 cells were used as a permeability model, biocompatibility and tolerability of developed VST-SEDDS and VST-S-SEDDS formulations on Caco-2 cells were important [29]. For this reason, formulations were assessed in Caco-2 cells by MTT assay to check their safety. In addition to the developed formulations, the effect of powder VST and commercial product on cell viability was investigated. In order to predict any cytotoxic effects that may arise from the formulation components, MTT studies were also conducted for the blank formulations and no change in cell viability was observed in blank formulations. In the cytotoxicity study, cell viability greater than 95% was obtained for powder VST, VST-SEDDS, VST-S-SEDDS, and the commercial product, 98.5%, 96%, and 96.5%, 98%, respectively. Therefore, there is no cytotoxic effect from formulations on Caco-2 cells.

In this study, stability evaluations and permeability studies of the developed SEDDS and S-SEDDS formulations containing the active ingredient VST, which shows low solubility depending on pH, were successfully carried out. No significant change was observed in the characterization results performed during the stability follow-ups of both SEDDS and S-SEDDS formulations. Moreover, in the permeability study conducted on the Caco-2 cell line, it was observed that the permeability increased in SEDDS and S-SEDDS formulations depending on the active ingredient. These results show that SEDDS and S-SEDDS formulations containing VST may be an alternative approach in antihypertensive treatment.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

EVALUATION OF GREENNESS PROFILES OF VARIOUS DEVELOPED METHODS FOR THE DETERMINATION OF COMMONLY USED NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs) IN ENVIRONMENTAL WATERS

SIK KULLANILAN NONSTEROİD ANTİ-İNFLAMATUVAR İLAÇLARIN (NSAİİ) ÇEVRESEL SULARDA TAYİNİ İÇİN GELİŞTİRİLMİŞ ÇEŞİTLİ YÖNTEMLERİN YEŞİLLİK PROFİLLERİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *In our study, it was aimed to make a comparative analysis of the environmental impact profiles of two approaches including Gas Chromatography (GC) and Liquid Chromatography (LC) methods, which are frequently used techniques for the determination of non-steroidal anti-inflammatory drugs (NSAIDs) and their metabolites in environmental water samples.*
Material and Method: *The evaluation of the methods' environmental impact was performed using National Environmental Methods Index Label (NEMI), Analytical Eco-scale, Analytical GREEnness Metric (AGREE), and Green Analytical Procedure Index (GAPI).*
Result and Discussion: *The routine analysis of NSAIDs in environmental waters is carried out, resulting in a significant volume of chemical waste. In recent times, there has been a growing significance attributed to environmentally conscious analytical methodologies and the evaluation of methodologies through a green lens to confront this challenge. There is no statistically significant difference in terms of environmental impact profile was observed between the two methods compared.*
Keywords: *Environmental waters, green chemistry, greenness assessment, nonsteroidal anti-inflammatory drugs (NSAIDs)*

ÖZ

Amaç: *Çalışmamızda, çevresel su örneklerinde steroid olmayan antiinflamatuar ilaçların (NSAİİ) ve bunların metabolitlerinin tayininde sıklıkla kullanılan teknikler olan Gaz Kromatografisi (GK) ve Sıvı Kromatografisi (SK) yöntemlerini içeren iki yaklaşımın çevresel etki profillerinin karşılaştırmalı bir analizinin yapılması amaçlanmıştır.*
Gereç ve Yöntem: *Yöntemlerin çevresel etkisinin değerlendirilmesi, Ulusal Çevresel Yöntemler İndeks Etiketi (NEMI), Analitik Eko-ölçek, Analitik Yeşillik Metriği (AGREE) ve Yeşil Analitik Prosedür İndeksi (GAPI) kullanılarak gerçekleştirilmiştir.*
Sonuç ve Tartışma: *Çevresel sularda NSAİİ'lerin rutin analizi gerçekleştirilmekte ve bunun*

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sonucunda önemli miktarda kimyasal atık ortaya çıkmaktadır. Son zamanlarda, çevreye duyarlı analitik metodolojilere ve bu zorluğun üstesinden gelmek için metodolojilerin yeşil bir mercekle değerlendirilmesine atfedilen önem giderek artmaktadır. Karşılaştırılan iki yöntem arasında çevresel etki profili açısından istatistiksel olarak anlamlı bir fark olmadığı gözlenmiştir.
Anahtar Kelimeler: *Çevresel sular, nonsteroid anti-inflamatuvar ilaçlar (NSAİİ), yeşil kimya, yeşillik değerlendirmesi*

INTRODUCTION

Pharmaceuticals used for various purposes, such as the treatment and prevention of diseases, along with improperly disposed waste medications, result in high concentrations of active pharmaceutical ingredients and their metabolites contaminating wastewater. Substantial quantities of these substances reach wastewater treatment plants, and some of these may also directed to surface waters. In fact, in the effluent from agricultural areas irrigated with treated wastewater, pharmaceuticals, including non-steroidal anti-inflammatory drugs (NSAIDs), have been detected [1-3]. While some of these substances can be completely removed by traditional treatment systems, some drugs, such as diclofenac, an NSAID drug, can only be partially removed [4].

Since the discovery of aspirin (acetylsalicylic acid), NSAIDs have become globally popular over-the-counter drugs, constituting approximately 5% of all prescription medications. While NSAIDs are classified based on their chemical properties, selectivity toward the inhibition of their target enzymes, and half-lives, their functions are relatively similar. NSAIDs are commonly used for the treatment of patients experiencing chronic pain, rheumatoid arthritis, menstrual cramps, postoperative surgical conditions, osteoarthritis, and other painful and inflammatory conditions. They are also widely used as analgesics and antipyretics. Studies have demonstrated the significant role of NSAIDs in a protective capacity against various cancers. Despite their non-addictive nature and broad therapeutic benefits, NSAIDs come with numerous serious side effects, such as cardiovascular risks, gastrointestinal toxicities, kidney injuries, and hypertension [5-7].

In wastewater treatment plants, the removal rate of acidic compounds such as NSAIDs is quite low due to their high solubility in water and poor degradability. While the individual concentrations of these residues in the aquatic environment may be low, the coexistence of drug combinations that share a common mechanism of action introduces the potential risk of synergistic effects. Therefore, the development of analytical methods that enable the determination of NSAIDs and their metabolites at low concentrations in the aquatic environment has become an important part of studies aimed at determining the formation, fate, and effects of pharmaceuticals [2,8].

NSAIDs and their metabolites are among the most frequently detected analytes among pharmaceuticals. Methods based on gas chromatography (GC) [3,9-18], liquid chromatography (LC) [19-31], and electrophoretic techniques [32-36] are used for the determination of NSAIDs and their metabolites in environmental water samples.

GC devices are primarily used due to their widespread availability and low cost in environmental laboratories and are often combined with mass spectrometry (GC-MS). Due to the polarity of NSAIDs, derivatization of the analyte is required for GC-MS analysis. The derivatization step brings disadvantages such as difficulties encountered in the case of many samples, loss of time, reproducibility problems, and possible formation of artifacts. With this technique, the carboxyl group of NSAID drugs can be converted to methyl ester with excellent yield using diazomethane, but due to the high toxicity and low stability of this reagent, LC and electrophoretic techniques that do not require derivatization can be preferred. LC-MS techniques have advantages such as low LODs, high sensitivity, and good linear range for quantitative analysis, but also disadvantages such as high acquisition and maintenance costs. Capillary electrophoretic techniques, on the other hand, are a good alternative for the determination of NSAIDs thanks to their high efficiency, rapid analysis, and the possibility of combining with an MS detector, but they have low analyte detectability, especially in the determination of trace levels, due to the small amount of sample that can be injected into the capillary [2,37,38].

As a result of analytical methods used in the determination of various analytes, including studies in environmental waters, millions of liters of chemical waste are generated, most of which are toxic and

ecologically hazardous [39,40]. Hence, researchers have directed their attention towards green analytical chemistry as a strategic approach to protect the environment and human health, and greenness evaluations of applied analytical methods have become an important parameter in method selection. In this context, various greenness evaluation methods have been developed [41-43]. In our study, greenness profiles were created and compared using various greenness assessment tools, for two different methods reported in the literature for the determination of NSAIDs in environmental waters.

MATERIAL AND METHOD

Greeness Assessment Tools

In the context of the study, an LC-MS [31] and a GC-MS [3] application have been chosen as exemplary analytical methods from the literature. Within the scope of this research, we employed four different tools to evaluate the greenness assessment of the selected methods. These tools include National Environmental Methods Index Label (NEMI) Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and Analytical GREEnness Metric (AGREE). Our objective was to conduct a thorough greenness assessment and evaluate the environmentally friendly characteristics inherent in the investigated chromatographic methods.

National Environmental Methods Index (NEMI)

NEMI is one of the earliest greenness analytical tools to evaluate the sustainability of analytical procedures. This method contains a circle symbol including four quarters (Pictogram) is drawn and each quarter illustrates an aspect of the method that may pose a potential environmental hazard. Achievement of the prescribed requirements results in the display of relevant fields in a green format. The determination of this state is contingent upon the fulfilment of the following conditions: firstly, a chemical utilized in the method must be identified as a PBT (persistent, bioaccumulative, and toxic) according to the Environmental Protection Agency's Toxics Release Inventory (EPA's TRI). Secondly, the chemical's inclusion on either the TRI or on the Resource Conservation and Recovery Act's (RCRA) D, F, P, or U hazardous waste lists signifies its categorization as hazardous. Corrosiveness is established if the pH level during analysis falls below 2 or exceeds 12. Lastly, the generation of waste surpassing 50 grams is a prerequisite for the classification of the method under the waste criterion [44].

Analytical Eco-Scale

The Analytical Eco-Scale assesses the environmental impact of an analytical method by deducting penalty points (PP) from a maximum score of 100. This scoring system provides a quantitative measure of the method's eco-friendliness, with a flawless green analysis achieving a perfect score of 100. The allocation of penalty points takes into account various factors (the types and quantities of solvents and reagents utilized, energy consumption, occupational hazards, waste generation, and strategies for waste management). The resulting numerical output from the eco-scale reflects the overall environmental performance, where a higher score indicates a more sustainable and environmentally friendly analytical approach, while a lower score suggests a greater negative impact on the environment [45].

The output of the eco-scale is a numerical value derived by subtracting the cumulative penalty points from 100 and its calculation is performed according to the "*Analytical Eco-Scale = 100 – total penalty points (PP)*" formula. The results are categorized on a scale as follows: an excellent green analysis (>75 points); an acceptable green analysis (>50 points); and an inadequate green analysis (<50 points) [45].

The Analytical Greenness Metric (AGREE)

AGREE is a metric system that enables the evaluation of an analytical procedure based on 12 green principles. This software offers advantages such as comprehensiveness, rapid, simplicity, flexibility, and ease of interpretation. The assessment result is visualized in the shape of a circular clock, with the total score displayed at the centre. This score present as a representation of the overarching environmental sustainability of the assessed process or methodology. Furthermore, a visual representation in colour (green, yellow, red colour scale) is used to depict the assessment result,

facilitating swift comprehension and comparison. The score is indicated on a 0-1 scale, with a higher score approaching 1 signifying a heightened degree of greenness. This suggests that the assessed process or methodology is more closely aligned with the principles of green analytical chemistry [46,47].

Green Analytical Procedure Index (GAPI)

GAPI, frequently used as one of the most important tools for assessing eco-friendliness, was employed to evaluate both analytical methods. GAPI is represented by a pictogram consisting of a total of 15 subcategories, each with 3 colour criteria for every stage. Red, yellow, and green colours are depicted on 5-pointed star symbols. The pictogram provides information about the extent to which the analytical procedures meet green requirements [48].

RESULT AND DISCUSSION

Greenness Assessment of the Methods

NEMI

The NEMI labeling of the examined methods is presented in Figure 1. The results show that the methods meet the same green requirements such as PBT, corrosive, and waste except for the hazardous criteria section.

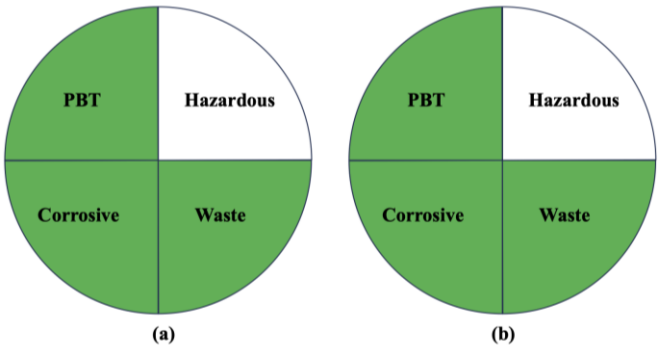

Figure 1. NEMI pictograms for the methods (a) LC-MS (b) GC-MS

Analytical Eco-Scale

A higher score denotes a more environmentally friendly analysis, signifying that the method has effectively minimized its ecological impact and inherently possesses greater sustainability. Conversely, a lower score indicates a more substantial negative impact on the environment. The Analytical Eco-Scale points of the investigated methods are shown in Table 1 and Table 2. Analytical Eco-Scale points were found to be as 76 for LC-MS method which is classified as excellent green analysis and 73 for GC-MS method which is classified as acceptable green analysis.

AGREE

The AGREE pictograms of the investigated analytical methods are given in Figure 2. AGREE scores are found to be as 0.52 and 0.48, respectively. The sections in the pictogram represent (1) sampling procedure, (2) amount of sample, (3) device positioning, (4) sample preparation steps, (5) degree of automation, (6) derivatization, (7) amount of waste, (8) number of analytes in a single run, (9) total power consumption, (10) type of reagents, (11) use of toxic reagents, (12) safety of the operator [49].

The weakest sections were determined as 3 and 9 for LC-MS method, 2, 3 and 9th section for GC-MS method. The sections marked in red, namely 2, 3, and 9, correspond to the minimum sample size, device positioning, and total power consumption ($\geq$ 1.5 kWh) of a single analysis, respectively. As a result, since the scores of both analytical procedures is below 0.6, it does not meet the greenness score in terms of AGREE [49].

Table 1. Analytical Eco-Scale penalty points for evaluated LC-MS method [31-45]

Components	Pictograms	Signal word	Used in method	Penalty Point
Reagent				
Ammonium acetate	No hazard pictogram	-	< 10 ml (g)	**0**
Methanol		Danger	< 10 ml (g)	**6**
Formic acid		Danger	< 10 ml (g)	**6**
Acetic acid		Danger	< 10 ml (g)	**4**
Monosodium phosphate	No hazard pictogram	-	< 10 ml (g)	**0**
Water	No hazard pictogram	-	< 10 ml (g)	**0**
Sodium hydroxide		Danger	< 10 ml (g)	**2**
Instruments				
LC-MS/MS			> 1.5 kWh per analysis	**2**
Sonicator			≤ 0.1 kWh per analysis	**0**
Centrifuge			≤ 1.5 kWh per analysis	**1**
Occupational hazard			Analytical process hermetization	**0**
Waste			1-10 ml (g) per analysis	**3**
Total Penalty Point				**∑=24**
Eco-Scale Score			**100 - ∑**	**76**

Table 2. Analytical Eco-scale penalty points for evaluated GC-MS method [3-45]

Components	Pictograms	Signal word	Used in method	Penalty Point
Reagent				
Trimethylsilyl		Danger	< 10 ml (g)	**4**
Methanol		Danger	< 10 ml (g)	**6**
Toluene		Danger	< 10 ml (g)	**6**
Water	No hazard pictogram	-	< 10 ml (g)	**0**
Helium		Warning	< 10 ml (g)	**1**
Instruments				
GC-MS			> 1.5 kWh per analysis	**2**
Occupational hazard			Emission of vapours and gases to the air	**3**
Waste			>10 ml (g) per analysis	**5**
Total Penalty Point				**∑=27**
Eco-Scale Score			**100 - ∑**	**73**

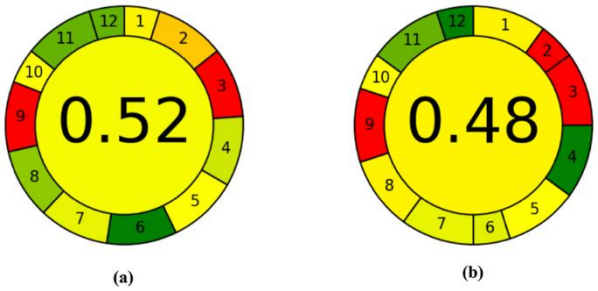

Figure 2. AGREE pictograms for the methods (a) LC-MS (b) GC-MS

GAPI

The GAPI pictograms for both analytical methods examined in this study are presented in Figure 3. The pentagrams of the examined HPLC methods reveal negative environmental impact due to the characteristics of its off-line sample collection, transport, require extraction procedure, use of non-green solvents or reagents, energy consumption (≥ 1.5 kWh) and no treatment for waste, in which case all coded red. Physical preservation, storage under normal conditions, apply micro extraction process, use moderately toxic reagent and highest NFPA flammability or instability score of 2 or 3, or a special hazard solvent and 1–10 ml (1–10 g) waste produce is represent the yellow part of the both methods and no additional treatments, use under 10 ml (or < 10 g) solvent are shows the green environmental impact of the analytical procedures.

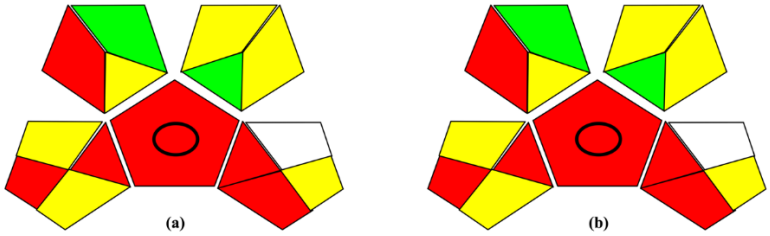

Figure 3. GAPI pictograms for the methods (a) LC-MS (b) GC-MS

The increasing prevalence of high concentrations of active pharmaceutical ingredients and their metabolites in environmental waters, as a result of the frequent use of non-steroidal anti-inflammatory drugs (NSAIDs), has become a significant area of research. The determination of these NSAID drugs in water samples has been widely investigated using various analytical methods such as LC/MS and GC/MS. However, in recent years, growing environmental concerns have directed researchers to examine the environmental impact of the conducted analyses. The environmental impact assessment of these analytical methods has gained attention due to the escalating environmental issues. This shift in focus reflects a broader awareness among researchers regarding the potential ecological consequences of pharmaceutical contamination in environmental water. As a result, the exploration of analytical techniques for NSAID detection now not only encompasses accurate and sensitive measurements but also considers the broader implications on the environment. This multidimensional approach aims to address both the analytical aspects of pharmaceutical detection and the environmental repercussions of these methods. The derivation of greenness profiles for the recommended methods in the literature should be an integral part of method development studies. Analytical methodologies should not only strive for accuracy, sensitivity, and efficiency but also consider their ecological impact, thereby contributing to the broader framework of green analytical chemistry.

AUTHOR CONTRIBUTIONS

Concept: B.S., M.S.; Design: B.S., M.S.; Control: B.S., M.S.; Sources: B.S., M.S.; Materials: B.S., M.S.; Data Collection and/or Processing: B.S., M.S.; Analysis and/or Interpretation: B.S., M.S.;

Literature Review: B.S., M.S.; Manuscript Writing: B.S., M.S.; Critical Review: B.S., M.S.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no actual, potential or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANTIDEPRESSANT CONSUMPTION IN TÜRKİYE DURING THE PANDEMIC

PANDEMİ DÖNEMİNDE TÜRKİYE'DE ANTİDEPRESAN TÜKETİMİ

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ABSTRACT

Objective: *This study aims to reveal how the restrictions, quarantines, social policies, and implemented measures during the COVID-19 pandemic have affected the consumption of antidepressant drugs in Türkiye.*
Material and Method: *The research was conducted based on the total consumption figures of antidepressant drugs between 2017 and 2022. The necessary data were obtained from IQVIA/Turkey and OECD official sources.*
Result and Discussion: *Our study has shown that antidepressant consumption in Türkiye during the pandemic has increased much more than expected. Additionally, alongside countries where consumption has increased in OECD countries, there are countries where consumption has not been affected. Examining countries' social and economic policies where consumption is unaffected during the pandemic would be beneficial. Türkiye's social and economic policies have proven inadequate in psychologically protecting the country's citizens during the pandemic.*
Keywords: *Antidepressant, COVID-19, pandemic, Türkiye*

ÖZ

Amaç: *Bu çalışmada COVID-19 Pandemisi döneminde kısıtlamaların, karantinaların, sosyal politikaların, uygulanan önlemlerin, vb. durumların Türkiye'de antidepresan ilaçların tüketimini nasıl etkilediğini ortaya çıkarmak amaçlanmıştır.*
Gereç ve Yöntem: *Araştırma Antidepresan ilaçların 2017-2022 yılları arasındaki toplam tüketim rakamları üzerinden gerçekleştirilmiştir. Gerekli veriler IQVİA/Türkiye ve OECD resmi verilerinden alınarak kullanılmıştır.*
Sonuç ve Tartışma: *Çalışmamız göstermiştir ki; pandemi sürecinde Türkiye'de antidepresan tüketimi beklenenden çok daha fazla artmıştır. Ayrıca OECD ülkelerinde tüketimin arttığı ülkelerin yanı sıra tüketimi hiç etkilenmeyen ülkeler de bulunmaktadır. Tüketimin etkilenmediği ülkelerin pandemi sürecindeki sosyal ve ekonomik politikaları incelenmelidir. Türkiye'nin sosyal ve ekonomik politikaları ülke vatandaşlarını psikolojik açıdan korumakta yetersiz kalmıştır.*
Anahtar Kelimeler: *Antidepresan, COVID-19, pandemi, Türkiye*

INTRODUCTION

The COVID-19 pandemic emerged in December 2019 when a group of people in Wuhan, China,

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exhibited respiratory symptoms such as fever, cough, and shortness of breath. The identification of the COVID-19 virus was made on January 13, 2020, following research conducted on a group of patients. While the virus originated in the local animal market, it later spread from person to person, first within the People's Republic of China and eventually globally, leading to a pandemic. There are variations in the symptoms of the COVID-19 virus; some infected individuals remain asymptomatic, while others experience severe cases, some of which result in death [1,2].

As of November 2, 2021, the COVID-19 virus has caused approximately five million deaths worldwide [3]. In Türkiye, during the same period, around 70 thousand people have died due to the COVID-19 virus [4]. Studies indicate that the risk of death in hospitalized patients ranges from 0.5% to 4%, while for critically ill patients, this rate varies between 5% and 15% [5].

There is a broad consensus that the COVID-19 pandemic affects physical, mental, and well-being [6]. Measures such as confinement, social and physical distancing, and stay-at-home orders contribute to increased incidents of violence and aggressive behavior. Similarly, restrictions on production and limited commercial activities negatively impact many individuals and businesses economically. These risk factors challenge societal health during the pandemic [7]. From a public health perspective, the pandemic leads to insecurity, confusion, emotional distress, and stigmatization in individuals, along with adverse effects such as insufficient resources during medical care. These effects have caused emotional changes, such as anxiety, depression, insomnia, and an increased desire for substance use, in individuals across various demographics [8].

It is assumed that the fundamental cause of anxiety during both past pandemics and the current COVID-19 situation is the uncertainty surrounding the exact cause of the disease. This uncertainty is believed to increase psychiatric morbidity [9,10]. Past pandemics, such as the SARS-CoV-1 in 2003, H1N1 influenza in 2010, the Ebola outbreak in 2014, and the MERS outbreak in 2015, demonstrated that uncertainty, isolation, interruption of social activities, and the atmosphere of a significant disaster contribute to deterioration in mental health during and after the pandemic [11-15]. Additionally, despite numerous studies evaluating the psychological effects and psychopharmacological aspects of the Covid-19 pandemic [1,16-19], there is limited research on the impact of the pandemic on psychological medication use and outpatient visits.

Therefore, this study aims to present the psychological effects of the COVID-19 pandemic on individuals in Türkiye with concrete and measurable data and to reveal how it has affected the antidepressant drug market.

MATERIAL AND METHOD

The study was conducted using sales and unit box data of drug molecules defined and used as antidepressants over the years. Data between 2017 and 2022, when all precautions were lifted entirely to observe the pre-pandemic consumption trend, were used. The data from Turkey were obtained from Iqvia/Turkey (IMS/Health), an institution that tracks and records market movements from drug production to end consumers. Data from OECD countries were obtained from the official OECD website. Additionally, statistics such as the number of outpatient clinics, prescription numbers, and diagnosis numbers for the years included in the study were formally requested from the Ministry of Health and the Social Security Institution. However, positive responses could not be obtained. Therefore, data on the number of psychiatry outpatient clinics, diagnosis distributions, and hospital admission rates for the years included were obtained from Hitit University Erol Olçok Training and Research Hospital with the approval of the ethics committee, considering that it would support and contribute to the study. Initially, it was considered to include antipsychotic drugs in the study. However, it was abandoned as no significant change in the consumption of this group of drugs was observed when examining the data. The study was conducted retrospectively based on consumption data of antidepressant drugs over the years and quantitative data obtained from the sample hospital.

RESULT AND DISCUSSION

In Table 1, data on the prevalence of depression and anxiety per population in Türkiye and worldwide are provided.

Between 2017 and 2019, corresponding to the pre-pandemic period in Türkiye and globally, there has not been a significant increase in the prevalence of depression and anxiety disorders (Table 1).

Table 1. Prevalence of depression and anxiety per population in Türkiye and worldwide [20]

Location	Diagnosis	2017	2018	2019
Türkiye	Depression	% 4.54	% 4.56	% 4.60
Worldwide	Depression	% 3.72	% 3.74	% 3.76
Türkiye	Anxiety	% 4.99	% 5.00	% 5.02
Worldwide	Anxiety	% 4.03	% 4.04	% 4.05

In 2017, 42.204 individuals applied to the psychiatry clinic, and the number of inpatients was 551. During the same year, 2.881 individuals were diagnosed with depression, 35.128 with anxiety, and 954 with Obsessive-Compulsive Disorder (OCD). In 2019, just before the pandemic, 56.333 individuals sought help from the psychiatry clinic, and the number of inpatients was 660. Within the same year, 2.055 individuals were diagnosed with depression, 44.649 with anxiety, and 1.169 with OCD. A significant and consistent increase in diagnoses, outpatient clinic visits, and inpatients was observed in the years leading up to the pandemic. However, starting from 2020, when restrictions began and the pandemic was widely felt, a notable decrease has been observed (Table 2).

Table 2. Data on individuals seeking psychiatry clinic in Çorum province for the years 2017-2022

Diagnosis	2017	2018	2019	2020	2021	2022
Depression (F33)	2.881	2.242	2.055	537	216	67
Anxiety (F41)	35.128	41.928	44.649	25.875	22.481	23.947
Obsessive Compulsive (F42)	954	1.167	1.169	681	767	559
Annual number of outpatient clinic visits	42.204	51.384	56.333	37.393	33.491	***
The annual number of inpatient-treated patients	551	604	660	406	527	***

***Due to changes in the hospital data system, data for this year could not be obtained

Before the pandemic, the consumption of antidepressant drugs was directly proportional to the prevalence of diseases (Table 1). Unexpectedly, starting in 2020, when restrictions began and uncertainties related to the disease were felt, consumption started to increase. In 2020 and 2021, the consumption of antidepressant drugs increased by approximately 20% compared to 2019. Consumption showed a lower increase in 2022, when all restrictions were lifted, compared to the previous year (Table 3). When the consumption of drug groups is examined, there is no significant change in the consumption of tricyclic and other antidepressants. The entire increase occurred in the consumption of SSRI and SNRI group drugs (Table 3).

When examining the data on the daily antidepressant dose per 1000 people in the workforce in OECD countries over the years, significant increases in antidepressant consumption were observed in 19 out of 31 countries (Table 4). Germany, Australia, Belgium, the Czech Republic, France, Hungary, Italy, Lithuania, Luxembourg, the Netherlands, and Norway did not experience a significant increase during the pandemic. On the other hand, Australia, Belgium, Canada, Denmark, Finland, Iceland, New Zealand, Portugal, Spain, Switzerland, and the United Kingdom had daily antidepressant doses per 1000 people that exceeded the OECD country average.

After the coronavirus was detected in China in December 2019, the first case in Türkiye was identified in March 2020 [23]. Depending on the effects of the coronavirus, severe symptoms can occur in some individuals, while others can fully recover without the need for treatment [24].

Table 3. Unit box consumption of antidepressant drugs in Türkiye by years [21]

ANTIDEPRESSANTS	2018	2019	2020	2021	2022
Escitalopram	10.529.619	10.144.714	11.744.525	12.869.289	13.728.810
Sertraline	8.148.558	8.733.827	9.852.838	11.253.207	11.803.761
Fluoxetine	5.552.252	5.539.824	5.925.600	6.876.494	7.469.335
Duloxetine	5.096.552	5.388.454	5.791.382	6.074.065	6.328.466
Paroxetine	3.978.307	4.127.676	4.842.143	5.086.292	4.898.636
Venlafaxine	3.942.414	4.210.894	4.445.134	4.606.524	5.026.684
Mirtazapine	2.068.344	2.169.126	2.460.106	2.623.603	2.641.811
Vortioxetine	802.571	994.818	747.482	770.555	857.893
Trazodone	2.030.914	2.069.637	2.115.082	2.291.318	2.132.211
Citalopram	1.928.396	1.790.880	1.897.539	1.928.562	1.865.714
Fluvoxamine	240.147	239.485	198.215	196.146	232.535
TOTAL SSRI and SNRI	**44.318.074**	**45.409.335**	**50.020.046**	**54.576.055**	**56.985.856**
Amitriptyline	1.580.025	1.305.409	1.441.041	1.651.184	1.623.194
Clomipramine	984.439	970.056	1.027.715	1.167.088	1.171.788
Lithium	450.415	563.760	539.668	559.555	536.249
Opipramol	712.549	551.584	553.432	508.978	464.895
Mianserin	327.279	335.846	372.811	384.284	236.678
Bupropion	186.855	265.806	232.860	336.825	352.290
Imipramine	170.035	180.070	160.607	158.384	160.242
Maprotiline	82.959	78.719	89.130	92.262	98.225
TOTAL TRICYCLIC and OTHERS	**4.494.556**	**4.251.250**	**4.417.264**	**4.858.560**	**4.643.561**
TOTAL MARKET	**48.812.630**	**49.660.585**	**54.437.310**	**59.434.615**	**61.629.417**
EXCHANGE		**1.6 %**	**9.6 %**	**9.2 %**	**3.7 %**

Table 4. Daily antidepressant dose per 1000 people in OECD countries by years [22]

OECD COUNTRIES	2017	2018	2019	2020	2021	2022
Australia	109.3	112.2	115.6	122.2	127.9	..
Austria	61	61.3	62	63.2	63.5	..
Belgium	78.8	79.7	81.9	83.8	86.2	..
Canada	104.4	108.4	114.1	122	130.1	134
Chile	42	40.1	52	67	90.7	94.3
Costa Rica	30.9	33.4	35.9	39	41.5	34.5
Czech Republic	59.9	61.4	64.4	65.7	69.4	..
Denmark	75.7	76.6	78.3	80.7	84.6	..
Estonia	28.8	31.9	34.8	37	40.7	44.5
Finland	70.5	74.9	78.3	81.5	85.2	..
France	51.3	51.5	54.4	54.5	57.6	..
Germany	56.9	58.5	60.3	62.2	64	..
Grecee	55.1	58	61	65.8	70.6	73.1
Hungary	28.8	29.3	29.5	30.4	29.8	30.3
Iceland	138.6	142	146	153.4	161.1	157.3
Israel	49.2	51.1	53.8	55.4	57.7	61.8
Italy	40.4	41.6	42.8	43.7	44.6	45.5
Korea	18.8	21	23.4	27.4	31.1	..
Latvia	15	16.1	17.6	19.8	21.5	24.3
Lithuania	32.1	31.3	35.4	36.8	37.4	..
Luxembourg	53	52.9	54.9	55.3	57.3	53.1
Netherlands	46.2	47.2	48	48.4	48.5	..

Table 4 (*continue*). Daily antidepressant dose per 1000 people in OECD countries by years [22]

OECD COUNTRIES	2017	2018	2019	2020	2021	2022
New Zealand	..	81.4	82.6	81.4	92	96
Norway	57.3	56.2	57.9	58.7	61.1	63.1
Portugal	103.8	109.3	123.7	131	138.8	150.5
Slovak Republic	39.4	41.1	42.4	42.9	44.7	..
Slovenia	59.5	61.5	63.3	63.6	66.1	68.4
Spain	77.2	80.4	83.6	86.9	92.7	98.4
Sweden	96.9	98.8	102.7	105.5	108.9	114.5
Türkiye	43.5	44.1	44.2	48.9	52.5	..
United Kingdom	107.9	116.5	123.9	131.7	138.2	..
Mean	59	61.5	64.6	67.6	71.75	

The COVID-19 pandemic, when contagious diseases rapidly spread and deaths increased, has negatively impacted health globally. Government policies also have similar effects, especially with psychological implications. Anxiety disorders, characterized by uncontrollable worry, are frequently reported diseases [25]. Before the pandemic, the prevalence of anxiety and depression disorders in Türkiye and worldwide was approximately the same and had not changed significantly (Table 1). However, it is believed that the prevalence changed in situations like the pandemic with uncertainties. Additionally, despite the Turkish population increasing by 2.97% from 80.8 million in 2017 to 83.2 million in 2019, there was no proportional increase in the frequency of depression and anxiety [26].

During the pandemic, measures were taken globally, including in Türkiye, to reduce and delay the spread of the virus [27]. Consequently, a series of measures have been implemented, including restrictions and prohibitions on international entries and exits, curfews, limitations on gatherings in crowded places such as restaurants, and the daily disclosure by the Ministry of Health of the number of individuals infected with and deceased from the virus, aiming to prevent misinformation. Moreover, many hospitals have been converted into pandemic hospitals [28]. These measures generally had adverse psychological effects on individuals [8]. The misinformation that emerged in the early stages of the pandemic was reported to increase stress and risk perception based on the fear of death. Even quarantine and isolation processes were considered a trauma, leading to cognitive disorders [29]. In a qualitative study conducted among individuals with psychiatric illnesses during the pandemic, participants mentioned an increase in the dosage of the medications they used. The study also included interviews where individuals reported a deterioration in their psychological state despite the prescribed medication, choosing not to stay in the hospital but continuing to take their medications [30]. However, an examination of applications to the psychiatric clinic in Çorum revealed a consistent increase in applications before the pandemic, followed by a sudden decrease in depression, anxiety, OCD, annual outpatient clinic numbers, and hospitalized patient numbers during the pandemic (Table 2). This could be attributed to the fact that the only state hospital in Çorum served as a pandemic hospital and people's fear of infection, leading them to avoid going to the hospital and the restrictions in place.

Before the pandemic, the consumption of antidepressant drugs was directly proportional to the prevalence of diseases (Table 1). However, during the pandemic, a significant increase was observed in the use of SSRI and SNRI group drugs, unlike the period before the pandemic (Table 3). However, during these years, the population of Turkey has increased by a rate of 1.8% [26]. This increase in drug consumption is not proportional to population growth; in fact, there was a decrease in the number of polyclinics in inpatient health institutions during this period (Table 2). Therefore, it is thought that the deaths and restrictions during the pandemic influenced drug consumption, and an increase in prevalence rates is expected in the future. In the future, it is anticipated that there will be a regression in prevalence rates. All the drugs included in the study are controlled medications that should be prescribed with a white prescription by the Ministry of Health of the Republic of Turkey. The increased consumption of drugs, particularly those belonging to the SSRI and SNRI groups, can be attributed to their more accessible prescription by family physicians [31]. Additionally, studies in the literature indicate the genotoxic effects of tricyclic group drugs. At the same time, there is evidence suggesting that drugs

belonging to the SSRI and SNRI groups are more reliable compared to tricyclic drugs [32]. On the other hand, the Social Security Institution has extended the durations of chronic medication usage reports during the pandemic, allowing community pharmacists to issue continuation prescriptions based on these reports [33]. A study indicated that such practices by community pharmacists generally lead to an excessive increase in drug consumption and give rise to unethical situations [34]. Furthermore, the observed decline in the consumption of tricyclics and other classes of antidepressants raises the possibility of prescription migration from inexpensive to more expensive drugs following manufacturers' marketing activities.

The COVID-19 pandemic has manifested its impact worldwide; however, these effects vary from individual to individual. As societies experience differing levels of impact internally, variations in these effects are also observed at the country level. This situation is evident in the significant increase in antidepressant drug consumption in some countries, while similar increases may not be observed in certain societies (Table 4). Furthermore, Chile, which had antidepressant consumption rates below the OECD average, has surpassed the OECD average in the year 2021 (Table 4). In a society like Italy, with a high incidence of deaths and lockdowns, the absence of any increase is noteworthy (Table 4). Studies indicate that the occurrence or absence of these increases may be influenced by factors such as the health systems of countries, policies implemented during the pandemic, death and infection rates, and cultural impact. Individuals can exhibit biological responses to diseases, and societies also have cultural responses. A virus pandemic affecting the entire world is anticipated to lead to significant social changes and the emergence of new normative orders. Furthermore, it can be mentioned that culture impacts the emergence of behaviors when threatened [35]. Therefore, it is considered that the impact of all mentioned factors may contribute to the emergence of differences. In this context, the support services provided to their societies during the pandemic by states such as Italy, Germany, Hungary, and Austria should be examined. When examining the data on the daily antidepressant dosage per 1000 people in OECD countries, another notable observation is the return to 2022 figures in the data for Costa Rica, Iceland, and Luxembourg. This regression is an expected phenomenon and is anticipated to occur in other countries in the coming years.

However, it should be noted that these consumption figures, especially for Turkey, represent only the data related to drugs prescribed and used upon consultation with a doctor. It is also considered that many individuals experiencing psychological problems during this period without seeking medical help or using medication are not accounted for.

In conclusion, processes such as pandemics that affect and restrict the daily lives of societies vary in terms of the psychological responses people give based on the socio-cultural structures of the societies, the content of the measures taken by governments, and the economic support provided. Antidepressant consumption in Turkey has been influenced during the pandemic period, and an increase has occurred beyond what was expected.

AUTHOR CONTRIBUTIONS

Consept: E.B., Design: H.D.K., E.B., E.K.; Control: E.B.; Sources: H.D.K.; Data Collection and/or Processing: H.D.K., E.B., E.K.; Analysis and/or Interpretation: H.D.K., E.B.; Literature Review: H.D.K., E.K.; Manuscript Writing: H.D.K., E.B., E.K.; Critical Review: E.B.; Others: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DETERMINATION OF DNA DAMAGE INDUCED BY BISPHENOL A AND BISPHENOL S IN MCF7 CELL LINE

BİSFENOL A VE BİSFENOL S'NİN MCF7 HÜCRE HATTINDA NEDEN OLDUĞU DNA HASARININ ARAŞTIRILMASI

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ABSTRACT

Objective: *This study aimed to determine the DNA damage induced by Bisphenol A (BPA) and Bisphenol S (BPS) on MCF7 cell line.*
Material and Method: *DNA damage was determined by COMET assay in MCF7 cell line at 0.1, 0.5, 1, 5, 10 and 50 µM concentrations of BPA and BPS.*
Result and Discussion: *All BPA and BPS concentrations studied (0.1, 0.5, 1, 5, 10 and 50 µM) significantly induced DNA damage on MCF7 cell line compared with control ($p<0.05$). BPS significantly induced DNA damage more than BPA at the 3 highest concentrations studied (5, 10 and 50 µM) ($p<0.05$). This study shows that bisphenol derivatives can also cause DNA damage like BPA.*
Keywords: *Bisphenol A, bisphenol S, COMET assay, DNA damage, MCF7 cell line*

ÖZ

Amaç: *Bu çalışmada, Bisfenol A (BPA) ve Bisfenol S (BPS)'nin MCF7 hücre hattında neden olduğu DNA hasarının belirlenmesi amaçlanmıştır.*
Gereç ve Yöntem: *DNA hasarı BPA ve BPS'nin 0.1, 0.5, 1, 5, 10 ve 50 µM konsantrasyonlarda MCF7 hücre hattında COMET yöntemi ile belirlenmiştir.*
Sonuç ve Tartışma: *Çalışılan tüm BPA ve BPS konsantrasyonları (0.1, 0.5, 1, 5, 10 ve 50 µM) MCF7 hücre hattında kontrole kıyasla önemli ölçüde DNA hasarına neden olmuştur ($p<0.05$). BPS, çalışılan en yüksek 3 konsantrasyonda (5, 10 ve 50 µM) DNA hasarını BPA'dan daha fazla indüklemiştir ($p<0.05$). Bu çalışma bisfenol türevlerinin de BPA gibi DNA hasarına neden olabileceğini göstermektedir.*
Anahtar Kelimeler: *Bisfenol A, bisfenol S, COMET testi, DNA hasarı, MCF7 hücre hattı*

INTRODUCTION

Bisphenol A (BPA) is among the most produced chemicals worldwide. BPA was first synthesized by Dianin in 1891 and was extensively researched in the 1930s during studies to produce synthetic

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estrogen. After this date, it was suggested that BPA could be used to make plastics, and in the 1940s it began to be used in resin production. The polymerization of BPA by Bayer and General Electric was discovered in 1957, leading to the use of polycarbonate for beverage and food packaging. This development led to a rapid increase in the use of BPA in plastic making, making it the most widely used commercial product in the world [1,2]. As a result, the widely used BPA monomers, polycarbonate (PC), were found to be released from plastics into the ecosystem and food [3,4]. Various reports indicate that BPA in plastic food containers, paper money, personal care products, and toys may cause reproductive, developmental, and carcinogenic effects. Many *in vivo* and *in vitro* studies demonstrated that BPA negatively affects human health with its endocrine-disrupting effects [5-7].

BPA acts as an endocrine disruptor that alters the histological structure of cells and causes biochemical and physiological changes that modify the functions of tissues and organs. When it is looked at its effects in the reproductive system, it is seen it shows a weak estrogenic effect by binding to estrogen receptors and that the main target is ovarian granulosa cells. Disruption of these cells by BPA plays an important role in fertility. As MCF7 is estrogen-positive, it is considered one of the most suitable cell lines to study bisphenols that mainly affect the reproductive system. In addition, it interacts with androgen receptors, peroxisome proliferator active receptors, and other endocrine system receptors [8,9]. BPA can bind to androgen receptors by acting like androgen and can cause specific changes in gene expression. Considering its effect on the androgen receptor, BPA is a known antagonist. It slows down nuclear transport and forms non-functional foci in the nucleus [10,11].

According to the report that FDA (Food and Drug Administration) released in 2010, fetuses, infants, and children may develop brain, behavioral, and prostate abnormalities if they are exposed to BPA in their early years. Several states have banned the use of BPA since then. In 2011, the European Union (EU) banned BPA-containing baby bottles and in 2013, a maximum allowable dose level (MADL) of 290 micrograms per day has been established for BPA exposure by the Office of Environmental Health Hazard Assessment (OEHHA) [12,13].

The demonstration of the toxicity of BPA in numerous studies has encouraged the industry to search for alternative chemicals. As a result, manufacturers started to remove this compound from their BPA-containing products and gradually transitioned to the use of bisphenol analogs, such as Bisphenol S (BPS), Bisphenol AF (BPAF), Bisphenol Z (BPZ), and Bisphenol F (BPF). However, these analogs are still bisphenols and have the potential to have toxic effects similar to BPA. Toxicological information on the endocrine-disrupting potential of these compounds is limited and little is known about their toxicity. Particularly in recent years, the use of the least toxic bisphenol compound in food contact products has been emphasized [14,15].

Among these analogs, the use of BPS has become increasingly common in recent years due to its resistance to high temperatures and sunlight and its lower toxicity. BPS is widely used in many industrial areas for cleaning purposes, in "BPA-free" thermal papers, as a primer, especially in pipes to increase thickness and durability, in industrial floors, on the tops of roads and bridges, and in epoxy resin construction and coatings. BPS is found in many personal care products used in daily life such as body gels, hair care products, make-up, lotions, and toothpaste, paper products such as money, tickets, flyers, airplane boarding cards, dairy products, vegetables, boxed foods, and human exposure to bisphenols continues [15,16].

Although products such as water bottles, baby bottles, toys, and personal care products have remarkable labels such as "BPA free", these products contain bisphenols. In addition, the toxicity profiles of these newly introduced bisphenol analogs have not been fully elucidated. There are many studies on BPA, but detailed toxicological investigations of other bisphenol analogs should be carried out and shown if they have similar toxic effects as BPA and should be regulated to the limit values of use by legal authorities. It is important to raise public awareness and inform the producers and consumers about this issue. For this purpose, in this study, DNA damage caused by BPA and BPS on MCF7, a breast cancer cell line, was studied by COMET assay.

MATERIAL AND METHOD

Chemicals

Hydrogen peroxide (H_2O_2), phosphate-buffered saline (PBS), bisphenol A (BPA), bisphenol S (BPS), and low melting point agarose (LMPA) were obtained from Sigma-Aldrich (Germany). Fetal bovine serum (FBS) was bought from Biological Industries (Israel). Dulbecco's Modified Eagle's Medium (DMEM) and trypsin were products of Sartorius (Israel). Dimethyl sulfoxide (DMSO) was bought from Serva (USA). Sodium hydroxide (NaOH) and triton-X 100 were purchased from Merck (Germany). Penicillin/Streptomycin (Pen/Strep) was obtained from Gibco (USA) and sodium chloride (NaCl) was from Zag Kimya (Türkiye). Disodium ethylenediaminetetraacetic acid (Na_2EDTA) and tris were purchased from VWR Chemicals (USA). Normal melting point agarose (NMPA), ethidium bromide and sodium sarcosinate were bought from Amresco (USA).

COMET Assay

As a preliminary study, cytotoxicity assays of BPA and BPS at concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, and 500 µM were performed in order to assess cell viability in MCF7 (ATCC® HTB-22™) cell line for 24 h [17].

The standard method [18] was the foundation for the alkaline COMET assay, with a few minor adjustments. COMET assay was performed in 6-well plates at 2 x 104 /2 ml cells/well. BPA and BPS solutions were prepared with sterile DMSO. Based on the preliminary study, concentrations below the IC_{50} values of 0.1, 0.5, 1, 5, 10, and 50 µM BPA and BPS were applied to wells. H_2O_2 at 50 µM served as the positive control. Since DMSO was used as a solvent for bisphenol compounds, a control containing 0.1% DMSO was used100 µl of melted LMPA (0.5%) was combined with 50 µl of cell suspension (1-2 x 104 cells/slide) at 37˚C. After spreading cell suspensions with 1% NMPA on the pre-coated slides, a coverslip was placed over them. The agar was left to firm for about five minutes on a flat, ice-cold tray. A cold lysing solution (10 mM Tris, 2.5 M NaCl, 100 mM Na_2EDTA, 1% sodium sarcosinate, 1% Triton-X 100, 10% DMSO, pH 10.0) was prepared ahead of time, and the slides were submerged in it for at least an hour at 4˚C. An adequate amount of cold electrophoresis solution (1 mM Na_2EDTA, 300 mM NaOH, pH=13) was added to the electrophoresis tank. The slides that had been taken out of the lysing solution were put in the electrophoresis tank, where they were electrophoresed for 20 minutes at 25 V and 300 mA after being left in this solution for 20 minutes to allow for denaturation. The electrophoresed slides were removed from the electrophoresis tank and then rinsed three times for five minutes each in a neutralizing solution (0.4 M Tris, pH 7.5). The cells were fixed on the slides with alcohol and stored in a humid condition until analysis. Following 10 minutes of staining with 60 µl (20 µg/ml) ethidium bromide solution, slides were analyzed under a microscope. A Leica DM 1000 fluorescent microscope was used to examine 100 randomly chosen cells per slide at 40x magnification., and the COMET Assay IV software (Perceptive Instruments, UK) was used to count the cells. One researcher scored the DNA damage, and the damage was reported as a mean tail intensity percentage. COMET assays were performed in duplicate at different times.

Statistical Analysis

The SPSS software (SPSS Windows Release 23.0, SPSS Inc., USA) was used to conduct statistical analyses. The findings were presented as mean ± standard error of mean (SEM). DNA Tail Intensity values of all 100 cell counts for each sample were used as control and test chemicals groups in the evaluation of COMET assay results and were evaluated by one-way analysis of variance (ANOVA). Fischer's least significant difference test (LSD) was utilized for post-hoc analysis (comparison among groups). p-value lower than 0.05 ($p<0.05$) was regarded as statistically significant.

RESULT AND DISCUSSION

In the preliminary study, BPA and BPS concentrations that reduce cell viability by 50% (IC_{50}) for MCF7 cell line were calculated as 45 µM and 450 µM, respectively [17].

To assess genotoxicity, one researcher randomly selected 100 cells per sample and scored them using COMET Assay IV Software. The mean % tail intensity values of BPS and BPA were used to evaluate the COMET assay (Figure 1).

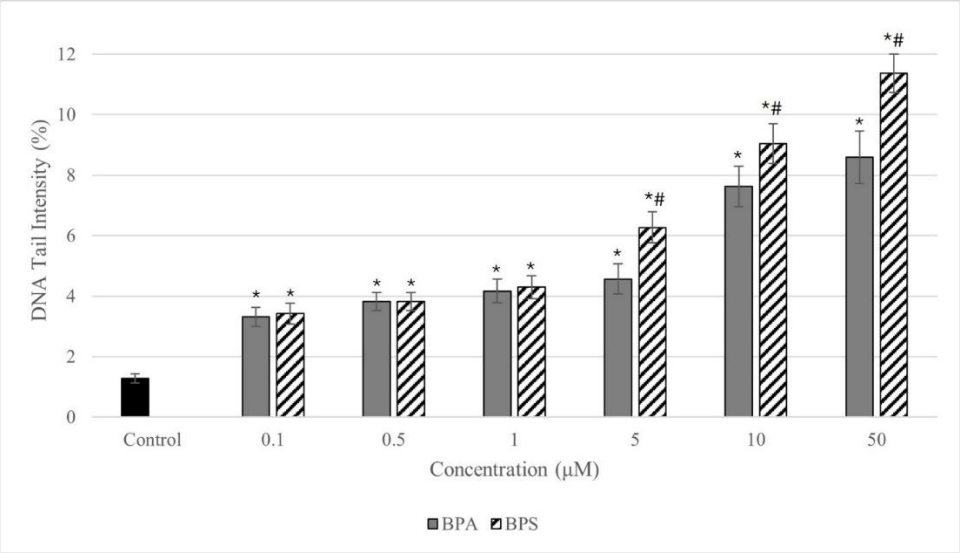

Figure 1. DNA damage in MCF7 versus increasing concentrations of BPA and BPS. The mean tail intensity of the groups was compared using a one-way ANOVA (n=100) (control: solvent (0.1%DMSO), BPA: Bisphenol A, BPS: Bisphenol S. *p<0.05: statistically significant vs control # p<0.05: statistically significant vs BPA at the same concentrations

Mean tail intensity results of cells that were exposed to BPA and BPS at increasing doses are shown in Table 1. When compared to the control, all studied doses of BPA, BPS, and 50 µM H_2O_2 significantly caused DNA damage in MCF7 (p<0.05). BPS significantly induced DNA damage more than BPA at the 3 highest concentrations studied (5, 10, and 50 µM).

Table 1. % DNA Tail Intensity results of chemicals on MCF7 cell line

Chemicals	Concentrations (µM)	% DNA Tail Intensity ± SEM
Control		1.28±0.1513
BPA	0.1	3.31±0.3132*
	0.5	3.82±0.3006*
	1	4.17±0.3898*
	5	4.57±0.4982*
	10	7.62±0.6732*
	50	8.59±0.8653*
BPS	0.1	3.42±0.3438*
	0.5	3.82±0.3005*
	1	4.30±0.3750*
	5	6.27±0.5177*#
	10	9.04±0.6559*#
	50	11.37±0.6394*#
H_2O_2		25.2166±9.4795*

*Concentrations that significantly induced DNA damage in MCF7 compared with control (p<0.05)
#Concentrations that significantly induced more DNA damage in MCF7 compared with BPA at the same concentrations (p<0.05)

In a study by He et al. investigating the role of brain-derived neurotrophic factor signaling pathway in BPS-induced cytotoxicity in human neuroblastoma cell line (SK-N-SH), cells were exposed to BPS at concentrations of 100, 200, and 300 µmol/L and cell viability was examined. It was observed that cell viability decreased in a dose-dependent manner and cell morphology was changed. As a result of 24 hours of BPS exposure in this cell line, IC_{50} value was calculated as 285.84 µmol/L. It was also observed that the apoptosis rate increased in cells in a dose-dependent manner [19]. Feng et al. investigated the potential endocrine-disrupting effects of bisphenol A and its derivatives on human adrenocortical carcinoma cell line (H295R) and exposed cells to BPA, BPS, BPF, and BPAF at increasing doses of 10-500 µM for 24 hours, 48 hours and 72 hours. The viability of cells was measured using the Cell Counting Kit-8 (CCK-8) and it was observed that cytotoxicity increased as exposure time and concentrations increased. After 72 hours of exposure, the LC_{50} (Lethal Concentration 50) value was calculated as 103.4 µM for BPA and 159.6 µM for BPS and it was reported that BPA was more cytotoxic than BPS [20]. These findings support our study which we also found that BPA was more cytotoxic than BPS. In a study investigating the levels of cytotoxicity, reactive oxygen species (ROS), and DNA damage due to BPA and BPS exposure in human bronchial epithelial cells (BEAS-2B), cells were exposed to BPA and BPS at concentrations of 12.5, 25, 50, 100 and 200 µM. To measure cell viability, MTS assay was performed after 24 hours of exposure and IC_{50} values were calculated above 200 µM for both bisphenol derivatives. In the COMET assay to examine DNA damage, tail intensities were measured, and it was found that BPA induced DNA damage more than BPS. They stated that this may be due to increased ROS production [21]. In another research, human peripheral blood mononuclear cells (PBMCs) were exposed to BPA, BPF, BPAF, and BPS, and alkaline and neutral COMET assays were performed as a result of 1-hour and 4-hour exposures. In the alkaline COMET assay, BPA induced DNA damage at concentrations of 0.1, 1, and 10 µg/ml after 1 h exposure and at concentrations of 0.01, 0.1, 1, and 10 µg/ml after 4 h exposure; whereas BPS induced DNA damage only at a concentration of 10 µg/ml after 4 h exposure. It was discovered that BPA damaged DNA in the neutral COMET assay at concentrations of 1 and 10 µg/ml following a 1-h exposure.; however, BPA and BPS only caused DNA damage at a dose of 10 µg/ml after 4-h exposure. As a result of these studies, it was stated that the genotoxic potential of BPA was higher than that of BPS [22]. However, in our study we found that BPS was more genotoxic than BPA, especially at 5, 10 and 50 µM. BPA, BPS and other bisphenol derivatives were used in a study investigating the mutagenicity and DNA damage of bisphenol derivatives on HepG2 cell line. Using Salmonella typhimurium strains TA98 and TA 100, the AMES test was used to examine the mutagenicity of bisphenol derivatives. At dosages of 0.004, 0.02, 0.1, and 0.5 mg, the test revealed no mutagenic activity. When using MTT as a test for cell viability at 24 hours of exposure, there wasn't any decrease in viability at concentrations of 12.5, 25, 50, and 100 µmol/L. Genotoxicity potentials were studied at concentrations of 0.1, 1, and 10 µmol/L by COMET assay, and DNA strand breaks were observed at all studied concentrations of BPA and 0.1 and 10 µmol/L concentrations of BPS in 24 h exposure [23]. In the study by Kose et al. on the toxicity of BPA, BPF, and BPS on prostate cell line (RWPE-1), cell viability was determined by MTT test, and IC_{50} values were calculated as 113.74, 249, and 380.90 µM, respectively. IC_{20} values for BPA, BPF, and BPS were calculated as 45, 65, and 108 µM, respectively, and these concentrations were used in the alkali COMET assay. Genotoxic potentials were observed as BPS > BPF > BPA [24]. These results support our findings that even though BPA is more cytotoxic than BPS, when DNA damage is investigated, BPS seems to be more genotoxic than BPA. In a study examining whether BPS causes epigenetic changes in MCF7 cell line, cells were exposed to 1 mM, 100 nM and 10 nM BPS for 24 hours. At the end of the experiment, it was observed that BPS induced DNA methylation [25]. However, it is seen that there were no studies examining the direct DNA damage caused by BPS on MCF7 cell line.

In conclusion, the chemical compound BPA is widely produced and used in plastics. After its use in food and beverage packages, BPA's widespread use increased, and it was later found to have endocrine-disrupting properties. After the increase in research on BPA and the confirmation of its toxic effects, the use of BPA was restricted and banned in most countries around the world. After these bans, the industry started to search for derivatives that could replace BPA. With the introduction of BPA derivatives into industrial use, it has been a matter of debate whether BPS, one of the most widely used derivatives, shows toxic effects like BPA. Our study aims to shed light on this issue. Our study showed

that although BPA was more cytotoxic than BPS, at high concentrations BPS was more genotoxic than BPA. Further studies on the toxicity of bisphenol derivatives are needed to determine whether they are safe to use.

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AUTHOR CONTRIBUTIONS

Concept: B.K.G., Y.D., A.U.; Design: A.U.; Control: B.K.G., Y.D., A.U.; Sources: E.E., A.U.; Materials: Y.D., A.U.; Data Collection and/or Processing: E.E., A.U.; Analysis and/or Interpretation: E.E., S.I.T.; Literature Review: E.E., S.I.T., A.U.; Manuscript Writing: E.E., A.U.; Critical Review: E.E., S.I.T., B.K.G., Y.D., A.U.; Other: E.E., S.I.T., B.K.G., Y.D., A.U.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that ethics committee approval is not required for this study.

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ÖZGÜN MAKALE / ORIGINAL ARTICLE

TOPLUM ECZANELERİNDE İŞ ANALİZİ ÜZERİNE BİR ÇALIŞMA: ANKARA ÖRNEĞİ

A STUDY ON JOB ANALYSIS IN COMMUNITY PHARMACIES: CASE OF ANKARA

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ÖZ

Amaç: *İş analizi; bir örgütteki işlerin görev ve sorumluluklarının sistematik olarak incelendiği ve araştırıldığı bir yöntemdir. Bu çalışmada amaç, toplum eczanelerinde; eczacının sorumluluklarından olan hasta danışmanlığı ile ilgili işler ile idari/yönetsel işleri belirlemek ve bu işlere ayrılan zaman ile işlerin kimler tarafından yapıldığını tespit ederek eczane yönetim verimliliğini artıracak tavsiyeler sunmaktır.*
Gereç ve Yöntem: *Bu çalışma betimleyici tipte tarama araştırmasıdır. Bu nicel araştırmada veri toplama aracı olarak anket yöntemi kullanılmıştır. Çalışma için öncelikle toplum eczacılarıyla görüşmeler yapılmış ve eczacıların günlük, haftalık ve aylık rutinde yaptıkları işler tespit edilerek oluşturulan görev envanteri anketi hazırlanmıştır. Anket az sayıda eczacı üzerinde uygulandıktan sonra son haline getirilerek Ankara'da bulunan toplum eczacılarına e-posta yoluyla ulaştırılmıştır.*
Sonuç ve Tartışma: *Çalışma sonuçlarına göre, eczacıların eczanede en çok vakit ayırdıkları işin hasta danışmanlığı olduğu belirlenmiştir. Eczanede çalışan personel, en çok idari işlerde katkı sunarken, eczacıya hasta danışmanlığında da yardımcı olmaktadır. Çalışma sonuçları, eczacıların ve özellikle toplum eczacılığı yapmayı planlayan eczacılık fakültesi öğrencilerinin eczanelerini verimli bir şekilde yönetebilmeleri için çok önemlidir. Öğrencilere, bir toplum eczanesinde ne tür işlerin kendilerini beklediğini, bu işlerin hangi sıklıkla gerçekleştiğini ve bu işlere ortalama olarak ne kadar süre ayrıldığını göstermektedir.*
Anahtar Kelimeler: *Eczacı, eczane personeli, iş analizi, toplum eczanesi*

ABSTRACT

Objective: *Job analysis is a method in which the duties and responsibilities in an organization are examined and researched systematically. The aim of this study is to determine both the workload of the pharmacist including the consultation to the patients and administrative works in community pharmacies, and the time allocated to these responsibilities. We are presenting advices which increase the productivity of the management.*
Material and Method: *This study is descriptive-survey research. In this research, the survey method was used as a data collection tool. A questionnaire was prepared for the study, which was created by conducting interviews identifying the tasks that the pharmacists do in their routine. The survey was applied to a small number of pharmacists. The final version was sent by e-mail to pharmacists located in Ankara.*

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Result and Discussion: *It was determined that pharmacists spend most of their time in the pharmacy on patient counseling. Pharmacy staff assist the pharmacist with counseling, but mostly with administrative work. This study is important for the students who plan to be community pharmacists to be able to manage their pharmacies efficiently. It shows that the work awaits them in a community pharmacy and the time to do the work in the pharmacy on average basis.*
Keywords: *Community pharmacy, job analysis, pharmacist, pharmacy staff*

GİRİŞ

İş analizi, bir meslek ile ilgili bilgi toplama ve bu bilgilerin analiz edilmesinin sistemli bir yöntemi olarak tanımlanmaktadır. İş analizi, işgören seçme sistemleri, eğitim programları, performans yönetimi ve tazminat sistemlerini de içeren birçok önemli insan kaynağı yönetim sistemini düzenlemesinde etkilidir [1]. Meslekleri incelemenin sistematik bir yöntemi ve mesleki bilgileri toplama aracı olan iş analizinde, iş ile ilgili bilgiler, işin mümkün olan en kısa sürede ve en verimli şekilde tamamlanması amacıyla da toplanmaktadır. Bir işletmede iş analizinden elde edilen veriler, etkili insan kaynakları yönetimi için tasarlanacak programların geliştirilmesinde ve yönetiminde kullanılmaktadır [2].

İş analizi, bir işi diğer işlerden ayıran özellikleri de ortaya çıkaran bir yöntemdir. Düzenli aralıklarla iş analizi yapmak, personelden daha iyi yararlanmayı sağlamaktadır [3]. İş analizleri bir kez yapılıp sistemde ya da dosyalarda unutulacak çalışmalar değildir ve sürekli yenilenmesi gerekmektedir [4].

İş analizi, işletmedeki çalışanların iş ortamındaki çalışmalarının tarafsız bir şekilde ölçümüyle yapılmalıdır. İşle ilgili bilgileri, araştırmacılar görüşme veya anket yoluyla doğrudan çalışan kişiden ya da yöneticisinden öğrenebilir veya gözlem yoluyla elde edebilirler. İşletmedeki birbirinden farklı tüm işlerin analizlerinin yapılması önemlidir. Aynı işi yapan farklı kişiler olduğunda, bir kişinin yaptığı işin incelenmesi genellikle yeterlidir. İş analizi yapılırken genellikle aşağıdaki aşamalar izlenmektedir [5].

- Analiz edilecek işlerin belirlenmesi,
- Soru formunun hazırlanması,
- Bilgi toplanması,
- Gözlem yapılması,
- Bilgilerin tasniflenmesi, değerlendirilmesi,
- İş analiz bilgilerinin kullanılması.

İş analizinden elde edilen veriler, çalışanların sorumlu oldukları işleri açık bir şekilde anlamalarında ve işleriyle alakalı yaşadıkları tereddütlerini azaltmakta da kullanılır. İş analizi personel yönetimi faaliyetlerinde teknik işlevlerin başında gelmektedir [6-7]. İstenen verimliliğin sağlanmasında, işletmelerin iyileştirme yöntemini belirlerken uygulamaya geçmeden, iş analizi sonuçları ve iş süreçlerinin her işletmede birbirinden farklı olacağını gözden kaçırmamaları gerekmektedir. İş analizi iş yükü çalışmalarının da temelini oluşturur. İşlerin organizasyonu insan kaynakları yönetiminin bir işlevi olarak ortaya çıkmıştır [8,9].

Personel yönetimi işlevleri toplum eczanelerinde son yıllarda artan sorumluluklar nedeniyle giderek önem kazanmaktadır. Hangi görevlerin hangi personelin sorumluluğunda olduğunun kesin olarak ortaya konması ile personelden beklentiler açık hale gelir ve eczanede sunulan hizmetin kalitesi artar. İş analizi ve iş yükü ile ilgili bilimsel çalışmalarının takibi ve toplum eczanesine uygun olanların uygulanması öncellikle toplum eczanesi mesul müdürü olan eczacının sorumluluğundadır ve bu çalışmaların eczanede çalışacak personel tarafından takip edilmesi önemlidir [8,9].

Bu bağlamda, bu çalışmanın amacı, toplum eczanelerinde Türk Eczacılık Mevzuatına göre, eczacının sorumlulukları içinde yapılan tüm mesleki ve idari/yönetsel işleri belirlemek, bu işlere ayrılan zamanı ve işlerin kimler tarafından yapıldığını ortaya çıkarmak, iş analizi yapmaktır.

GEREÇ VE YÖNTEM

Bu çalışma betimleyici tipte bir araştırmadır. Bu nicel araştırmada veri toplama aracı olarak anket yöntemi kullanılmıştır.

Bu çalışma için öncelikle toplum eczacılarıyla görüşmeler yapılarak, eczacıların günlük, haftalık ve aylık rutinde yaptıkları işler tespit edilmiştir. Görüşmeden elde edilen bilgilerle bir iş analizi yöntemi olan görev envanteri anketi hazırlanmıştır. Anket soruları, Türk eczacılık mevzuatı da dikkate alınarak araştırmacılar tarafından hazırlanmış, soruların hazırlanması öncesi ve sonrasında uzman kişilere danışılmıştır.

Anket formu dört bölümden oluşmaktadır. Birinci kısımda eczacıların demografik bilgileri, ikinci kısımda eczane personeli ile ilgili sorular, üçüncü kısımda eczacıların hasta danışmanlığı kapsamında yaptıkları işler ve son kısımda toplum eczanelerinde yapılan idari işler ile ilgili sorular yer almaktadır.

Ankara Üniversitesi Sağlık Bilimleri Etik Kurulu'nun 29.01.2018 tarih ve 38 sayılı kararı ile etik izin alındıktan sonra, 2018 yılı şubat ayında anket 25 eczacı üzerinde uygulanmış ve son haline getirilmiştir. Son halini alan anket, Türk Eczacıları Birliği Eczacı Bilgi Sistemi'nde yer alan verilere istinaden Ankara merkezde faaliyet gösteren 2300 eczacının e-posta adreslerine gönderilmiştir. Geriye dönen 104 anket formu değerlendirmeye alınmıştır. Elde edilen veriler; SPSS Versiyon 23.0'da değerlendirilerek incelenmiştir. Eczacıların toplum eczanelerinde yaptıkları hasta danışmanlığı ile ilgili işler ve idari işlerin (reçete kontrolü, stok kontrolü, miad kontrolü vb) eczane konumuna göre karşılaştırılması Ki-kare testi ile yapılmıştır.

SONUÇ VE TARTIŞMA

Bu çalışmada 104 eczacının anket formları değerlendirmeye alınmıştır. Eczacıların %56'sı kadın, %44'ü erkeklerden oluşmaktadır. Çalışmaya katılan eczacıların çoğu 11-20 yıl (%31) veya 20 yıl üzerinde (%30) çalışma deneyimine sahipken, diğer kısmı 0-5 yıl (%24) ve 6-10 yıl (%15) deneyime sahiptir. Çalışmaya katılan eczacıların büyük bir kısmı Aile Sağlık Merkezi yakınında (%38.5) ya da semt eczanesi (%35.6) olarak hizmet verirken, geriye kalan eczacılar cadde üzerinde (%24), büyük sağlık kuruluşu yakınında (%16.3) ve alışveriş merkezi içinde (%3.8) hizmet vermektedir.

Ankete katılan eczacıların çoğunun eczanesinde 1 (%32.7) ya da 2 (%35.6) personel çalışırken, bir kısmında 3 (%14.4), 4 (%7.7) ve 5 (%5.8) personel çalışmakta, pek azının (%3.8) eczanesinde ise personel çalışmamaktadır. Ankete katılan eczacıların çoğu (%41.3) personelleri arasında görev dağılımı olmadığını, bir kısmı (%29.8) esnek bir görev dağılımı olduğunu, diğer kısmı (%28.8) ise görev dağılımı olduğunu belirtmiştir. Görev dağılımı bulunan eczanelerde, reçete karşılama, ilaç dışı ürün, temizlik vb. işlerin farklı personel tarafından yürütüldüğü belirtilmiştir.

Eczacıların pek çoğu (%86), çalışma saatleri içinde vakit buldukça personeline sorumlu oldukları işler konusunda eğitim verdiğini, bir kısmı ise (%11.5) eğitim vermediğini ifade etmiştir. Ufak bir kısım (%5.8) zaman zaman eksik görülen noktalarda süre farketmeksizin eğitim verdiğini, pek azı ise (%2) çalışma saatleri dışında özel vakit ayırarak personeline eğitim verdiğini belirtmiştir. Personel eğitimine ayrılan günlük ortalama sürenin; %44.2 oranında 5-15 dakika, %26.9 oranında 16-30 dakika, %6.7 oranında 31-45 dakika, %4.8 oranında 46 dakika-1 saat olduğu belirlenmiştir.

Bir eczacının gün içerisinde en fazla zaman ayırdığı iş, hasta danışmanlığıdır. Çalışmaya katılan eczacıların hizmet sunarken hangi hastalık gruplarında ve konularda danışmanlık yaptıkları ve bu iş için bir günde ayırdıkları süreler Tablo 1'de sınıflandırılmıştır.

Eczanede hasta danışmanlığı konusunda eczacıya yardımcı olan kişi büyük oranda (%67.3) eczane yardımcı personeli ve az oranda (%2.9) ikinci eczacı olmakla beraber; bir kısmı ise (%29.8) bu konuda kendisine yardımcı olan bir personel olmadığını ifade etmiştir.

Çalışmaya katılan eczacıların çoğu haftada bir (%31.7) ve ayda bir (%24) bir kısmı 2-3 günde bir (%22.1), az bir kısmı her gün (%12.5) ve pek az bir kısmı çok az majistral ilaç hazırladığını (%4.8) ve majistral ilaç hazırlamadığını (%4.8) belirtmiştir. Majistral bir ilaç hazırlamanın ortalama olarak; eczacıların çoğunun (%47.1) 15-30 dakika ve (%34.6) 15 dakikadan az, bir kısmının (%10.6) 31-45 dakika, pek azının ise (%2.90) 46 dakika- 1 saat zamanını almakta olduğu belirlenmiştir. Veteriner ve tarım ilaçları hakkında eczacıların çoğunun (%88,5) danışmanlık vermediği ortaya çıkmıştır. Danışmanlık verenlerin ise; bir kısmı (%1.9) 31 dakika-1 saat, bir kısmı (%1) 15-30 dakika ve pek çoğu da (% 8.7) danışmanlığa 15 dakikadan az zaman ayırdığını belirtmişlerdir.

Anketimizin son bölümünde ise eczanede yapılan idari işlemler ile ilgili sorular sorulmuştur. Eczanede rutin olarak yapılan idari işler reçete kontrolü, SGK'ya (Sosyal Güvenlik Kurumu) aylık fatura

kesimi, stok kontrolü, miad kontrolü, temizlik ve ilaç ve ilaç dışı ürünlerin siparişleri olup bu işleri yapan kişilerin dağılımı Tablo 2'de belirtilmiştir. Bu soruda eczacılar birden fazla seçenek işaretleyebilmişlerdir.

Tablo 1. Eczacıların hizmet sunarken hangi hastalık gruplarında ve konularda danışmanlık yaptıkları ve bu iş için ayırdıkları sürelerin dağılımı

	Danışmanlık Vermiyoruz		30 dakikadan az		30-59 dakika		1-2 saat		2-3 saat	
Hastalık Grupları ve Danışılan Konular	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**
Kronik Hastalıklar	3	%2.9	17	%16.3	24	%23.1	31	%29.8	28	%26.9
Akut Hastalıklar	5	%4.8	20	%19.2	23	%22.1	33	%31.7	20	%19.2
Sürekli Hastalar	5	%4.8	26	%25	31	%29.8	17	%16.3	24	%23.1
Geleneksel ve Alternatif Tedaviler	52	%50	37	%35.6	7	%6.7	3	%2.9	3	%2.9
Hafif Rahatsızlıklar	2	%1.9	32	%30.8	31	%29.8	28	%26.0	9	%8.7
Yaşam Kalitesi	7	%6.7	57	%54.8	26	%25	9	%8.7	5	%4.8
Tıbbi Cihaz ve Malzeme	27	%26.0	56	%53.8	15	%14.4	4	%3.8	1	%1
Dermokozmetik	25	%24.0	44	%42.3	21	%20.2	5	%4.8	6	%5.8

Tablo 2. Eczanede rutin olarak yapılan idari işlerin dağılımı ve sorumluları

	Reçete Kontrolü		SGK'ya Aylık Fatura Kesimi		Stok Kontrolü		Miad Kontrolü		Temizlik		Günlük Sipariş		Toplu Sipariş	
SORUMLU PERSONEL	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**	**n**	**Yüzde**
Sadece Eczacı	42	%40.4	77	%74	26	%25	14	%13.5	4	%3.8	26	%25	85	%81.7
Sadece Yardımcı Eczacı	1	%1	1	%1	3	%2.9	-	-	-	-	-	-	-	-
Sadece Eczane Yardımcı Personeli	6	%5.8	6	%5,8	-	-	25	%24	83	%79.8	19	%18.3	3	%2.9
Eczacı+Eczane Yardımcı Personeli	51	%49	19	%18.3	71	%68.3	62	%59.6	12	%11.5	56	%53.8	16	%15.4
Eczacı+Yardımcı Eczacı	1	%1	1	%1	-	-	-	-	-	-	1	%1	-	-
Yardımcı Eczacı+Eczane Yardımcı Personeli	1	%1	-	-	-	-	1	%1	-	-	-	-	-	-
Hepsi	2	%1.9	-	-	3	%2.9	2	%1.9	2	%1.9	2	%1.9	-	-

Çalışmaya katılan eczacılara; reçete kontrolü, stok kontrolü, miad kontrolü ve temizliğin hangi periyotlarla yapıldığı sorulmuş olup yapılan bu işlerin periyotlarına göre dağılımı Tablo 3'de belirtildiği şekildedir. Bu soruda eczacılar birden fazla seçenek işaretleyebilmişlerdir.

Tablo 3. Eczanede yapılan rutin bazı işlerin periyotlarına göre dağılımı

	Günlük	Haftalık	Aylık	Yıllık
Reçete Kontrolü	%46.2	%40.4	%51	-
Stok Kontrolü	%34.6	%19.2	%44.2	%37.5
Miad Kontrolü	%9.6	%18.3	%69.2	%10.6
Temizlik	%88.5	%47.1	%14.4	-

Eczacıların bir kısmı, günlük reçete kontrolünün; (%15.4) 15 dakikadan az ve (%19.2) 15-30 dakika, diğer bir kısmı ise (%7.7) 31-45 dakika ve (%10.6) 46 dakika-1 saat zaman aldığını belirtmişlerdir.

Eczacılar tarafından SGK'ya aylık fatura kesiminin; çoğunlukla (%64.4) mesai saatleri içinde herhangi bir zaman diliminde gerçekleştiği belirtilirken, bir kısmı (%27.9) cumartesi günleri, diğer bir kısmı tarafından ise (%6.7) eczane kapanınca mesai saatleri dışında yapılmakta olduğu ifade edilmiştir. Eczanede önemli bir zamana ihtiyaç olan SGK'ya aylık fatura kesiminin çoğunlukla (%28.8) ortalama 1 saat ve (%32.7) ortalama 2 saat, bir kısmının (%21.2) ortalama 3 saat, kalan kısmının ise (%13.5) ortalama 4 saat zaman aldığı tespit edilmiştir.

Eczanede günlük stok kontrolü yapanların bir kısmı bu iş için (%15.4) 15 dakikadan az, bir kısmı (%18.3) 15-30 dakika, kalan kısmı ise (%8.7) 31 dakika-1 saat zaman ayırmaktadır.

Eczanede günlük miad kontrolü yapanların bir kısmı bu iş için; (%9.6) 15 dakikadan az, bir kısmı (%6.7) 15-30 dakika, kalan kısmı ise (%3.8) 31 dakika-1 saat zaman ayırmaktadır.

Diğer sağlık çalışanları (tıbbi mümessil, hekim, tıbbi sekreter vb.) ile günlük görüşmeler; çoğunlukla (%45.2) 15-30 dakika, (%27.9) 31 dakika- 1 saat sürerken, bir kısmında ise (%22.1) 15 dakikadan az zaman almaktadır. Eczacıların pek azı ise (%4.8) diğer sağlık çalışanları ile görüşme yapmadıklarını belirtmişlerdir.

Eczanede günlük temizlik yapanların büyük bir kısmı bu iş için (%54.8) 15-30 dakika, bir kısmı (%20.2) 15 dakikadan az ve (%16.3) 31 dakika-1 saat zaman ayırmaktadır. Az bir kısım ise (%8.7) günlük temizlik yapılmadığını belirtmiştir.

Eczanede günlük siparişleri vermek; %37.5 oranında 15-30 dakika, %36.5 oranında 31-45 dakika, %17.5 oranında 46 dakika- 1 saat, %8.7 oranında 15 dakikadan az zaman almaktadır.

Eczanede ilaç imhası için; eczacıların çoğu (%32) ilaçları kendi imkanlarıyla imha ettiklerini, bir kısmı (%26) ilaç imhasına gerek kalmadığını, bir kısmı (%23) Eczacı Odası aracılığıyla ve kalan kısmı ise (%17) firmalar aracılığıyla imha edildiğini belirtmiştir. Türkiye'de bir toplum eczacısının eczanesinde yapılan günlük işler ve bu işler için ayırdıkları ortalama süreler Tablo 4'te verilmiştir.

"Eczanede iş akışının daha verimli hale gelebilmesi için neler yapılmalıdır?" açık uçlu sorusuna gelen yanıtların değerlendirilmesi ile ortaya çıkan öneriler aşağıda verilmiştir:

- Eczanede yapılacak işler planlanmalı ve eczane işleyişi standarda bağlanmalıdır.
- Eczacılık ile ilgili gelişmeler takip edilmeli ve buna bağlı olarak eczane işleyişi düzenli olarak revize edilmelidir.
- Personele düzenli aralıklarla eğitimler verilmelidir.
- Görevli personelin görev tanımları anlaşılır ve doğru bir şekilde yapılmalıdır. Aynı zamanda rutin görevler için yöntem (Standart işlem prosedürleri) belirlenmesi ve bu yöntemin izlenmesi gereksiz işgücü ve zaman kaybını önleyecektir.
- Personeller arasında iş bölümü olmalı ve kişilerin yetenekleri doğrultusunda görevler verilmelidir.
- Reçete karşılama konusunda iş akışı şeması oluşturularak; bu işlem belirli standarta bağlı hale getirilmelidir.
- MEDULA Provizyon Sistemi (Medikal Ulak), Sağlık Uygulama Tebliği'ne uyumlu hale getirilmeli, böylelikle eczanedeki sürenin daha verimli kullanılması sağlanmalıdır.
- Hastalara özellikle muayene ücretleri gibi konular hakkında kamu spotları vs. hazırlanması onlara ilaç veya hastalıkları hakkında bilgi vermek için daha fazla zaman ayrılmasını sağlayabilir.

Tablo 4. Bir toplum eczanesinde yapılan işler ve sürelerinin dağılımı

Eczanede yapılan günlük işler	Ortalama Süre
Akut hastalığı olan, raporsuz reçeteli hastalara danışmanlık	1-2 saat
Dermokozmetik ürünler hakkında danışmanlık	30 dakikadan az
Diğer sağlık çalışanları (tıbbi mümessil, hekim, tıbbi sekreter vb.) ile görüşmeler	15-30 dakika
Eczacılık uygulamaları dışında (ailesel problemler, meslek hakkında sorular, adres sorma vb.) danışmanlık	15-30 dakika
Eczane temizliği	15-30 dakika
Geleneksel ve alternatif tedaviler (homeopati, akupunktur vb.) ile bitkisel ürünlerle ilgili danışmanlık	30 dakikadan az
Hekime başvurmayı gerektirmeyen hafif rahatsızlıklar hakkında ilaç danışmanlığı	30 dakika
İlaç kullanımı dışında yaşam kalitesini arttıracak uygulamalar	30 dakikadan az
İlaç ve ilaç dışı ürünlerin siparişi	15-30 dakika
Kronik hastalığı olan, raporlu reçeteli hastalara danışmanlık	1-2 saat
Majistral ilaç hazırlamak	15-30 dakika
Miad kontrolü	15 dakikadan az
Ödeme işlemleri ile ilgili açıklamalar (Katılım bedeli, fiyat farkı, muayene ücreti)	30 dakikadan az
Reçete kontrolü	15-30 dakika
SGK Provizyon Sistemi, Renkli Reçete Sistemine giriş	2-3 saat
Stok kontrolü	15-30 dakika
Sürekli gelen hastaların sağlık durumunu (ilaç kullanımı ve yararlanımının izlenmesi) izlemek için danışmanlık	30-59 dakika
Tıbbi cihaz ve malzemeler hakkında danışmanlık	30 dakikadan az
Veteriner ve Tarım ilaçları hakkında danışmanlık	15 dakikadan az

Ankara'da toplum eczanesi eczacıları ile yapılan bu çalışmada, eczacıların en temel görev ve sorumluluğu olan hasta danışmanlığı yanında idari ve yönetsel işleri de incelenmiştir. Sipariş verme, gelen ürünlerin eczanede yerleştirilmesi, reçete kontrolü, majistral ilaç yapımı, SGK reçetelerinin provizyon sistemine girişi ve reçetelerin faturalandırılması, stok kontrol, ilaçların miad kontrolleri, miadı geçen ilaçların imhası, diğer sağlık çalışanları ile görüşmeler, eczaneye gelip, ilaç dışı çeşitli konularda bilgi isteyenlere cevap verme ve eczane temizliği toplum eczanelerinde en fazla yapılan idari ve yönetsel işler arasında yer almaktadır.

Toplum eczanelerinde yapılan sipariş verme, gelen siparişin kontrol edilmesi, yerleştirilmesi, stok kontrol gibi işlerin bir kısmının diğer işletmelerde de yapıldığı söylenebilir. Etkili stok yönetimi, toplum eczanelerinde ürün kalitesinden ödün vermeden kaliteli hizmetin sürdürülmesini sağlarken, aynı zamanda eczanelerin sürdürülebilirliğine ve eczane sermayesinin en uygun şekilde kullanılmasına katkı sunmaktadır [10].

Toplum eczaneleri, ilaçla tedavi hakkında bilginin hastaya aktarıldığı ve ilacın hastaya sunulduğu son sağlık hizmet basamağıdır. İlaç, doğru kullanılmadığında veya doğru muhafaza edilmediğinde hastaya geri dönüşü mümkün olmayan zararlar verebilir. Bu nedenle ilaçların siparişi ve depolanması diğer pek çok ürüne benzemeyen ve dikkat gerektiren bir süreçtir. Yanlış depolanan veya son kullanım tarihi geçen ilaçların hasta tarafından kullanılması çok tehlikeli olabilir. Bu nedenle saklanması zor, bozulması kolay ve son kullanma tarihi yakın ilaçların eczanelerde stoklanmasında azami özenin gösterilmesi gerekmektedir. İlaçlar stoklanırken, hastanın ihtiyacı olduğu an ilacına ulaşması için doğru ilacın gerekli zamanda, gerekli miktarda eczanelerde bulunması gerektiği göz önünde bulundurulmalıdır [11]. Belli aralıklarla stok kontrolü yapılması ilaçların hastalara daha güvenli bir şekilde ulaştırılmasını sağlayacaktır.

Türkiye'de toplum eczanelerinde verilen hizmetler hakkında hasta memnuniyeti ile ilgili yapılan bir çalışmada, hastaların eczacıların danışmanlıklarından orta derecede memnun oldukları sonucuna

varılmıştır [12]. Bu durum çalışmamızda eczacıların eczanelerinde en fazla zaman ayırdıkları iş olarak ortaya çıkan hasta danışmanlığının yeteri kadar kaliteli bir şekilde yerine getirilmediğini göstermektedir. Bu nedenle eczacıların her geçen gün daha da gelişen hasta odaklı eczacılık hizmetlerini göz önünde bulundurarak, danışmanlık rollerini geliştirmeleri ve personellerini de bu konuda eğitmeleri önemlidir.

Eczanede yapılan rutin işlerden biri de reçetelerin Sağlık Uygulama Tebliği'ne (SUT) uygunluğunun kontrolüdür. SUT'a uygun olmayan reçetelerin karşılanması büyük maddi yaptırımlar içerdiğinden eczanelerde her reçete SUT'a uygunluk açısından özenle incelenmektedir. Bu durum hastanın reçetede olabilecek hatalardan zarar görmemesi için dolaylı da olsa ikinci bir kontrol mekanizması oluşturmaktadır [13]. MEDULA'nın SUT hükümleri ile uyumlu hale getirilmesi, eczacıların reçete kontrolleri için ayırdıkları zamanı azaltarak, hasta danışmanlığına ayırdıkları süreyi arttıracaktır.

İş analizinden elde edilen veriler, örgüt yapısı oluşturulurken alınan kararlar için esastır. Ayrıca personel ihtiyacı için gereken işgücü niteliklerini belirler. Toplum eczanelerinde yapılan işlerle ilgili detaylı bilgi sahibi olmak, aynı zamanda uygun personelin, uygun sayıda işe alınmasını ve personel ücretlerinin uygun şekilde belirlenmesini de sağlayacaktır. İş analizleri, işlerin gerektirdiği personel niteliklerini ortaya koyarak, adil bir ücret politikası oluşturmaya yönelik çabalara da katkıda bulunur. Böylelikle hangi nitelikteki personelin hangi işleri yerine getireceği tespit edilir.

Toplum eczaneleri çok farklı demografik özelliklere sahip bölgelerde hizmet verdiklerinden, eczanede hizmet veren personelin iş başında eğitimi önemli bir konudur. Toplum eczanelerinde yapılan işlerin belirlenmesi ve analiz edilmesi, personelin ihtiyacı olan eğitimlerin belirlenmesi ve verilmesi açısından da önem taşımaktadır. Yapılan işlerin analiz edilmesi ile eczane personelinin işlevsel ve verimli kullanılabilmesi mümkün olur.

İş analizinin önemli bir diğer kazanımı işteki araç ve gereçlerin, iş ortamına uygun olarak düzenlenmesidir. Örneğin bir toplum eczanesinde, personelin kullanacağı bilgisayar, internet, yazar kasa, pos cihazı, terazi, havan vb. araç gereçler bellidir. Eczacının idari işlerle ilgili eğitimi içinde bu araç gereçlerin kullanımı ile ilgili eğitimin önceden personele verilmesi, ya da bu araç ve gereçleri kullanmayı bilen personelin istihdam edilmesi, hem hastalara verilen hizmetin kalitesini arttıracak hem de eczacıya zaman kazandıracaktır.

Toplum eczanelerinin sağlık hizmeti veren kuruluşlar olmaları nedeniyle özel temizlik kurallarına uymaları gerekir ve toplum eczanelerinde diğer pek çok işletmeden daha detaylı temizlik yapılır. Mevzuat gereği eczanede yer ve laboratuvar banko döşemeleri, kolay temizlenir, hijyenik malzemelerle kaplı olması gerekmektedir. En az 35m^2 olması gereken eczaneler daha çok hastaların başvurduğu bir işletme olarak bulaş riskinin de mümkün olduğunca ortadan kalkması için olabildiğince sık, düzenli ve uygun temizlik malzemeleri (antiseptikler, dezenfektanlar vb.) ile temizlenmelidir [11].

Semt eczanelerinin, geleneksel ve alternatif tedaviler ile bitkisel ürünler hakkında danışmanlığı daha fazla yaptığı belirlenmiştir. Ancak ülkemizdeki eczanelerde bulunan Sağlık Bakanlığı tarafından onaylanmış bitkisel ürünlerin sayısı ve çeşitliliğinin özellikle Avrupa Birliği ülkeleriyle karşılaştırıldığında çok düşük olduğu görülmektedir [14]. Ayrıca eczaneler dışında aktar, market ve internette gıda takviye edici olarak satılan bitkisel ürünlerin sayı ve çeşitliliği her ne kadar fazla olsa da maalesef bu ürünlerin standartlara uygunluğu ve güvenirliğiyle ilgili çekinceler bulunmaktadır. Bu nedenle toplum eczanelerinde yer alan bitkisel ürün çeşitliliğinin standartlara uygun bir şekilde arttırılması toplum sağlığı açısından da önem arz etmektedir.

Büyük sağlık kuruluşu yakınında bulunan eczanelerin akut hastalığı olan, raporsuz ve reçeteli hastalara danışmanlığı daha az yaptığı belirlenmiştir. Bunun sebebi olarak, hastane karşısı eczanelerde hasta sirkülasyonunun fazla olması nedeniyle, akut hastalara yeteri kadar vakit ayrılamaması olabileceği öngörülebilir.

Ankete katılan eczacıların büyük çoğunluğu (%88.5) veteriner ve tarım ilaçları ile ilgili danışmanlık vermediklerini söylemişlerdir. Veterinerlerin, bu tip ilaçları hem yazıp hem de satabiliyor olmaları nedeniyle eczanelere böyle bir talep gelmemesi ve anketin il merkezindeki eczacılara uygulanmış olması nedeniyle şehir merkezinde bu konuda bilgiye ihtiyaç olmaması bu durumun sebepleri olabilir.

Toplum eczanelerinde, eczacının ilaç dışı konularda da başvuranlara danışmanlık verdiği belirlenmiştir. Yapılan bir başka çalışmada toplum eczanelerinde eczacıya, ilaç dışında pek çok konu danışıldığını göstermektedir [15]. Bunun nedeni eczaneye başvuranların eczacıya güven duymaları olabilir. Ancak bu konulara zaman ayırmak zorunda kalmak eczacıların hastalara ayırdıkları zamanı azaltmaktadır.

Türkiye'de toplum eczanelerinde sunulan mevcut hizmetler dışında pek çok ülkede uygulanan farklı eczacılık hizmetleri de bulunmaktadır. Türkiye'deki toplum eczacılarının zamanlarını daha iyi planlamasıyla ilaç verme sırasında ilaçların güvenliği, etkinliği ve uyumu proaktif olarak ele alınarak ilaç tedavisinin optimizasyonu desteklenebilir. Bu konuda eczacılara sorumluluk verilebilir ve bunun karşılığında meslek hakkı olarak ödeme yapılabilir [16].

Toplum eczaneleri için yapılan iş analizi hem çalışanlar hem de eczacılar için yararlı bilgiler sağlar. Eczacılar için, iş analizinden elde edilen bilgiler iş tanımlarının yazılmasında, iş adaylarıyla görüşülürken, adayların taranmasında ve performans kriterlerinin belirlenmesinde kullanılır. Çalışanlar için, iş analizinden elde edilen bilgiler, çalışanlara işin nasıl yapılacağını ve beklenen sonuçları anlatır [17].

Toplum eczanelerinde her hastaya özel olarak hizmet verilmesi gereken, titizlikle ve özenle yapılması gereken işler bulunmaktadır. Bu çalışmada özellikle bir toplum eczanesinde yapılması gereken asgari işler ve bu işlerin kimler tarafından hangi süre ile yapıldığı belirlenmeye çalışılmıştır. Bundan sonraki çalışmalarda her iş kaleminin tek tek ayrıntılı analizi yapılarak, her işin nasıl yapılacağı, her basamağın algoritmasının belirlenmesi toplum eczanelerinde hizmet kalitesini arttıracaktır. Bu çalışma özellikle toplum eczanelerinin vizyon ve misyonlarını belirlemek için çok önemlidir. Bu çalışma sonuçlarına göre, toplum eczanelerinde eczacının hizmet kalitesini arttırmak ve eczacıların, eczanelerini daha verimli bir şekilde yönetmeleri için sunulan öneriler aşağıdaki şekildedir.

- Eczacılar, eczane personelinin işlerini yapmaları için gerekli olan eğitimleri sağlamalı ya da vermelidir.
- Eczacılar geleneksel ve alternatif tedaviler (homeopati, akupunktur vb.) ile bitkisel ürünlerle ilgili kendilerini geliştirmeli ve bu tedavileri ile ilgili hastalarına kanıta dayalı bilgi sağlamalıdır.
- Eczacılar gerekli durumlarda hastaları ile yaptıkları görüşmeleri planlamalıdır.
- Eczacıların hasta danışmanlığına daha çok vakit ayırabilmesi için, eczane personeli eğitim ve becerilerine uygun olarak yetkilendirilmeli ve eczanedeki bürokratik/yönetsel işlerin eczacı üzerinde olan yükü azaltılmalıdır.
- Eczacılık uygulamaları ile ilgili toplumu bilgilendirmek amacıyla kamu spotu gibi araçların kullanılması sağlanmalıdır.

YAZAR KATKILARI

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

A TALE OF CAPTOPRIL DETECTION BASED ON AN ELECTROCHEMICAL MIP SENSOR

KAPTOPRİL TESPİTİ İÇİN ELEKTROKİMYASAL BİR MIP SENSÖRÜNÜN HİKAYESİ

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ABSTRACT

Objective: *In this study, it was aimed to develop a voltammetric method using sensors prepared with the molecular imprinting technique for the detection of Captopril, an antihypertensive drug.*
Material and Method: *With the molecular imprinting method, molecularly imprinted polymers were formed on the surfaces of glassy carbon electrodes. The analysis of Captopril was carried out using the differential pulse voltammetry method, and the performance of the sensor was examined.*
Result and Discussion: *A linear analysis was performed up to 50 pM Captopril with a limit of detection value of 2.62 pM. Selectivity studies have shown that Captopril has a higher electrochemical response than other interfering substances, such as paracetamol, ascorbic acid, and L-proline.*
Keywords: *Biomimetic sensors, buffer effect, captopril, molecularly imprinted polymers*

ÖZ

Amaç: *Bu çalışmada antihipertansif bir ilaç olan Kaptoprilin tespitine yönelik moleküler baskılama yöntemi ile hazırlanmış sensörler kullanılarak voltametrik bir yöntem geliştirilmesi amaçlanmıştır.*
Gereç ve Yöntem: *Moleküler baskılama yöntemi ile camsı karbon elektroların yüzeylerinde moleküler baskınlanmış polimerler oluşturulmuş ve differansiyel puls voltammetri yöntemi ile Kaptoprilin analizi gerçekleştirilmiş, sensörün performansı incelenmiştir.*
Sonuç ve Tartışma: *2,62 pM teşhis sınırı değeri ile 50 pM Kaptopril seviyesine kadar doğrusal bir analiz gerçekleştirilmiştir. Seçicilik çalışmaları, Kaptoprilin, diğer girişim yapabilecek, parasetamol, askorbik asit ve L-prolin gibi maddelere göre daha yüksek elektrokimyasal cevaba sahip olduğunu göstermiştir.*
Anahtar Kelimeler: *Biyomimetik sensörler, kaptopril, moleküler baskılanmış polimerler, tampon çözelti etkisi*

INTRODUCTION

Cardiovascular diseases (CVD), a group of disorders of the heart and blood vessels, are the leading cause of death worldwide [1]. In 2019 it was estimated that 17.9 million people died due to the CVDs. 85% were due to heart attack and stroke [1].

Hypertension (elevated blood pressure), defined using specific systolic and diastolic blood pressure levels, increases the risk of heart, brain, kidney, and other diseases. Globally 1.4 billion people

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have been estimated to have high blood pressure, only 14% of whom have it under control [2]. Captopril (CAP, (2S)-1-[(2S)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid) is an antihypertensive drug, which is an angiotensin-converting enzyme inhibitor. It is widely used to treat congestive heart failure [3-5]. However, it has possible side effects like proteinuria, neutropenia, or skin rashes. Therefore, its determination in biological fluids and pharmaceutical dosage is necessary [6-8]. In addition to spectroscopical and chromatographical methods, a spectrum of electrochemical sensors has been developed for CAP detection [9]. Electrochemical methods provide some advantages, such as simplicity, cost-effectiveness, and sensitivity.

In this study, an electrochemical sensor for CAP determination based on a molecularly imprinted polymer (MIP) developed. Molecular imprinting stands out as a promising technique to replace biological recognition elements such as antibodies and enzymes in the field of bioanalysis and biosperation. Initially introduced by Wulff and Mosbach, this method involves the polymerization of functional monomers, either with or without cross-linkers, in the presence of a target analyte (template). The subsequent removal of the template results in the production of binding cavities that mirror the template's size, shape, and functionality. This process aims to generate artificial antibodies, often called plastibodies, which exhibit molecular recognition capabilities in bioanalytical applications [10–13]. Although MIPs for low-molecular-weight substances like pharmaceuticals, narcotic drugs, amino acids, toxins, sugars, and pesticides have been successfully prepared, it is still challenging for biomacromolecules like proteins or viruses due to their structural complexity and fragility under MIP preparation conditions [14-29]. However, increasing attention has been paid to the fabrication of MIPs due to their ease of preparation, cost-effectiveness, and stability under various conditions like extreme pHs, high temperatures, or organic solvents.

Herein, we describe a simple approach to fabricate an electrochemical MIP sensor to detect CAP. The MIPs were prepared in situ on a glassy carbon electrode using *o*-phenylenediamine (o-PD) as a functional monomer. All steps of MIP preparation were studied using the gate effect on the redox marker ferricyanide/ferrocyanide. Moreover, the sensor was applied in pharmaceutical dosage, and the effect of interfering substances was investigated.

MATERIAL AND METHOD

Reagents

o-Phenylenediamine dihydrochloride (*o*-PD), captopril, paracetamol (acetaminophen), and L-Proline were purchased from Sigma (Steinheim, Germany), ascorbic acid, ferricyanide and ferrocyanide from Merck (Germany). Kapril® 25 mg was obtained from GENSENTA Ilaç Sanayi ve Ticaret A.Ş. All reagents were of analytical grade and used without further purification.

Instruments

Electrochemical experiments were conducted in a homemade cell with a three-electrode system using PalmSens potentiostat (Utrecht, The Netherlands). A glassy carbon electrode (GCE, diameter: 3 mm), an Ag/AgCl electrode (in 3 M KCl), and a platinum wire served as the working electrode, the reference electrode, and the counter electrode, respectively.

Preparation of CAP-MIP/GCE and working solutions

The MIP-sensor was fabricated by electrodepositing the polymer directly on a GCE in a solution of 0.5 mM CAP and 5 mM *o*-PD (in 100 mM acetate buffer, pH 5.2) utilizing cyclic voltammetry (Figure 1). The potential was swept between 0 and 0.8 V with a scan rate of 50 mV/s with different scan numbers. For the removal of template molecules, different strategies have been applied. Rebinding and removal of CAP were followed by changes in DPV responses of 5 mM $[Fe(CN)_6]^{3-/4-}$ solution (in 100 mM KCl) by applying respective conditions: The potential range: -0.2 - 0.7 V, potential step: 5 mV, pulse amplitude: 25 mV, and pulse duration: in these measurements 0.7 s [14].

For the electrochemical analysis, an aliquot from the stock solution of CAP was prepared as 500 ppm, and then desired concentrations were obtained by diluting the stock solution, used for the rebinding studies.

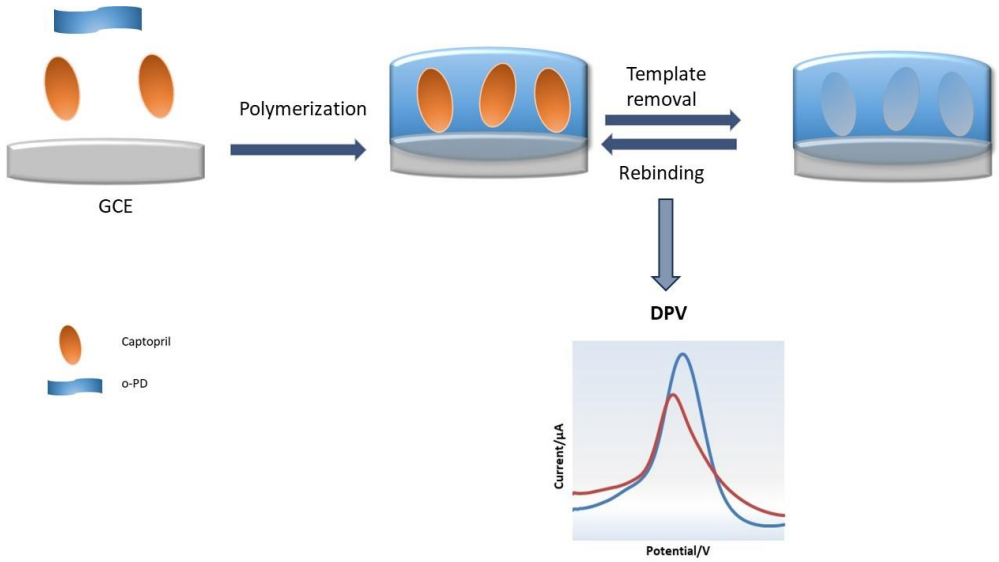

Figure 1. Workflow of the CAP-MIP/GCE

CAP stock solution from pharmaceutical dosage forms Kapril® was prepared by crushing ten tablets of Captopril film-coated tablets containing 25 mg CAP and, after homogenized, weighing an amount that is equal to one tablet, was dissolved in the working buffer solution, sonicated for 10 min and filtered. Following than, desired concentrations were obtained by diluting the stock solution.

RESULT AND DISCUSSION

Polymerization Step

Electropolymerization is one of the elegant ways to prepare MIPs with controlled thickness in situ on the surface of transducers. Moreover, it provides faster preparation time and eliminates the use of a cross-linker. Based on former experiences *o*-phenylenediamine was chosen as an electroactive monomer, and captopril was the target analyte (Figure 1) [24,26].

As seen from the CVs, an irreversible peak observed at around 450 mV during the first cycle decreased in successive cycles, indicating the formation of a non-conducting polymer film (Figure 2). Non-imprinted polymer (NIP) was also prepared like MIP but without the template captopril, had a similar behavior during electropolymerization (data not shown).

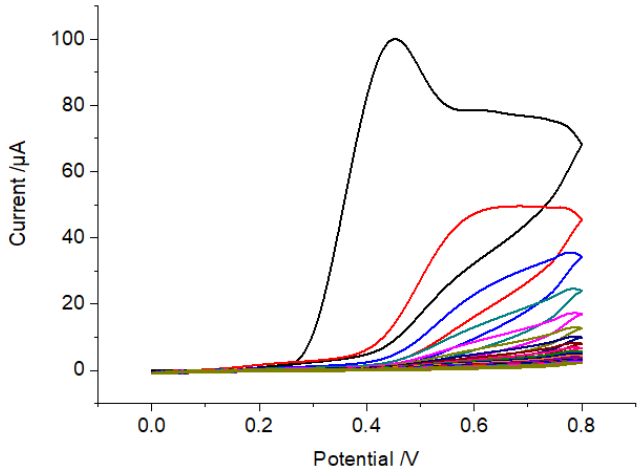

Figure 2. Cyclic voltammograms during electropolymerization of 5 mM *o*-PD in the presence of 0.5 mM Captopril in 50 mM acetate buffer, pH 5.2 (Scan rate: 50 mV/s, 20 cycles)

We followed the steps of MIP sensor fabrication and application by the most common technique assessing the diffusional permeability of the polymer layer to a redox marker, ferricyanide/ferrocyanide (Figure 3). This method is known for its simplicity, cost-effectiveness, and high sensitivity. It allows for the characterization of each step of MIP synthesis and the measurement of target-rebinding to the MIP, applicable to low-molecular-weight targets, (bio)macromolecules, and (nano)particles [11,30]. In the case of low-molecular-weight molecules, like CAP, the cavities formed after template removal exhibit diameters comparable to that of the redox marker (Figure 3) [11]. Various mechanisms have been proposed to explain the impact of target binding on the current signal of the redox marker. These include alterations in the MIP film's porosity or the marker's diffusion rate, doping-dedoping effects, and changes in the electric double layer [31]. This concept is known as the "gate effect," initially introduced by Yoshimi et al. for a theophylline-imprinted polymer [31]. However, the complex mechanism behind the "gate effect" is not yet fully understood.

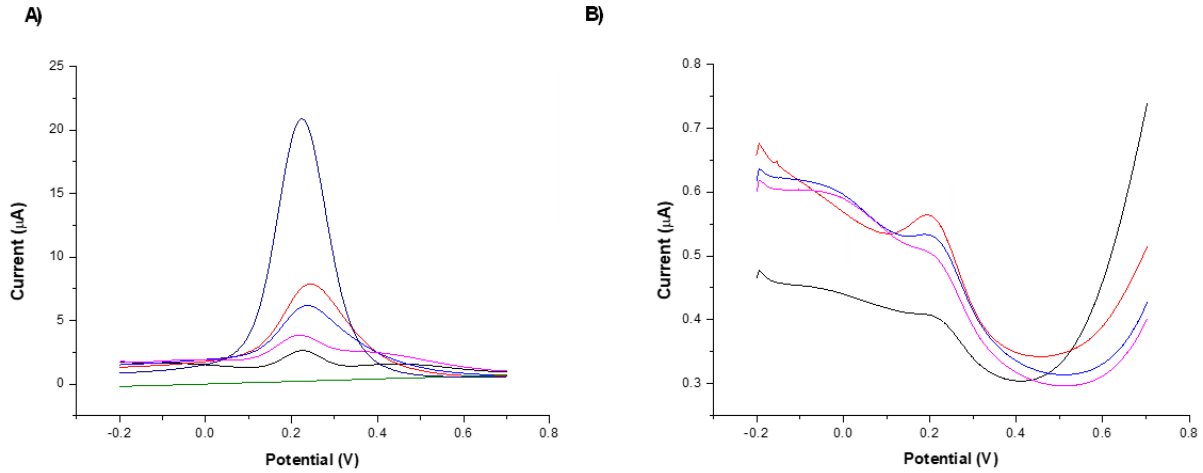

Figure 3. Differential pulse voltammograms A) Bare GCE: Dark purple. CAP-MIP/GCE after electropolymerization (black), template removal (red), 1 h incubating in buffer (blue), 40 pM CAP binding (pink) and B) NIP/GCE after electropolymerization (black), template removal (red), 1 h incubating in buffer (blue), 10 pM CAP binding (pink)

Template Removal

Template removal is one of the most crucial steps in fabricating MIP sensors [32]. Harsh conditions may cause deformation of the structure of the polymeric film, whereas milder conditions might not be strong enough for the complete removal of the template molecules. As a result, the binding capacity of the MIP is low. In this study, several procedures were applied to remove the captopril molecules from the polymer.

Milder acidic conditions, e.g., 50 mM glycine-HCl buffer, pH 2.2 (overnight incubation), cannot remove the template molecules from the polymeric film. By contrast, incubation in 0.1 M H_2SO_4 caused the observation of 75% of the ferricyanide signal at the bare electrode. Moreover, the combination of acidic (0.3 M H_2SO_4) and electrochemical procedure (CV, scanning between -1.0 V and 1.0 V) caused a complete loss of the polymeric film. On the other hand, template molecules were successfully removed in 0.1 M NaOH solution by applying the cyclic voltammetric technique between -1.0 V and 1.0 V (10 scans).

Analytical Performance of the MIP-Sensor

Rebinding studies have been conducted in acetate buffer containing different amounts of captopril. DPV was used to indicate the change in the signal of the redox marker. Under optimum conditions, a calibration plot for the determination of was obtained. (Figure 4). The sensor responded linearly up to 50 pM (R^2= 0.977) with a limit of detection of 2.62 pM and limit of quantification of 7.94 pM. The comparison of the binding affinities of CAP on MIP- and NIP-modified electrodes indicated

specific binding to the cavities formed after template removal in the polymer. As seen from Figure 3, the ferricyanide signal was completely suppressed at the NIP-modified electrode upon 10 pM CAP binding, whereas 40 pM CAP led not a complete suppression. Therefore, further calibration studies were not performed with NIP-modified electrodes. As seen in Table 1, the sensitivity of the developed sensor is higher than that of the sensors described in the literature. In contrast, the linear dynamic range is narrow, which might be improved by incorporating nanomaterials into the electrode [33].

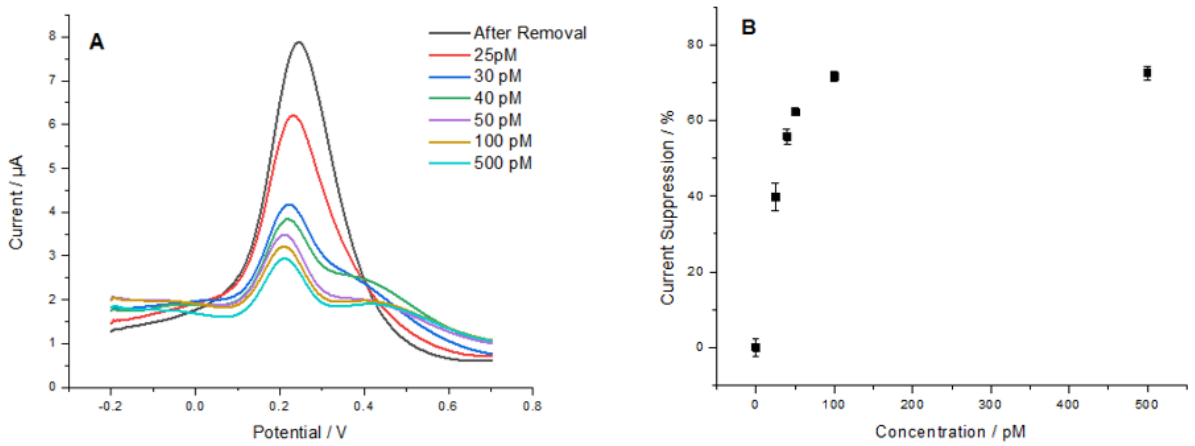

Figure 4. A) Concentration dependence of the DPV peak on the concentration of Captopril on CAP-MIP/GCE and B) Calibration graph

Table 1. Examples of Electrochemical Sensors for the Detection of Captopril

Electrode	Detection Method	Linear Dynamic Range	LOD	Reference
GSPE	CV	up to 0.8 mM	4.27 µM	[34]
CuBTC/GCE	DPV	0.5-7 µM	0.20 µM	[35]
CuO/ITO electrode	DPV	0.01 to 3.43 µM	2 nM	[36]
Au@Cu/BTC	CV	0.5-7 µM; 10-2500 µM	0.047 µM	[37]
SnO_2/rGO/Pt electrode	DPV	1-700 nM; 10-100 µM	0.061 nM; 0.0018 µM	[38]
CAP-MIP/Modified CPE	Potentiometry	3 nM-0.1 M	1 nM	[39]
CAP-MIP/GCE	DPV	up to 50 pM	2.62 pM	This work

GSPE: Graphite Screen-Printed Electrode; Cu-BTC: $C_{18}H_6Cu_3O_{12}$ (copper metal-organic framework); rGO: Reduced graphene oxide; CPE: Carbon Paste Electrode; GCE: Glassy Carbon Electrode

One notable drawback of this MIP-approach arises when dealing with low concentrations of the target substance. Detection of minute amounts causes only small decreases of a large baseline current. This difficulty is exacerbated by the fluctuation of the background current, leading to high uncertainties in determinations, which might be caused by variations in the polymer film's volume due to changes in the ionic strength and/or pH of the sample solution [40]. Just incubating in a buffer solution can also provoke a suppression of the ferricyanide signal, as seen in Figure 3. It was observed that at least one hour of pre-incubation in the buffer solution was needed to prevent false lower-concentration measurements.

In an effective therapeutic process, it is essential to quantify the amount of drug used. For this purpose, the MIP-sensor was used to determine CAP in Captopril film-coated tablets. For 40 pM CAP containing sample solution, 101.95 % recovery results were obtained with Bias% of -1.95.

Cross-reactivity Studies

Since selectivity is the most essential validation parameter, cross-reactivity of potentially

interfering substances paracetamol, L-proline, and ascorbic acid was investigated. The highest suppression of the ferricyanide signal was obtained upon the rebinding of the template molecule, captopril, while paracetamol had a 4.8-fold lower while ascorbic acid had almost no effect on the ferricyanide signal of the MIP sensor. On the other hand, L-proline exhibited 1.6-fold lower suppressions than captopril, which may be caused by the fact that captopril is a derivative of L-proline (Figure 5).

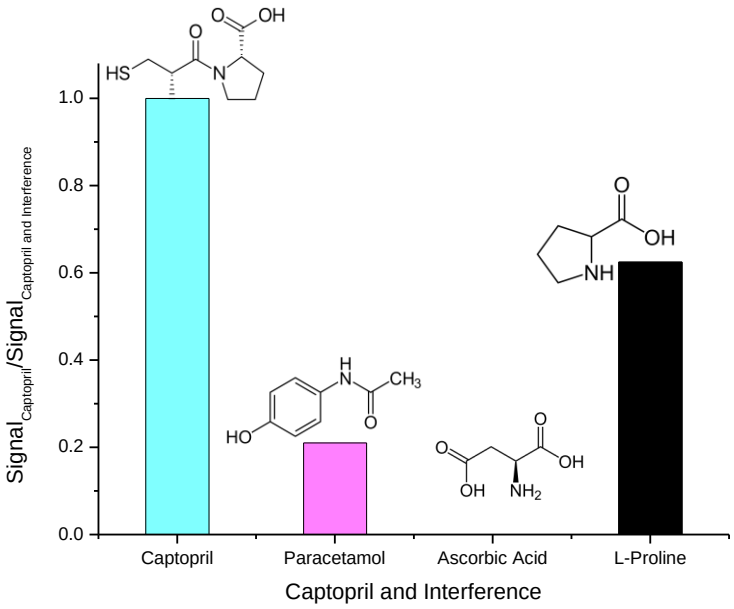

Figure 5. Cross-reactivity of interfering substances binding on the CAP-MIP/GCE

Conclusion

In this work, the first voltammetric MIP sensor was developed to detect the antihypertensive drug captopril. Evaluating the gate effect on the redox probe ferricyanide/ferrocyanide. The sensor responded linearly up to 50 pM captopril (R^2= 0.977) with a limit of detection of 2.62 pM and limit of quantification of 7.94 pM. The sensor was further successfully applied in captopril detection in pharmaceutical dosage forms. It showed high discrimination towards paracetamol, ascorbic acid, and L-Proline. One crucial issue was that one hour of pre-incubation of the MIP sensor in the buffer solution was needed to prevent false lower-concentration readout.

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AUTHOR CONTRIBUTIONS

Concept: A.Y.; Design: A.Y.; Control: A.Y., S.K.; Sources: A.Y., S.K.; Materials: A.Y., S.K.; Data Collection and/or Processing: A.Y.; Analysis and/or Interpretation: A.Y., S.K.; Literature Review: A.Y., S.K.; Manuscript Writing: A.Y., S.K.; Critical Review: A.Y., S.K.; Other: -

CONFLICT OF INTEREST

The authors declare that this article has no real, potential, or perceived conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that this study does not require the ethics committee's approval.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INVESTIGATION OF THE INHIBITORY POTENTIAL OF SOME ANTIVIRAL AGENTS ON HUMAN TELOMERASE BY MOLECULAR DOCKING AND MOLECULAR DYNAMICS SIMULATION STUDIES

BAZI ANTİVİRAL AJANLARIN İNSAN TELOMERAZ ENZİMİ ÜZERİNDEKİ İNHİBİTÖR POTANSİYELİNİN MOLEKÜLER KENETLENME VE MOLEKÜLER DİNAMİK SİMÜLASYON ÇALIŞMALARI İLE ARAŞTIRILMASI

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ABSTRACT

Objective: *This study investigated the anticancer effects of nucleoside and non-nucleoside reverse transcriptase inhibitors drugs by computational methods. The study aimed to evaluate the binding capacity of these drugs on the telomerase essential N-terminal (TEN) domain of telomerase reverse transcriptase (TERT). Molecular docking was used to assess the drugs' binding potential to the TEN domain. The stability of the protein-drug combination obtained from the docking method was assessed using molecular dynamics (MD) simulation.*
Material and Method: *The TEN domain of TERT's crystal structure was obtained from the Protein Data Bank (PDB). The crystal structure identified by the PDB code 2B2A has a resolution of 2.2 Å. The molecular docking was performed using AutoDock Vina. The complexes were visualized using Biovia Discovery Studio. The MD simulation was conducted using GROMACS 2020 as indicated. An MD simulation was conducted for 200 ns on both the complexes and the free protein. The RMSD (root mean square deviation) of the backbone protein and the molecules in relation to the backbone protein, RMSF (root mean square fluctuation), and Rg (radius of gyration) were shown via Qt Grace.*
Result and Discussion: *Doravirine, Etravirine, Rilpivirine showed higher binding affinity to the TEN domain compared to the reference TERT inhibitor, BIBR1532, based on the docking investigation. The MD simulation analysis showed that the protein-Doravirine complex had the highest stability in remaining within the protein's binding pocket. On the contrary, the protein-Rilpivirine complex decreased stability, potentially causing the ligand to not to stay within the binding site. Doravirine was found to inhibit the TEN domain in the computational study. Therefore, the design and synthesis of novel doravirin derivatives is being considered because of the potential anticancer activity of doravirin in inhibiting the TEN domain of TERT.*
Keywords: *MD simulation, molecular docking, NNRTI, NRTI, TERT*

ÖZ

Amaç: *Bu çalışmada, nükleozid ve non-nükleozid ters transkriptaz inhibitörü ilaçların, antikanser etki potansiyeli hesaplamalı yaklaşımlar kullanılarak araştırılmıştır. Bu amaçla, bu ilaçların*

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telomeraz ters transkirptaz (TERT)'ın telomeraz temel N-terminal (TEN) alanına bağlanma potansiyeli araştırılmıştır. İlaçların TEN alanına bağlanma potansiyeli için moleküler yerleştirme çalışması yapılmıştır. Moleküler yerleştirme sonucu elde edilen protein-ilaç kompleksinin kararlılığı moleküler dinamik (MD) simülasyonu ile değerlendirilmiştir.

Gereç ve Yöntem: *TERT'in TEN alanı kristal yapısı için Protein Veri Bankası (PDB) kullanılmıştır. 2,2 Å çözünürlüğe sahip PDB kodu 2B2A kristal yapı kullanımıştır. Moleküler yerleştirme çalışması için AutoDock Vina programı kullanılmıştır. Kompleksler Biovia Discovery Studio kullanılarak görselleştirilmiştir. MD simülasyonu GROMACS 2020 kullanılarak gerçekleştirilmiştir. Hem kompleksler hem de serbest protein üzerinde 200 ns boyunca bir MD simülasyonu gerçekleştirilmiştir. Omurga protein ve moleküllerin, omurga yapısına göre RMSD (kök ortalama kare sapması), RMSF (kök ortalama kare dalgalanması) ve Rg (dönme yarıçapı), Qt Grace ile gösterilmiştir.*

Sonuç ve Tartışma: *Moleküler yerleştirme çalışması sonucunda, Doravirin (bileşik 3), Etravirin (bileşik 6) ve Rilpivirin'in (bileşik 9) referans TERT inhibitörü BIBR1532'ye kıyasla TEN alanına daha yüksek bağlanma potansiyeli ile bağlandığını ortaya koymuştur. MD simülasyon çalışması ile, protein-Doravirin kompleksinin proteinin bağlanma cebindeki en yüksek stabiliteye sahip olduğu gösterilmiştir. Öte yandan, protein-Rilpivirin kompleksinin kararlı olmaması nedeniyle bağlanma cebinde kalmama ihtimali bulunmaktadır. Yapılan çalışma, Doravirin'in TEN'i inhibe edebileceğini göstermiştir. Bu nedenle, Doravirin'in TERT'in TEN alanını inhibe ederek antikanser potansiyel gösterebilme ihtimali nedeniyle Doravirin türevi yeni bileşiklerin tasarlanması ve sentezlenmesi düşünülmektedir.*

Anahtar Kelimeler: *MD simülasyon, moleküler yerleştirme, NNRTI, NRTI, TERT*

INTRODUCTION

People with HIV infection may have earlier development of age-related diseases due to accelerated aging processes caused by the virus. The list of HIV-associated non-AIDS disorders is expanding. Many non-AIDS disorders are linked to both increasing age and chronic inflammation. These disorders comprise cardiovascular disease, several malignancies, osteoporosis, liver disease, renal disease, and neurocognitive decline [1]. It is uncertain if the increased risk of these consequences is due to a precipitated aging process, issues emerging at earlier stages of life, or a caused emphasized aging process [2].

Antiretroviral drugs may lead to telomere shortening, which could contribute to accelerated aging in HIV-infected patients [3]. Shortened telomere length in peripheral blood mononuclear cells (PBMCs) is closely associated with age-related disorders such as cardiovascular diseases and dementia [4,5].

Telomerase is an enzyme that exists in organisms that exist that generates new DNA repeats at the termini of linear chromosomes [6]. The telomerase reverse transcriptase (TERT) and telomerase RNA (TER) both constitute the catalytic center of the telomerase enzyme [7,8]. A special region of its intrinsic RNA known as TER acts as a template for nucleotide incorporation by TERT. A comprehensive array of biochemical and cell biology studies have been conducted to evaluate the inhibitory effects of reverse transcriptase (RT) inhibitors for HIV, with particular focus on the structural similarity between the RT domains of HIV RT and TERT [9]. By adding TTAGGG sequences repeatedly to chromosomal ends, telomerase inhibits the progressive degradation of telomeres that occurs during cell division. Due to their structural and molecular similarity with HIV reverse transcriptase [10], NRTIs may inhibit telomerase.

Previous studies have demonstrated that Zidovudine inhibits the activity of telomerase and induces telomere shortening in human breast cancer cells [11], colon cancer cells [12], and leukemia cells [13]. The evaluation of Zidovudine-induced telomerase inhibition was conducted on a human hepatoma cell line [14], while cervical cancer cells exhibited telomere shortening [15].

Previous research has demonstrated that NRTIs can inhibit human telomerase utilizing the same approaches as they use to inhibit HIV RT. Repeated exposure of telomerase-positive human cells to NRTIs can lead to inadequacies in maintaining telomere length because of the inhibition of telomerase by these drugs.

This work involved constructing molecular docking and molecular dynamics simulation studies to investigate the inhibitory effects of NNRTIs/NRTIs on the human telomerase enzyme.

MATERIAL AND METHOD

Molecular Docking

The crystal structure of the TEN domain of TERT was retrieved from the protein data bank (PDB). The crystal structure with a PDB code of 2B2A has a resolution of 2.2 Å [16]. The possible binding pocket of the structure was predicted through CASTp first [17]. Then, the grid box for the molecular docking was specified based on the estimated pocket. The molecules were downloaded from the PubChem database [18]. The molecular docking was undertaken by using AutoDock Vina as used earlier. The resulting complexes were visualized through Biovia Discovery Studio [19,20].

Molecular Dynamics Simulation

The stabilities of the TEN domain-drug complexes, which were procured from the docking, were explored through MD simulation. Then, the stability of the complexes was compared to the stability of the unbound protein. The MD simulation was undertaken by using GROMACS 2020 as described in earlier studies. For the complexes and the unbound protein, MD simulation was run for 200 ns. Thereafter, RMSD (root mean square deviation) of the backbone protein and the molecules in relative to the backbone protein, RMSF (root mean square fluctuation), and Rg (radius of gyration) were drawn via qt grace. Then, the resulting plots were analyzed accordingly [21,22].

RESULT AND DISCUSSION

Molecular Docking

The binding potential of the selected antiviral drugs to the TEN domain of TERT was explored through molecular docking. Before proceeding to the docking, the binding pocket was estimated via CASTp (Figure 1). Thereafter, the binding of a TERT inhibitor, BIBR1532, to the crystal structure (2B2A) was investigated. This was performed to validate the docking protocol and set a benchmark for the interaction of the antiviral drugs under investigation. BIBR1532 is a selective potent TERT inhibitor with an IC_{50} value of 0.093 μM [23,24]. The docking study showed that the ligand interacted with the TEN domain via three conventional hydrogen bonds (Arg16(2), Ser28), pi-pi (Ser28), pi-sigma (Ala29), pi-ion (Asn191), and pi-alkyl (Lys31) interactions (Figure 1). The binding energy was found to be -8.1 kcal/mol (Table 1). The binding energy and the formation of three conventional hydrogen bonding with the crystal structure implied that the ligand could bind to the TEN domain and remain inside the binding pocket.

The investigated antiviral drugs interacted to the TEN domain of TERT (2B2A) with at least two conventional hydrogen bonds and at least three more other types of interactions (Table 1, Figure 1, Figure S1). Some of the drugs had better binding potential than the reference TERT inhibitor, BIBR1532. In this regard, Doravirine, Etravirine, and Rilpivirine had binding energy of -8.7 kcal/mol, -8.9 kcal/mol, and -9.0 kcal/mol, respectively. The binding energy of these drugs was lower than that of BIBR1532 (-8.1 kcal/mol). Therefore, these drugs are expected to have higher binding affinity towards the TEN domain (2B2A). Doravirine and Etravirine had higher number of conventional hydrogen bonds than BIBR1532. Doravirine had five more other types of interactions than the reference. Similarly, Etravirine had two more other types of interactions. On the other hand, Rilpivirine had similar number of conventional hydrogen bonds (3) and other types of interactions (4) with the reference (Table 1, Figure 1). Hence, Doravirine and Etravirine are expected to have higher binding affinity and strength than the reference to the TEN domain. Together with this, Rilpivirine is expected to show higher binding affinity than the reference. From the docking study, it is possible to infer that Doravirine, Etravirine, and Rilpivirine would exhibit higher binding potential to the TEN domain in relative to BIBR1532 as well as the other antiviral drugs.

Table 1. Binding residues of the antiviral drugs and BIBR1532 to the TEN domain of TERT

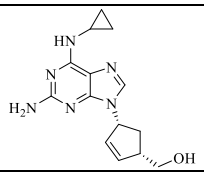
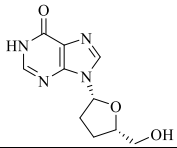
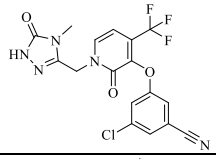
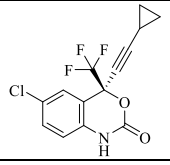
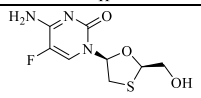
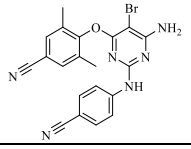
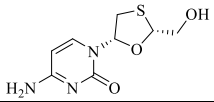
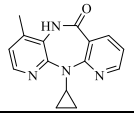
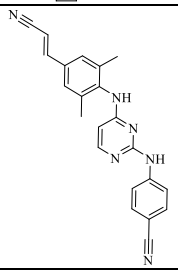
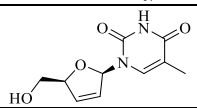
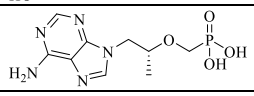
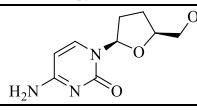
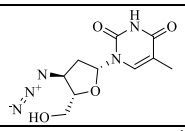
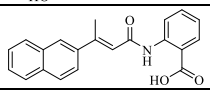
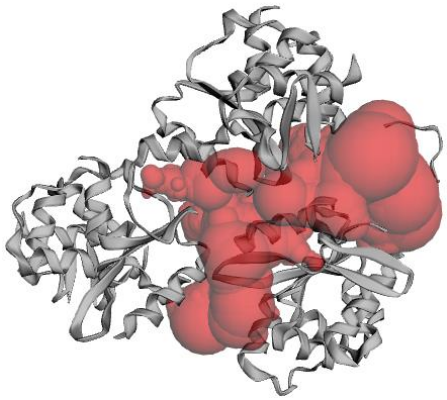
Ligands	Structure	Classification	Binding energy (kcal/mol)	Conventional hydrogen bonding residues	Other interaction residues
Abacavir		NRTI	-6.1	Asp154, Gln176, Tyr177	Glu68(2)[a], Leu71[b], Asp154[c], Val172(2)[b]
Didanosine		NRTI	-6.6	Asp19, Gln163	Leu14[b], Arg16(2)[a], Ala29[b], Lys31[b], Glu162[c]
Doravirine		NNRTI	-8.7	Leu174, Asn175, Trp187, Asn191	Leu20[b], Val23[b], Phe158[b], Leu174[b], Leu174[d], Asn175(2)[c], Phe178[b], Asn191[a]
Efavirenz		NNRTI	-6.1	Thr15, Asn175	Met13[b], Val23[b], Phe158[b], Leu174[b] Asn175[c], Asn191[e], Asn191[a]
Emtricitabine		NRTI	-5.5	Leu14, Arg16	Thr15[c], Arg16[a], Asp19[e], Ala29[b], Lys31[b], Lys31[a]
Etravirine		NNRTI	-8.9	Met13(2), Arg16, Asn70	Val23[b], Lys31[a], Leu174[b], Phe178[b], Asn191(2)[a]
Lamivudine		NRTI	-5.9	Gln69, Glu162	Ala29[b], Ala29[d]
Nevirapine		NNRTI	-6.3	Leu174, Asn175	Gln168[c],Cys173[f], Leu174(2)[b]
Rilpivirine		NNRTI	-9.0	Leu14, Thr15, Gln168	Thr15[d], Lys31[a], Leu174[b], Asn191[a]
Stavudine		NRTI	-6.6	Gln69, Glu162, Gln163	Leu14[b], Ser28[g], Ala29[d]
Tenofovir		NRTI	-5.8	Asn135(2), Tyr177	Ser133[c], Lys160[c]
Zalcitabine		NRTI	-6.2	Arg16(2), Gln69	Leu14[b], Arg16[a], Ala29(2)[b], Asn164[c]

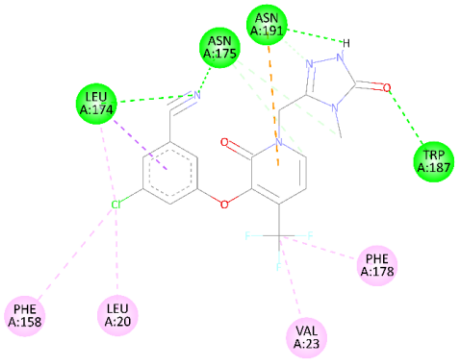
Table 1 (*continue*). Binding residues of the antiviral drugs and BIBR1532 to the TEN domain of TERT

Ligands	Structure	Classification	Binding energy (kcal/mol)	Conventional hydrogen bonding residues	Other interaction residues
Zidovudine		NRTI	-5.3	Gln91, Val119(2)	Asp95[a], Tyr118[g]
BIBR1532			-8.1	Arg16(2), Ser28	Ser28[g], Ala29[d], Lys31[b], Asn191[a]

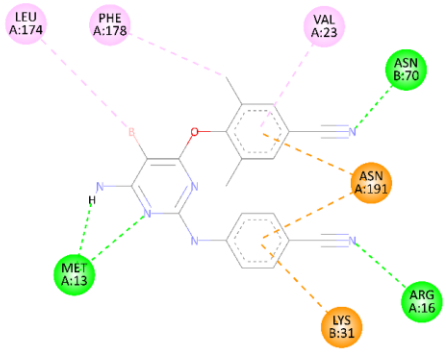
[a]pi-ion, [b]alkyl/pi-alkyl, [c]carbon-hydrogen bond, [d]pi-sigma, [e]halogen, [f]pi-sulfur, [g]pi-pi



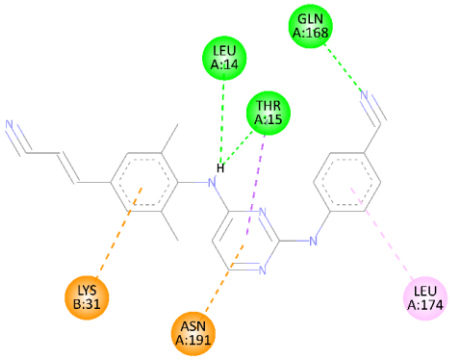
Predicted binding pocket



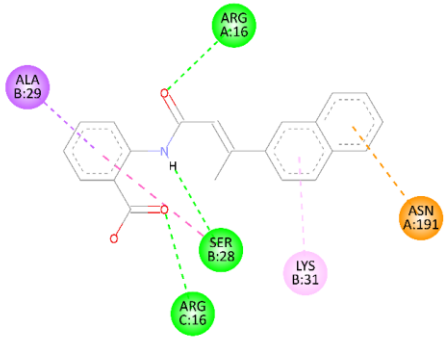
Doravirine



Etravirine



Rilpivirine



BIBR1532

Figure 1. Binding pocket of the target protein and binding profiles of Doravirine, Etravirine and Rilpivirine and BIBR1532 with the TEN domain of TERT (2B2A)

In the docking analysis, the antiviral drugs with the highest binding potential had common interaction residues with the BIBR1532. Doravirine had common interaction points at Asn191 amino acid residue. Etravirine had also common interaction points at Arg16, Lys31, and Asn191 residues. Similarly, Rilpivirine had common interaction points at Lys31 and Asn191 residues. The interaction of the antiviral drugs and the reference ligand implicated that the interaction of the compounds with Asn191 residue played a role for achieving effective binding. A previous computational study revealed that some catechin derivatives had interactions through Gln168 residue as observed in the binding of Rilpivirine in this study. The same study reported that the interaction through Gln162 residue was essential for effective binding of catechins to the TEN domain [25]. In this study, Didanosine, Lamivudine and Stavudine had interactions through this residue but the binding affinity and/or strength of these compounds was less than the three highest binding compounds. Conventional hydrogen bonds were formed between Gln162 and the hydroxyl group of the catechins. Similarly, conventional hydrogen bonds were formed between Gln162 and hydrogen bond donor groups (hydroxyl, amine) of the highest binding compounds in this study.

Structure-activity relation analysis of Doravirine, Etravirine and Rilpivirine revealed that the cyanide (-C≡N) functional group in their structures contributed for effective binding of these drugs to the TEN domain. Two conventional hydrogen bonds of Doravirine and Etravirine were formed between the cyanide functional group and the amino acid residues. Similarly, a conventional hydrogen bond was formed between this functional group of Rilpivirine and the TEN domain (Figure 1). Conventional hydrogen bonds were formed between the amino group of the compounds and various amino acid residues of the target. In this regard, Etravirine had one and Rilpivirine had two conventional hydrogen bonds through their amino group (Figure 1). Similarly, conventional hydrogen bonding was formed with the hydrogen on the nitrogen of the triazole ring for Doravirine and the nitrogen of the pyrimidine ring for Etravirine.

Earlier research performed by scientists has shown that Zidovudine inhibits the action of telomerase and induces the shortening of telomeres. The findings from our molecular docking and molecular dynamics investigation demonstrated that the binding potentials of Doravirine, Etravirine, and Rilpivirine, which are non-nucleoside reverse transcriptase enzyme inhibitors, were higher with binding energies of -8.7 kcal/mol, -8.9 kcal/mol, and -9.0 kcal/mol, respectively. The binding potential of the nucleoside reverse transcriptase enzyme inhibitor Zidovudine was found to be less as its binding energy was higher (-5.3 kcal/mol).

Molecular Dynamics Simulation

The molecular docking study demonstrated that some of the antiviral drugs would have better binding affinity than the reference TERT inhibitor. The stabilities of the complexes formed between the TEN domain and these drugs (Doravirine, Etravirine and Rilpivirine) were investigated by performing MD simulation analysis. To this end, RMSD of the protein, RMSD of the ligands, RMSF, and Rg plots were drawn. A general notion about the effect of ligand binding to the protein's stability was measured by drawing the changes in the RMSD value of the backbone structure. The state of remaining inside the binding pocket of the protein for the ligands during the simulation period was evaluated by using the ligand RMSD value [26,27].

The overall stability of the backbone protein for the compound bearing structures was lower than the unbound structure. The unbound protein structure had the lowest RMSD value after the 18 ns and gave the least RMSD variation during the simulation period (Figure 2). Etravirine bearing complex had lower RMSD value than the other two compound bearing structures. However, it had sharp rises and declines in some time intervals. Doravirine bearing complex had similar RMSD value with Etravirine bearing complex after the 150 ns time but a higher RMSD value till then. Together with this, its RMSD value variation was not as sharp as that of Etravirine bearing complex. Rilpivirine bearing complex had the highest RMSD value and variations during the simulation period. Especially, in the 20-100 ns time interval, it gave high rises and falls that implicated instability for this complex (Figure 2).

The state of the compounds to remain inside the binding pocket of the target was evaluated by drawing the RMSD plot of the compounds in relative to the protein structure. The ligand RMSD plot demonstrated that Doravirine could remain inside the binding pocket during the simulation period. In

general, the RMSD value of Doravirine was below 0.5 nm (Figure 2). Etravirine had above 0.5 nm RMSD value with some exception time intervals. In addition to this, it had a sharp RMSD value rise in the 132-139 ns time interval. The compound might fly out of the binding pocket in this interval. Rilpivirine had generally high RMSD value that went up to 2.5 nm and also high variations throughout the simulation period. The high degree of changes in the RMSD value implicated that the compound would move in and then out of the binding pocket. Therefore, this would hinder its binding potential to form a stable complex with the protein. The RMSD plots demonstrated that Doravirine had the highest potential to remain inside the binding pocket and thus retain its binding potential to form a stable protein-compound complex.

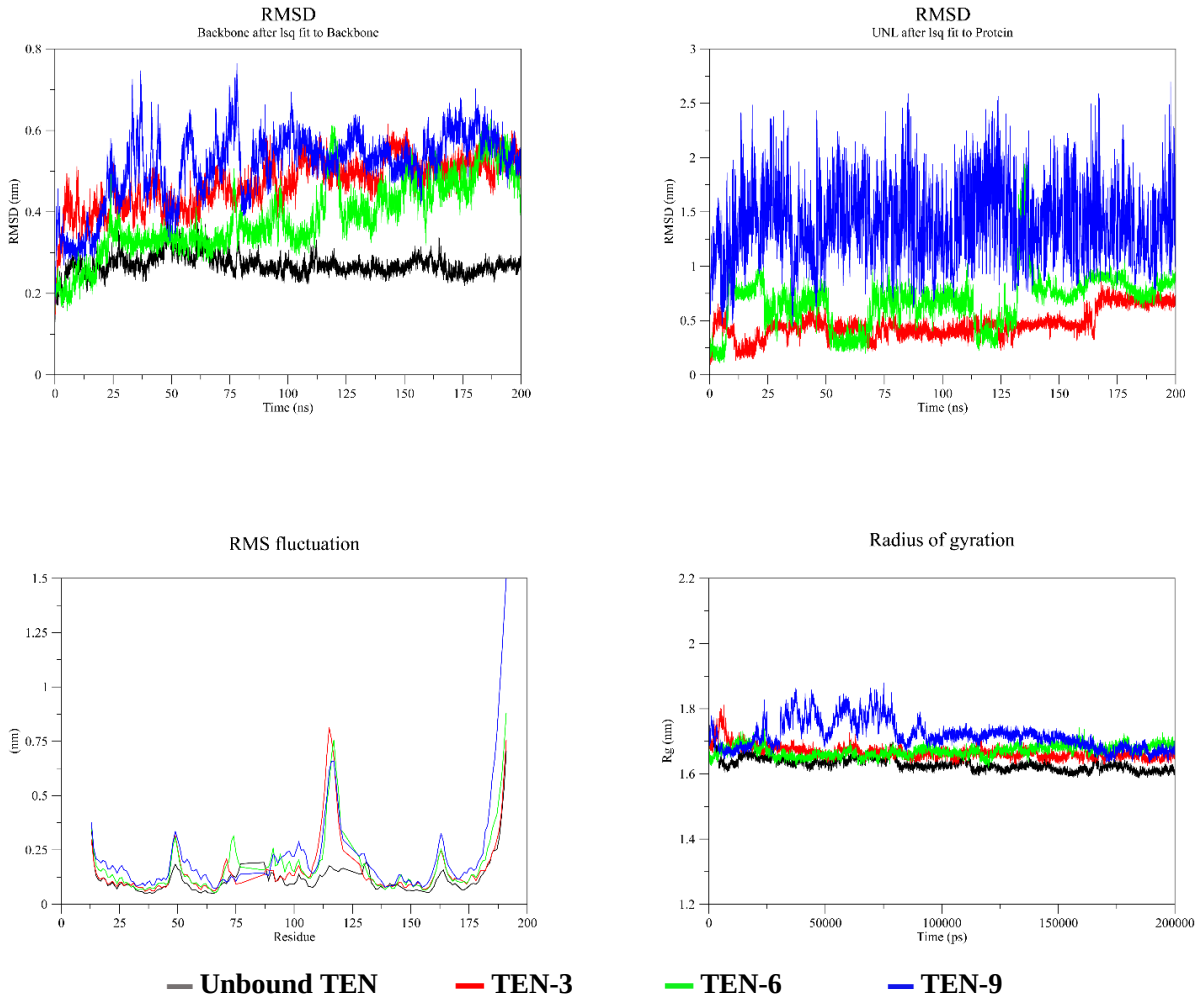


Figure 2. RMSD, RMSF, and Rg plots from the MD simulation trajectories

The per-residue fluctuations of the unbound protein and complex structures were evaluated by the RMSF plot. In general, all of the evaluated structures had similar RMSF plots throughout the simulation period. A significant RMSF value change was detected in the C-terminal for all of the structures. Similarly, all of the structures except the unbound protein had significant RMSF value changes in the 110-130 amino acid intervals (Figure 2). The effect of compound binding on the compactness of the structures was evaluated via Rg plot [28]. The compactness of the protein structure has decreased by the binding of Rilpivirine on it. Especially, in the 20-95 ns time interval, the complex was less compact than the other complexes and the unbound protein. The effect of the binding of Doravirine and Etravirine on the firmness of the protein was similar as they gave similar Rg values

during the simulation period. Furthermore, the change in the firmness of the protein structure by the binding of Doravirine and Etravirine was insignificant.

Conclusion

Antiviral drugs were evaluated for their potential to inhibit cancer by targeting the TEN domain of TERT. Doravirine, Etravirine and Rilpivirine showed higher binding affinity to the TEN domain compared to BIBR1532, the reference TERT inhibitor, according to the results of the molecular docking study. Molecular dynamics simulation showed that Doravirine formed a stable combination with the protein. Thus, Doravirine was able to persist in the binding pocket of the TEN domain and maintain its binding ability throughout the duration of the simulation. In short, Doravirine was able to bind to the TEN domain and inhibit it. The potential of Doravirine as an anticancer agent by inhibiting the TEN domain of TERT should be confirmed by further *in vitro* and *in vivo* investigations based on computational studies. Based on this investigation, we will design the structures of telomerase enzyme inhibitor compounds to be developed, using the structures of these 3 drugs as a reference.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflicts of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

CHEMICAL COMPOSITION AND BIOACTIVITIES OF ESSENTIAL OIL FROM AN ENDEMIC *SALVIA ABSCONDITIFLORA* GREUTER & BURDET

ENDEMİK SALVIA ABSCONDITIFLORA GREUTER & BURDET UÇUCU YAĞININ KİMYASAL İÇERİĞİ VE BİYOAKTİVİTELERİ

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ABSTRACT

Objective: *The study aimed to determine the chemical composition and antibacterial, antibiofilm, and anti-quorum sensing activities of the essential oil of Salvia absconditiflora Greuter & Burdet (an endemic species) growing wildly in Türkiye.*
Material and Method: *The essential oil from the aerial parts of the plant was obtained by hydro-distillation (0.4%) and analyzed by GC-FID and GC-MS. In addition, the broth microdilution method was used to determine antibacterial activity. The crystal violet assay was performed for antibiofilm activity, and the reporter bacteria Chromobacterium violaceum ATCC 12472 was used in the anti-quorum sensing activity test.*
Result and Discussion: *The major components of the essential oil were identified as 1,8-cineole (32.2%), camphor (13.6%), α-pinene (7.6%), camphene (5.5%), and viridiflorol (5.1%). The essential oil showed the best antibacterial activity against Gram-positive test bacteria, with a minimum inhibitory concentration (MIC) of 0.0078 (v/v) against Staphylococcus aureus strains. The percentage biofilm inhibition value of the essential oil was determined as 84.4%. The inhibition of violacein production by the essential oil in Chromobacterium violaceum ATCC 12472 indicated the possibility of anti-quorum sensing activity. The results of this study show that the essential oil of S. absconditiflora could be a promising alternative in fighting bacterial infections.*
Keywords: *Antibacterial activity, antibiofilm activity, anti-quorum sensing activity, essential oil, Lamiaceae, Salvia absconditiflora*

ÖZ

Amaç: *Bu calışma, Türkiye'de doğal olarak yetişen Salvia absconditiflora Greuter & Burdet (endemik) uçucu yağının kimyasal bileşimini ve antibakteriyel, antibiyofilm ve anti-quorum sensing aktivitelerini belirlemeyi amaçlamıştır.*
Gereç ve Yöntem: *Bitkinin toprak üstü kısımlarından su distilasyonu yöntemi ile elde edilen uçucu yağın verimi (%0.4) belirlenmiş, GC-FID ve GC-MS cihazları ile kimyasal içeriği tayin edilmiştir. Ayrıca S. absconditiflora'dan elde edilen uçucu yağın antibakteriyel aktivitesi mikrodilüsyon yöntemi ile belirlenmiştir. Antibiyofilm aktivite için kristal viyole yöntemi ve anti-quorum sensing*

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aktivite için raportör bakteri Chromobacterium violaceum ATCC 12472 kullanılmıştır.
Sonuç ve Tartışma: *Uçucu yağın ana bileşenleri 1,8-sineol (%32.2), kafur (%13.6), α-pinen (%7.6), kamfen (%5.5) ve viridiflorol (%5.1) olarak belirlenmiştir. Uçucu yağ, Gram-pozitif bakterilerine karşı en iyi antibakteriyel aktiviteyi, Staphylococcus aureus suşlarına karşı 0,0078 (h/h) minimum inhibitör konsantrasyonu (MIC) ile göstermiştir. Ayrıca uçucu yağın biyofilm inhibisyon değeri %84.4 olarak belirlenmiştir. Chromobacterium violaceum ATCC 12472 suşunda viyolasin üretiminin engellenmesi de, olası anti-quorum sensing aktivitesinin varlığını göstermiştir. Yapılan çalışmaların sonucunda, S. absconditiflora uçucu yağının bakteriyel enfeksiyonlarla mücadelede umut verici bir alternatif olabileceği belirlenmiştir.*
Anahtar Kelimeler: *Antibakteriyel aktivite, antibiyofilm aktivite, anti-quorum sensing aktivite, Lamiaceae, Salvia absconditiflora, uçucu yağ*

INTRODUCTION

Plants and their secondary metabolites have an important place in traditional medicine. People use herbs to improve their health. Herbs will continue to play a significant role as a tool in health related to current research and investments [1]. Plant bioactive compounds have been an attractive target due to their biological effects and potential for biotechnological use [2]. Volatile oils are produced in various plant organs and have numberless healing effects in human diseases [3]. The Lamiaceae (Labiatae) family, which includes about 7200 species in 237 genera, mainly spreads in the Mediterranean and Central Asia [4]. A reputable aromatic and medicinal plant, *Salvia* L. (sage), a relatively large genus in Lamiaceae, comprising over 900 species in the world [5], is represented naturally by 89 species (with 50% endemism) in the "Flora of Turkey." Anatolia is an important gene center in Asia [6-8]. The name *Salvia* is derived from "salvare," meaning "to heal" and "to save" in Latin [9]. Most *Salvia* species are native to the Mediterranean region and are traditionally used for bronchitis, cough, asthma, digestive and circulatory disorders, excessive sweating, angina, inflammation, memory problems, and depression [10]. These species are rich sources of polyphenols, diterpenes, and triterpenes [9-12] and are also rich in essential oils. The *Salvia* species mainly exhibits antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antiviral, hepatoprotective, anticancer, and antidepressant effects [9,13-15].

Salvia absconditiflora Greuter & Burdet (Syn: *Salvia cryptantha* Montbret & Aucher ex Benth), which has been used in folk medicine for many years, is endemic to Türkiye. *S. absconditiflora* called "tapir" in Turkish, is a perennial herb that grows at an altitude of 700 to 2500 m on rocky lands, dry places, calcareous hills, and fallow lands [6]. According to regional knowledge, *S. absconditiflora*, is a wild plant in Central Anatolia, and its flowers in dry form are used as herbal tea [16]. Studies have mostly been done on this species regarding essential oil composition collected from different localities [17,18]. In a study, *S. absconditiflora* was collected from eight different provinces of Türkiye, and its phytogeographic effects on essential oil composition were examined. The main components of all species were demonstrated as *α*-pinene, camphene, 1,8-cineole, camphor, and borneol [18]. In a different study investigating the antimicrobial effects of essential oil of six species growing in Türkiye, including *S. absconditiflora*, the activity of this species was observed at low or medium levels [19]. Additionally, the antifungal and bioherbicidal properties of *S. absconditiflora* essential oil have been reported to use as a natural fungicide and herbicide due to its potent activity [20].

Antibiotic resistance is one of the most critical health concerns worldwide, as new strains of resistant bacteria have been reported from different countries. Infections are presently a significant cause of these bacteria for morbidity and mortality. All these consequences bring us to find urgently new compounds with antibacterial effects to treat these diseases that emerged from resistant bacteria. The difficulties in discovering new antibacterials and the need for long research have led researchers to discover new molecules that can inhibit other mechanisms involved in pathogenicity [21,22]. One of those mechanisms, Quorum Sensing (QS), effectively synthesizes virulence factors contributing to pathogenicity and biofilm formation. Scientists have recently focused on antibiofilm and anti-quorum sensing molecules as alternative compounds to treat bacterial infections. Based on the current research, anti-quorum sensing and antibiofilm compounds, especially by scanning natural resources, would effectively control the resistance problem [23-26]. Since the beginning of human history, plants and essential oils have been used for different purposes. One of the most important effects of many essential

oils is their antimicrobial potential. They seem to be a potential alternative to synthetic compounds, mainly due to the increasingly developed resistance of pathogenic microorganisms [27].

Therefore, in this current study, the essential oil composition of *Salvia absconditiflora* was investigated for the first time collected from the indicated locality, as well as a comprehensive evaluation of its antibacterial, antibiofilm, and anti-quorum sensing activity.

MATERIAL AND METHOD

Plant Material

An endemic species, *Salvia absconditiflora* Greuter & Burdet (Syn: *Salvia cryptantha* Montbret & Aucher ex Benth) from the Lamiaceae family was collected from the Ankara-Eskişehir Road in Türkiye on 12/06/2021. Taxonomic identification of the plant material was confirmed by Prof. Dr. Hayri Duman from the Department of Biology, Gazi University. The voucher specimen was deposited at the Herbarium of Ankara University (AEF) with the registered number AEF 28898.

Essential Oil Distillation

The air-dried aerial parts (with flowers) of the plant in the flowering period were subjected to hydro-distillation for 3-4 hrs. using a Clevenger-type apparatus. The essential oil was dehydrated on anhydrous sodium sulfate, filtrated, and kept at +4°C until tested and analyzed. The oil yield was 0.4% *v/w* (200 g of the plant gave 0.8 ml, 455 mg oil).

Gas Chromatography (GC) Analysis

An Agilent 6890N GC system was used for GC analysis. The FID detector temperature was adjusted to 300ºC, and simultaneous automatic injection was performed to replicate the same column, applying the same process conditions to achieve the same elution order as by GC-MS. The relative percentage amounts of the separated components were determined with the help of FID chromatograms, and the analysis results are given in Table 1. The essential oil components were identified by comparing their relative retention times with the original samples or their relative retention indices (RRI) with a range of n-alkanes. Commercial libraries are known as Wiley GC/MS Library, and MassFinder Software 4.0 [28,29] was used to identify components. In addition, a computer comparison with the in-house "Baser Essential Oil Components Library" consisting of original compounds and components of known oils was also used to determine the essential oil composition.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min, and then programmed to 240°C at a rate of 1°C/min. The split ratio was kept to 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. The mass range was from *m/z* 35 to 450.

Antibacterial Activity

The essential oil's MIC (Minimum Inhibitory Concentration) values were determined by the broth microdilution method. *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* ATCC 43300 (MRSA), *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 were used as test bacteria [26,30]. Serial two-fold dilutions of essential oil (0.25 to 0.002 *v/v*) were prepared in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA) supplemented with Tween 80 (Merck, Germany) to adjust a final concentration of 0.5% (*v/v*). The final test concentration of the bacteria was adjusted to 5×10^5 CFU/ml. After the incubation at 35°C for 18-24 hrs., the last well that completely inhibited visual bacterial growth was noted as the MIC value. A set of wells containing only inoculated broth supplemented with Tween 80 was used as the negative control.

Antibiofilm Activity

Before performing the antibiofilm activity test, the MIC value of the essential oil against *Pseudomonas aeruginosa* PAO1 was determined. Antibacterial activity was not observed. The antibiofilm activity test was performed by *in vitro* microplate-based biofilm model against *P. aeruginosa* PAO1 using the crystal violet assay [26,31-33].

For biofilm formation, *P. aeruginosa* PAO1 was incubated for 24 hrs at 37ºC in Brain Heart Infusion (BHI) Broth. The final inoculum suspension containing *P. aeruginosa* (~10^6 CFU/ml) was prepared in BHI enriched with 2% sucrose. 100 µl of the inoculum suspensions were added to 96-well microtiter plates for all test conditions. The plates were incubated at 37°C for 72 hrs. to form mature biofilm.

After forming the mature biofilm layers, the medium was aspirated, and non-adhered cells were removed by washing the wells with sterile phosphate-buffered saline (PBS, pH 7.2). 100 µl of essential oil was transferred into each well containing mature *P. aeruginosa* biofilms. The plates were incubated at 37°C for 24 hrs. After incubation, the content of the wells was poured off, and the wells were washed with PBS. The plates were then dried at room temperature for 1 hr. 100 µl of 0.5% crystal violet solution was added to each well for staining the biofilm cells. After 30 min, the wells were washed three times with PBS. Then, acetone-alcohol (30:70 *v/v*) solution was added to the wells to dissolve the bound dye within the biofilm matrix. BHI Broth enriched with 2% sucrose was used as a control. The optical density of the dissolved crystal violet dye was measured by a microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Vantaa, Finland) at 620 nm (OD 620 nm). The percentage biofilm inhibition values were calculated according to the following formula:

% Biofilm inhibition = [(OD (Growth control) – OD (Sample))/OD (Growth control)] x 100

Antiquorum Sensing Activity

The disc diffusion method was used to perform the anti-quorum sensing activity test. *Chromobacterium violaceum* ATCC 12472 was used as the reporter bacteria [26,34,35]. The density of the overnight bacterial culture was adjusted to 1.5x10^8 CFU/ml. Then, the bacterial suspension was inoculated on Luria Bertani Agar, and a sterile blank disc (6 mm diameter; Bioanalyse®, Ankara, Türkiye) impregnated with twenty microliters of the essential oil (0.15 mg/µl) was placed on the medium. Luria Bertani Broth was used as the negative control. After incubation at 30°C for 24 hrs, the plates were observed for a zone of violacein inhibition. The formation of an inhibition zone around the disc was noted as the potential anti-quorum sensing activity.

RESULT AND DISCUSSION

The present study reported the essential oil composition of *Salvia absconditiflora* with antibacterial, antibiofilm, and anti-quorum sensing activities. Traditionally, people have used *Salvia* species for various ailments, which may be the main components responsible for their biological properties.

Ordinarily, in *Salvia* species, essential oils of all aerial parts found in the glandular trichomes were an average concentration of 1.3-3.6% by dry weight, almost all. In comparison, the essential oil rate is maximum in leaves, low in flowers, and lowest in branches. In previous studies, both aerial parts and flower essential oils of *S. absconditiflora;* have the major components as 1,8-cineole (30.38 and 36.28%), valencene (24.34 and 26.53%), and camphor (12.29 and 14.72%) [36]. In addition, valencene (31.80%) was also determined as the leading compound from the aerial parts of this plant in native and field-grown species [37]. Moreover, the seed oils of several *Salvia* species, including *S. absconditiflora* were investigated and the *γ*-tocopherol was abundant component in most of the seed oils [38]. The significant components in *S. absconditiflora* from Southern Turkish forms were determined as camphor (25.6%) and 1,8-cineole (20.3%), which could be used as flavor and fragrance agents in some products [39].

In the current study, the essential oil concentration was 0.4% in *S. absconditiflora*. The composition of hydrodistilled essential oil from aerial parts almost consisted of flowers of *S.*

absconditiflora was determined by Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) simultaneously. The study of the volatiles of *S. absconditiflora* revealed 48 different components, representing 93.5% of its chemical composition. The main components were 1,8-cineole (32.2%), camphor (13.6%), *α*-pinene (7.6%), camphene (5.5%), and viridiflorol (5.1%) in the essential oil of *S. absconditiflora* (Table 1).

Table 1. The composition of essential oil from *Salvia absconditifolia* Greuter & Burdet

No	RRI	Components	%	IM
1	1014	Tricyclene	0.2	RRI, MS
2	**1032**	***α*-Pinene**	**7.6**	RRI, MS
3	**1076**	**Camphene**	**5.5**	RRI, MS
4	1118	*β*-Pinene	3.8	RRI, MS
5	1132	Sabinene	0.3	RRI, MS
6	1174	Myrcene	0.8	RRI, MS
7	**1213**	**1,8-Cineole**	**32.2**	RRI, MS
8	1246	(*Z*)-*β*-Ocimene	0.3	MS
9	1255	*γ*-Terpinene	0.5	RRI, MS
10	1266	(*E*)-*β*-Ocimene	0.1	MS
11	1280	*p*-Cymene	0.3	RRI, MS
12	1290	Terpinolene	0.1	RRI, MS
13	1452	1-Octen-3-ol	0.1	MS
14	1474	*trans*-Sabinene hydrate	0.4	MS
15	1466	*α*-Cubebene	2.0	MS
16	1493	*α*-Ylangene	tr	MS
17	**1532**	**Camphor**	**13.6**	RRI, MS
18	1535	*β*-Bourbonene	0.7	MS
19	1590	Bornyl acetate	0.5	RRI, MS
20	1612	*β*-Caryophyllene	2.5	RRI, MS
21	1611	Terpinen-4-ol	1.0	RRI, MS
22	1719	Borneol	2.1	RRI, MS
23	1704	*γ*-Muurolene	4.3	MS
24	1740	*α*-Muurolene	0.6	MS
25	1742	*β*-Selinene	0.3	MS
26	1744	*α*-Selinene	0.9	MS
27	1776	*γ*-Cadinene	2.3	MS
28	1796	Selina-3,7(11)-diene	0.2	MS
29	1799	Cadina-1,4-diene	0.2	MS
30	1804	Mrytenol	0.3	MS
31	1807	*α*-Cadinene	0.1	MS
32	1849	Calamelene	0.6	MS
33	1900	*epi*-Cubebol	0.2	MS
34	1941	*α*-Calacorene	0.2	MS
35	1953	Palustrol	0.4	MS
36	1984	*γ*-Calacorene	0.1	MS
37	2008	Caryophyllene oxide	0.8	RRI, MS
38	2057	Ledol	0.3	MS
39	2088	1-*epi*-Cubenol	0.1	MS
40	**2104**	**Viridiflorol**	**5.1**	MS
41	2145	Valeranone	0.7	MS
42	2257	*β*-Eudesmol	0.7	MS
43	2324	Caryophylladienol II	0.1	MS
44	2392	Caryophyllenol II	0.1	MS
45	2500	Pentacosane	0.1	RRI, MS
46	2622	Phytol	0.1	MS

Table 1 (*continue*). The composition of essential oil from *Salvia absconditifolia* Greuter & Burdet

No	RRI	Components	%	IM
47	2600	Hexacosane	0.1	RRI, MS
48	2670	Tetradecanoic acid	tr	RRI, MS
Total			**93.5**	

RRI: Relative retention indices calculated against *n*-alkanes; % calculated from FID data; tr: Trace (< 0.1 %)
IM: Identification method, RRI, identification based on the relative retention times of genuine compounds on the HP Innowax column; MS, identified based on computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.
Monoterpene hydrocarbons 19.5 %, Oxygenetaed monoterpenes 50.1 %
Sesquiterpene hydrocarbons 15.0 %, Oxygenetaed sesquiterpenes 8.5 %
Fatty acid+esters (tr), Diterpenes 0.1 %, Others 0.3 %

Herbs and essential oils have many pharmacological properties that are used in medicine because of their health properties. Essential oils have been used in many fields for significant antimicrobial effects [27]. Many recent articles have reported the antimicrobial properties of extracts, essential oils, resins, and various plant phytochemicals. However, detailed studies on plant-derived antimicrobial agents used in practical applications to improve human health are still incomplete [40]. In a previous study, the methanolic extract of *S. absconditiflora* showed inhibitory activity against three strains of HRoV (EC_{50} values ranged from 5.8 μg/ml to 25.5 μg/ml) in a dose-related manner. Moreover, it was inactive or hardly active against other RNA viruses called human rhinovirus and respiratory syncytial virus [41]. The ethanolic extract *S. absconditiflora* exhibited marked results in wound healing activity in rats [42]. The essential oil of *S. absconditiflora* also inhibits the growth of pathogenic microorganisms as scavenging free radicals [43]. A study also concluded that *S. absconditiflora* (black weed) extract protects against liver damage caused by CCl_4-induced and may be helpful as a hepatoprotective agent to combat the toxic effects caused by CCl_4 and other chemicals [44]. *S. absconditiflora* is also in a formulation called YXFMs (Yixin-Fumai granules), used in Chinese traditional medicine to treat bradyarrhythmia and increase heart rate besides being effective as a remedy for sick sinus syndrome [45]. In another study, the significant component of *S. absconditiflora* oil, 1,8-cineole, is a component of many drugs due to its anti-inflammatory, mucolytic, antiseptic, and antimicrobial properties [46,47].

On the other hand, camphor possesses many biological activities, including antiviral and antimicrobial activities [48]. Essential oils containing 1,8-cineole have been reported to be used as folk remedies for decades. In addition, several studies have revealed that 1,8-cineole has been used as an active component in various diseases [47]. Gum Arabica, obtained from Acacia species, is a commercial natural product that contains many active bio components. The extracts of this product inhibited violacein production at MIC and sub-MIC concentrations in *Chromobacterium violaceum* CV12472 and nucleation detection under externally supplied acyl-homoserine lactone. The extracts also exhibited antimicrobial activity (MIC = 0.1562 mg/ml-2.5 mg/ml), especially at MIC and lower MIC concentrations, showed excellent biofilm inhibition against *E coli* [49]. Propolis is one of the trendy traditional medicines due to its antimicrobial activity and antioxidant effects. The extract had a notable activity with an inhibition diameter zone of 18.0±1.0 mm, and its active components were also determined. Samples blocked *P. aeruginosa* PA01 herd motility at the three concentrations (50, 75, and 100 g/ml) in a dose-dependently. This completed study reported that Propolis extract and its components inhibit biofilm formation [50].

In this context, we investigated the antibacterial (Table 2), antibiofilm, and anti-quorum sensing activities of *S. absconditiflora* essential oil. In the present study, 1,8-cineole or eucalyptol is the major component (32.2%) in the essential oil of *S. absconditiflora*. The current analysis of the essential oil of *S. absconditiflora* exhibited marked antibacterial activity against Gram-positive test bacteria with MIC values between 0.0078 and 0.0625 (*v/v*). For this reason, the notable antimicrobial effect of the *S. absconditiflora* essential oil is attributed to these major components, especially the 1,8-cineole.

Biofilms are defined as intense populations of sessile bacterial cells adhering to the surface and forming a protein, DNA, and exopolysaccharide matrix. The biofilm formation mechanism of this matrix, called extracellular polymeric substance, occurs by providing stability to the cells holding it. It also assisted in the pathogenesis of biofilm-associated infections and resistance by providing nutrients.

Biofilms promote bacterial persistence by resisting host immune responses and antibiotic treatment [51]. This study determined the percentage biofilm inhibition value of tested essential oil to be 84.4%. According to the results, the essential oil has notable antibiofilm activity (Figure 1).

Table 2. Minimum inhibitory concentration (MIC) values of essential oil (*v/v*) against tested bacteria.

Essential Oil	Gram-positive Test Bacteria			Gram-negative Test Bacteria		
	S. aureus **ATCC 25923**	***S. aureus*** **ATCC 43300 (MRSA)**	***E. faecalis*** **ATCC 29212**	***E. coli*** **ATCC 25922**	***K. pneumoniae*** **ATCC 13883**	***P. aeruginosa*** **ATCC 27853**
EO	0.0078	0.0078	0.0625	0.0625	0.125	-
DMSO (10%)	-	-	-	-	-	-

'-': represents no activity

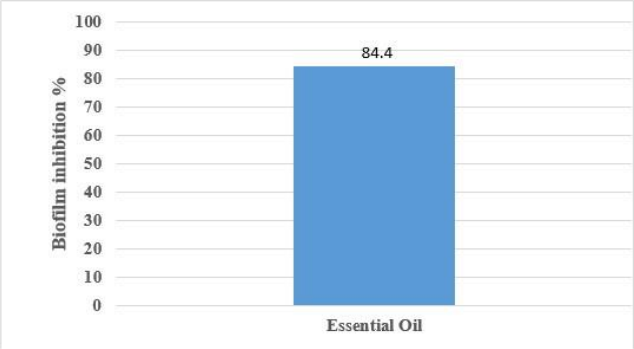

Figure 1. Antibiofilm activity of essential oil of *Salvia absconditiflora*

The global threat of antimicrobial resistance development leads to a constant demand to discover new antimicrobial drugs and antivirus agents. The so-called 'quorum-sensing' is a signaling system that regulates cellular processes such as bacterial cell-to-cell communication [52]. Thus, objection to the bacterial pathogenic potential through quorum-sensing inhibition is welcomed as a new strategy to struggle with microbial resistance [53,54]. The anti-quorum sensing activity inhibited the quorum-sensing-controlled violacein pigment production by the sensor bacteria. The disc produces a transparent inhibition zone representing the potency of quorum-sensing's inhibitory effect. This effect tested on the essential oil is shown in Figure 2.

Figure 2. QS inhibitory activity of essential oil of *Salvia absconditiflora*
SAEO: *Salvia absconditiflora* essential oil, C: Control, LB Broth

For all these reasons, quorum sensing inhibition is new hope in tackling multi-antibiotic-resistant bacteria. Instead of bactericidal or bacteriostatic strategies, inhibition of bacterial nucleotide detection systems is thought to find applications in medicine, agriculture, and food technology [55,56].

The random use of antimicrobial drugs has led to the rise of resistant bacteria, fungi, and viruses. Developing more effective antimicrobial agents with the help of new mechanisms on activities is necessary to overcome the increased resistance of pathogenic microbes. Medicinal and aromatic plants that treat various diseases appear to be the source of secondary metabolites and essential oils. For this reason, studies on the screening of these metabolites of plants in terms of antimicrobial activities are increasing. Because of their effects, essential oils can be considered an alternative to antibiotics. The results support the medical application of these oils to prevent and treat certain infections and diseases and suggest further studies. In conclusion, the essential oil of *Salvia absconditiflora* may represent a new source of antibacterials in the future treatment of bacterial infection-related ailments in the pharmaceutical and food industry.

AUTHOR CONTRIBUTIONS

Concept: A.O.; Design: A.S.C.K., A.O.; Control: A.O.; Sources: A.S.C.K., A.O.; Materials: A.O.; Data Collection and/or Processing: A.S.C.K., S.S.R., M.E., B.D., A.O.; Analysis and/or Interpretation: M.E., B.D.; Literature Review: A.S.C.K., A.O.; Manuscript Writing: M.E., A.O.; Critical Review: A.S.C.K., S.S.R., M.E., B.D., A.O.; Other: -

CONFLICTS OF INTEREST

The authors declare that this article has no real, potential, or perceived conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DEVELOPMENT OF A FAST LIQUID CHROMATOGRAPHY METHOD WITH A CHEMOMETRIC APPROACH BASED ON BOX-BEHNKEN DESIGN FOR THE DETERMINATION OF ANTIDEPRESSANTS IN PHARMACEUTICAL FORMULATIONS

FARMASÖTİK FORMÜLASYONLARDAKİ ANTİDEPRSANLARIN TAYİNİ İÇİN BOX-BEHNKEN TASARIMINA DAYANAN KEMOMETRİK YAKLAŞIM İLE HIZLI SIVI KROMATOGRAFİ YÖNTEMİNİN GELİŞTİRİLMESİ

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ABSTRACT

Objective: *The objective of this work was to develop a liquid chromatographic method for the quantification of antidepressants, namely duloxetine (DXN), fluoxetine (FXN), citalopram (CIT), paroxetine (PXN), and sertraline (SRN), by a chemometric approach based on Box-Behnken design.*
Material and Method: *After initial experiments to determine significant parameters, a Box-Behnken design consisting of 17 experiment sets was carried out. All separations were conducted using an Agilent Poroshell 120 EC-C18 analytical column (75 mm × 4.6 mm × 2.7 µm).*
Result and Discussion: *The optimum levels of pH, acetonitrile ratio, and flow rate were determined with the desirability function as 2.7, 38%, and 1.1 ml/min, respectively. The differences (<8%) between predicted optimum responses and experimentally obtained results proved the model's suitability. Limits of detection and limits of quantification values were in the ranges of 0.17-0.29 µg/ml and 0.53-0.89 µg/ml, respectively. The feasibility of the technique was proven by analyzing PXN and DXN formulations.*
Keywords: *Antidepressants, design of experiments, liquid chromatography*

ÖZ

Amaç: *Bu çalışmanın amacı, duloksetin (DXN), fluoksetin (FXN), sitalopram (CIT), paroksetin (PXN) ve sertralin (SRN) adlı antidepresanların tayini için Box-Behnken tasarımına dayalı kemometrik bir yaklaşımla sıvı kromatografik bir yöntem geliştirmektir.*
Gereç ve Yöntem: *Önemli parametreleri belirlemek için yapılan ilk deneylerden sonra, 17 deney setinden oluşan bir Box-Behnken tasarımı gerçekleştirilmiştir. Tüm ayırma işlemleri bir Agilent Poroshell 120 EC-C18 analitik kolon (75 mm × 4.6 mm × 2.7 µm) kullanılarak gerçekleştirilmiştir.*
Sonuç ve Tartışma: *pH, asetonitril oranı ve akış hızının optimum seviyeleri, arzu edilebilirlik fonksiyonu ile sırasıyla 2.7, %38.2 ve 1.1 ml/dak olarak belirlenmiştir. Tahmin edilen optimum yanıtlar ile deneysel olarak elde edilen sonuçlar arasındaki farklar (<%8) modelin uygunluğunu kanıtlamıştır. Tespit ve tayin limitleri sırasıyla 0.17-0.29 µg/ml ve 0.53-0.89 µg/ml aralığındadır. Yöntemin uygulanabilirliği, PXN ve DXN'nin formülasyonlarının analiz edilmesiyle kanıtlanmıştır.*
Anahtar Kelimeler: *Antidepresanlar, deneysel tasarım, sıvı kromatografisi*

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INTRODUCTION

Depression, a chronic disorder with substantial social and economic effects, is often characterized by a lack of interest, reduced energy levels, guilt-ridden emotions, changes in sleep or eating patterns, and impaired concentration. Chronic, recurring, and widespread challenges in psychosocial and occupational functioning are commonly linked to major depressive disorder (MDD) and can have severe consequences such as long-term incapacity and potentially fatal illness [1]. On a global level, there are over 320 million individuals affected by MDD. The lifetime prevalence is 26.1% and 14.7% for female and male adults in the US, respectively [2]. In this picture, due to its rapid dissemination, MDD is expected to rank as the second leading cause of work incapacity for individuals of all genders and age ranges [3,4].

While the exact origins and processes of MDD remain unclear, several theories have been proposed to elucidate the underlying molecular mechanisms, including the monoamine, neuroplasticity, glutamate, cholinergic/adrenergic, and stress-induced hypothalamic-pituitary-adrenal axis hypotheses [5].

Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), classified as second-generation antidepressants, are now the most commonly prescribed antidepressant groups worldwide. The advent of these drugs has brought about a major change in the way depression is treated, as they are both highly effective and less likely to produce negative side effects, unlike their predecessors with poor tolerability profiles [6]. SSRIs target serotonin transporters to prevent the reabsorption of serotonin, resulting in higher levels of active serotonin in the synapses. Their impact on norepinephrine and dopamine transporters is minimal. The best-known members of this group are citalopram (CIT), escitalopram, fluoxetine (FXN), fluvoxamine, paroxetine (PXN), sertraline (SRN), and vilazodone. Through selective inhibition of the reuptake process, SNRIs effectively prolong the presence of serotonin and norepinephrine in the synaptic cleft, thus modulating their respective physiological effects. Venlafaxine, desvenlafaxine, milnacipran, levomilnacipran, and duloxetine (DXN) belong to this group. Elevated extracellular serotonin and norepinephrine concentrations result in improved neurotransmission, thus relieving central nervous system dysfunctions [1,7].

Due to the widespread use of SNRIs and SSRIs, reliable techniques are required for rapid and accurate quality control (QC) of their formulations. Liquid chromatography and spectrometry are the most widely used analytical methodologies for quantifying SSRIs and SNRIs [1]. In addition, gas chromatography [8], thin-layer chromatography [9], electrochemical [10], and electrophoretic methods [11] have been reported.

The most commonly used approach in developing HPLC methods is trial and error, which involves altering one variable at a time while maintaining the others constant. Though this approach may achieve the desired separation, there is no assurance of attaining the actual optimum conditions. Conventional stepwise optimization is not only time, money- and labor-consuming but also unpredictable and even unsuccessful in correcting errors [12]. In order to surmount this challenge, a considerable amount of factors must be meticulously determined, examined, and managed. In addition, the experimental variables are interdependent, and the step-by-step approach generates a large amount of raw data that is highly challenging to interpret to achieve optimum conditions [13]. In this context, chemometric tools combined with appropriate statistical analysis methods have gained widespread acceptance nowadays as they provide numerous benefits, including fewer experiments, reduced use of reagents, and decreased time spent in the laboratory [13,14].

Design of Experiments (DoE) is an experimental setup in which several factors are evaluated simultaneously by conducting a predefined number of experiments at predetermined levels [12]. DoE is frequently used to optimize the operating conditions of various analytical processes, achieve high extraction yields, and improve separation efficiency with minimum effort, time, and resources [14]. Chromatographic optimization typically involves utilizing response surface methodology that relies on various approaches, including Box-Behnken design (BBD) and central composite design (CCD) [15,16].

Despite the proven efficiency of DoE in chromatographic method optimization, there is a scarcity of research on this topic in existing literature. Carlsson and Norlancler developed an HPLC method for

the chiral separation of CIT, desmethyl-CIT, and didesmethyl-CIT using a surface-centered CCD [17]. The effects of methanol ratio, buffer amount, oven temperature, and pH on separation were successfully optimized. Hasnain et al. optimized an HPLC method for the determination of FXN in plasma by BBD [18]. The effect of organic solvent ratio, mobile phase pH, and flow rate on peak area and separation power were investigated. In another study, Houbart et al. optimized LC parameters using DoE to determine FXN and norfluoxetine in rat plasma, considering resolution (*Rs*), run time, and sensitivity [19]. To enhance the *Rs* between FXN and norfluoxetine while minimizing analysis time, a D-optimal experimental design was employed to optimize the chosen chromatographic factors.

To our knowledge, no study has employed the DoE approach to optimize the chromatographic separation of DXN, FXN, CIT, PXN, and SRN. Therefore, this work aims to optimize a reliable HPLC technique for the quantification of DXN, FXN, CIT, PXN, and SRN. DoE approach based on BBD with desirability function was used for the first time to optimize the chromatographic separation of these substances.

MATERIAL AND METHOD

Chemicals and Materials

All chemicals used were of analytical grade. Acetonitrile, methanol, phosphoric acid, sodium dihydrogen phosphate, and NaOH were products of Sigma Aldrich (St. Louis, MO, USA). DXN, FXN, CIT, PXN, and SRN were kindly supplied by companies Santa Farma (Şişli / İstanbul), Abdi İbrahim (Sarıyer / İstanbul), Nobel (Ümraniye / İstanbul), Ali Raif (Kağıthane / İstanbul), and Sanovel (Silivri, İstanbul), respectively. Paxera 10® tablets, which were labeled to contain 10 mg PXN, and Duloxx 30® capsules, labeled to contain 30 mg DXN, were purchased from a local pharmacy.

Preparation of Standard Solutions

Using methanol as the solvent, separate stock solutions were prepared for each analyte at 1000 µg/ml. Mixed standard solutions between 2 and 50 µg/ml were prepared by appropriately diluting stock solutions with water. The solutions were stored in a refrigerated environment at a temperature of 4°C and shielded from any light exposure.

Instrumentation and Apparatus

A Prominence-20 series HPLC instrument with an SPD-20A diode array detector (DAD) was used for all experiments. LCsolution 1.25 (Shimadzu, Japan) software was utilized for the system control and data acquisition. Sample and solution preparation involved an HI 2211 pH meter from Hanna Instruments, a magnetic stirrer, a vortex mixer, and an ultrasonic bath from Isolab Laborgerte.

Chromatographic Conditions

Agilent Poroshell 120 EC-C18 analytical column (75 mm × 4.6 mm × 2.7 µm) was utilized for analyses. A mobile phase of acetonitrile and phosphate buffer (20 mM, pH 2.7) (38.2:61.8 %v/v) was used. Flow rate was adjusted to 1.1 ml/min. All analyses were carried out at 25ºC. DAD was operated at 220 nm. 10 µl of standard solutions or samples were injected into the system.

Analysis of Commercial Formulations

For the assay of the pharmaceutical products, five Paxera 10® tablets or five Duloxx 30® capsules were randomly selected and weighed. Afterward, these tablets or capsules were homogenized, and an amount equivalent to 12.5 mg of PXN or 25 mg of DXN was transferred to a 25 ml volumetric flask and completed to volume with methanol. The suspensions were ultrasonicated for 10 min to dissolve the analytes. The tablet and capsule suspensions were diluted 25 and 50-fold, respectively, with the mobile phase to achieve an analyte concentration of 20 µg/ml. The final solution was filtered using a 0.45 µm nylon syringe filter and transferred to a vial for further HPLC analysis.

Optimization Approach

The optimization approach focused on evaluating the influence of 3 variables: pH, acetonitrile

ratio, and flow rate utilizing the BBD. The desirability function was employed to simultaneously optimize *Rs* between critical peak pairs, capacity factor (*k*) of the first peak in the chromatogram, and peak symmetry. The experimental data were evaluated by the software Design Expert Version 11.1.2 (Stat-Ease, USA).

Method Validation

Validation studies were carried out following the recommendations of the International Conference on Harmonization (ICH) and official pharmacopeias. A 10 µg/ml mixed standard solution of all analytes was analyzed 11 times for the system suitability test (SST). Linearity was verified by quadruplicate analysis of standard solutions prepared at six levels (2, 5, 10, 20, 35, 50 µg/ml). QC solutions at three different concentrations (15, 20, and 25 µg/ml) were analyzed to examine the intra- and inter-day accuracy and repeatability of the method. Four analyses were performed in the same day at each level for intra-day experiments, whereas twelve analyses were carried out on three days for the evaluation of inter-day experiments. Relative standard deviation (RSD) and % accuracy were used to express the results of repeatability and accuracy, respectively. The limits of detection (LOD) and quantification (LOQ) were statistically estimated as previously reported [20]. Peak purity values obtained for formulation analyses were evaluated to demonstrate the selectivity of the method.

RESULT AND DISCUSSION

Optimization of Chromatographic Conditions

Conventional HPLC method development typically relies on a step-by-step methodology, which can be time-consuming, solvent-intensive, and costly due to the large number of experimental runs required. In contrast, the DoE enables the concurrent manipulation of numerous variables and rapid optimization of chromatographic conditions by considering the interactions between significant factors and their collective effects on the response. For this work, the DoE approach using the BBD was preferred as it requires fewer experiments, avoids edge parameter combinations, and provides flexibility in exploring quadratic response surfaces [16,21]. BBD was used to identify the shortest run time that allows for satisfactory separation of antidepressants and proper retention of the first-eluting analyte (CIT). Initial studies showed that gradient elution is not necessary to obtain the baseline separation of analytes in acceptable run times. In this manner, isocratic elution was preferred due to its ease of application and not requiring conditioning between consecutive injections.

Acetonitrile and methanol are the most frequently utilized strong organic solvents in reversed-phase liquid chromatography separations. Instead of methanol, acetonitrile was opted as the strong organic solvent, considering the relatively smaller particle size of the employed analytical column, which could have generated high backpressures exceeding the pressure limit of conventional HPLC systems when methanol with high viscosity was used in the mobile phase composition. Following initial experiments, the main parameters affecting the separation were identified as the acetonitrile ratio (%B), pH, and flow rate. Since the initial experiments showed limited effects of temperature and buffer concentration, they were excluded from the DoE to reduce the number of experiments. *Rs* of FXN-SRN, *Rs* of CIT-PXN, *k* of CIT, and average *T* were selected as the responses.

Table 1 displays the BBD matrix and the corresponding experimental results. In order to calculate the experimental error, the experiments were repeated five times at the center point, while all other runs were randomly conducted without duplication.

Multiple regression analysis was used to fit the experimental data to a quadratic polynomial model. The resulting equations, representing the corresponding relationships, are as follows:

$$Y1 = 2.9534 - 0.06625A - 4.61425B + 0.14425C + 0.02325AB - 0.01025AC + 0.12975BC + 0.147925A^2 + 2.13742B^2 - 0.159575C^2$$

$$Y2 = 1.479 + 0.18975A - 0.098125B + 0.121375C + 0.02675AB + 0.00325AC - 0.0145BC + 0.22725A^2 - 0.419B^2 - 0.044C^2$$

$$Y3 = 0.8838 + 0.04625A - 1.4585B + 0.01625C - 0.03925AB - 0.00225AC - 0.03575BC + 0.073475A^2 + 0.940475B^2 - 0.018525C^2$$

$$Y4 = 1.62476 + 0.1672A + 0.061325B + -0.004575C + 0.0249AB + 0.0011AC + 0.03525BC + 0.060995A^2 - 0.104655B^2 - 0.011355C^2$$

where Y1, Y2, Y3, and Y4 are the responses of *Rs* between CIT and PXN, *Rs* between FXN and SRN, *k* of CIT, and average *T*, respectively. The three chromatographic parameters are pH, the percentage of acetonitrile in the mobile phase, and flow rate, represented by A, B, and C, respectively.

Table 1. The experimental results for BBD

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
Std order	**Run order**	**A: pH**	**B: B%**	**C: Flow rate (ml/min)**	***Rs* of CIT-PXN**	***Rs* of FXN-SRN**	***k* of CIT**	**Average *T***
11	1	3.75	30	1.2	9.59	1.33	3.30	1.45
6	2	5	40	0.6	2.73	1.76	0.98	1.85
4	3	5	50	0.9	0.62	1.45	0.42	1.87
7	4	2.5	40	1.2	3.17	1.55	0.90	1.49
9	5	3.75	30	0.6	9.57	1.02	3.16	1.50
15	6	3.75	40	0.9	2.92	1.47	0.88	1.62
13	7	3.75	40	0.9	2.93	1.47	0.88	1.62
8	8	5	40	1.2	3.00	1.96	0.97	1.81
3	9	2.5	50	0.9	0.70	1.04	0.39	1.46
2	10	5	30	0.9	9.73	1.47	3.48	1.64
14	11	3.75	40	0.9	2.97	1.48	0.88	1.62
17	12	3.75	40	0.9	2.97	1.48	0.88	1.62
16	13	3.75	40	0.9	2.95	1.47	0.88	1.62
5	14	2.5	40	0.6	2.85	1.36	0.89	1.53
1	15	2.5	30	0.9	9.90	1.17	3.29	1.34
10	16	3.75	50	0.6	0.01	0.72	0.38	1.49
12	17	3.75	50	1.2	0.55	0.98	0.37	1.58

ANOVA was used for the statistical evaluation of the models, and the results are displayed in Tables S1-4. The p-value for all models was equal to or less than 0.0001, indicating statistical significance. The goodness of fit of the proposed equation can be evaluated by the regression coefficient (R^2), which helps estimate the predictive power of the model [22]. The fit of the data was considered adequate considering high R^2 and adjusted R^2 values (Tables S1-4).

In DoE, lack-of-fit (LOF) is a statistical measure that assesses how well a model fits the observed data. The use of LOF can help determine whether the model predictions significantly differ from the observed data. The LOF F-value is determined by dividing the discrepancy between the observed measurements and the model-predicted values by the variability among replicate measurements. In this manner, a statistically significant LOF may occur because of the improved precision of the central points and the presence of error at axial points [23]. The RSD values of 5 replicates calculated for 4 responses at the central point were ≤0.76% (Table 1). Results show that the LOF was due to the low variability at the center point.

Figure 1 depicts the response surface plots as defined by the regression model. Results indicate that the most significant factor for *Rs* of CIT-PXN was %B, which was strongly associated with a substantial decrease in the response (Figure 1A). The critical peak pair in the chromatogram was FXN-SRN. The mobile phase pH was the most significant parameter for the *Rs* of FXN-SRN, which increased with the increase in the pH from 2.5 to 5. The parameter %B had a dual effect on *Rs* of FXN-SRN. Figure 1B shows that *Rs* of FXN-SRN initially increased with %B up to 40%, and then slightly decreased with further increase in acetonitrile ratio. The *k* of CIT was most significantly affected by the %B in an inversely proportional manner, while the effects of pH and flow rate were limited (Figure 1C). A severe peak tailing was observed for all analytes with the increase in the mobile phase pH (Figure 1D), which

can be attributed to interactions between the negatively charged free surface silanols of the silica stationary phase and the positively charged analytes [24].

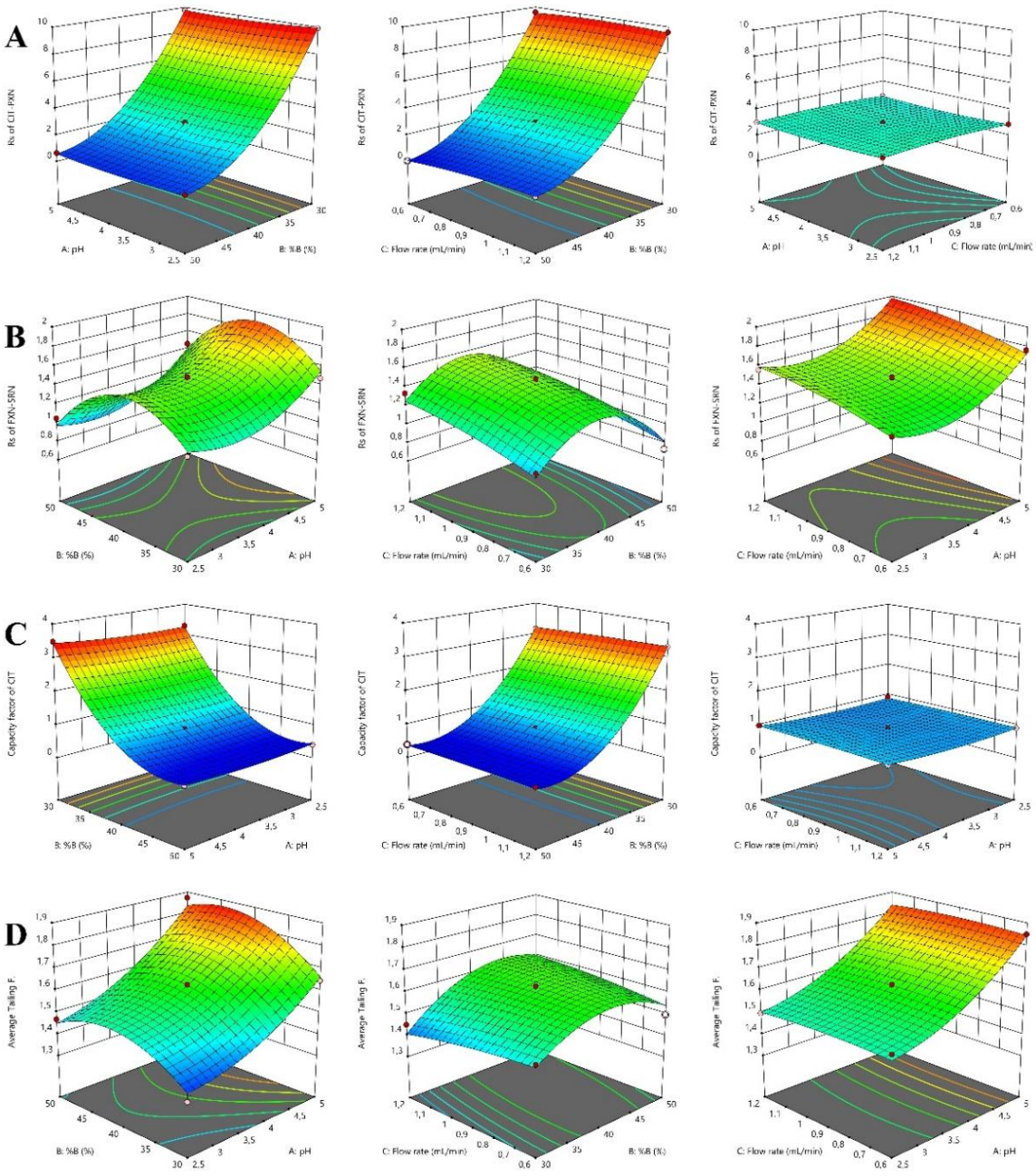

Figure 1. Response surfaces for (A) *Rs* between CIT and PXN, (B) *Rs* between FXN and SRN, (C) *k* of CIT, and (D) average *T*

The Design-Expert software's optimization tool, based on the Derringer and Suich desirability function [25], was employed to predict the most suitable conditions for the separation of antidepressants. The optimization aimed to achieve *Rs* values greater than 1.5 for CIT-PXN and FXN-SRN and peak pairs, a *k* greater than 1 for CIT, and an average *T* smaller than 1.5. The optimum values of pH, acetonitrile ratio, and flow rate were determined as 2.7, 38.2 %, and 1.1 ml/min, respectively. The optimal results for responses Y1, Y2, Y3, and Y4 were calculated as 4.1, 1.55, 1.22, and 1.5, respectively. The differences between predicted and experimentally obtained values for Y1, Y2, Y3,

and Y4 were 2,7%, 2.6%, 8.3%, and 0.61%, respectively, confirming the validity of the utilized models. Figure 2 shows the chromatogram recorded under the optimized chromatographic conditions. The retention times for CIT, PXN, DXN, FXN, and SRN were approximately 1.6, 1.9, 2.3, 2.9, and 3.2 min, respectively, resulting in a total run time of 5 min.

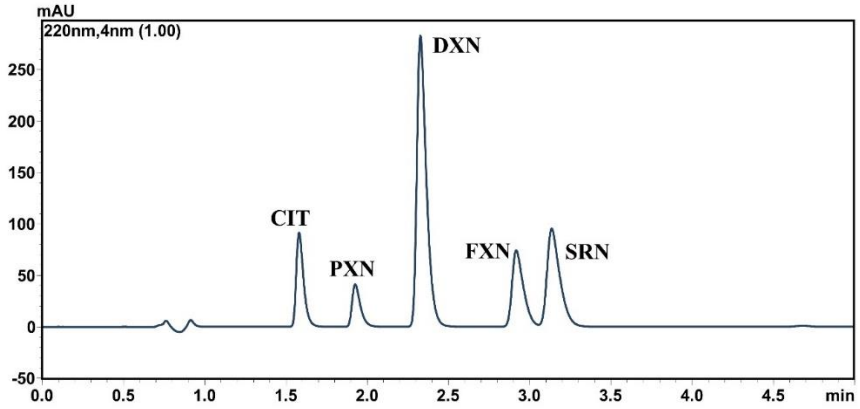

Figure 2. Chromatogram of a standard solution (10 µg/ml) of CIT, PXN, DXN, FXN, and SRN under optimized conditions at 220 nm

Method Validation

Validation studies were conducted compliant with the recommendations of ICH to demonstrate that the reported method was suitable for practical analysis. An SST was performed at the 10 µg/ml level (Table 2). The *k* of the first peak in the chromatogram, i.e., CIT, was 1.1, providing an adequate retention window for the elution of hydrophilic interferences originating from the matrix. *Rs* values obtained for all peak pairs were higher than the recommended value (1.5), except the FXN-SRN pair, for which the *Rs* was calculated as 1.4. It should be noted that peak purity index values calculated for both FXN and SRN were higher than 0.9999, demonstrating that acceptable resolution was achieved for the critical peak pair. Additionally, since the combined dosage form of these drugs is not commercially available, there is no risk of co-elution, even if a decrease in column efficiency occurs over time. The *T* values were in the range of 1.4-1.5, affirming the formation of symmetric peaks. Additionally, the RSDs were below 1%, demonstrating high precision.

The results of linearity and sensitivity experiments are shown in Table 3. The developed analytical method displayed acceptable linearity across the calibration range of 2-50 µg/ml for all five analytes, with correlation coefficients (r) ≥ 0.999. LODs were in the range of 0.17-0.29 µg/ml, while LOQ values ranged from 0.53 µg/ml to 0.89 µg/ml.

Intra-day accuracies ranged from 97.7 to 102.8 %, while inter-day accuracies were between 97.1% and 102.9%, with RSD values lower than 2.4% (Table 4). The results show that the proposed technique exhibits sufficient precision and accuracy for the determination of the antidepressants.

Table 2. Results of SST for antidepressants (n = 11)

	CIT	PXN	DXN	FXN	SRN	Recommended value
Retention time (min)	1.57	1.93	2.34	2.94	3.16	-
Tailing factor (T)	1.5	1.5	1.5	1.4	1.5	<2
Capacity factor (*k*)	1.1	1.5	2.1	2.9	3.2	>1
Resolution (Rs)	-	3	3.2	4.3	1.4	>1.5
Theoretical plates (N)	2963.2	3934.5	4998.3	6410.9	5814.8	>2000
Selectivity factor (*a*)	-	1.36	1.4	1.4	1.1	>1.05
RSD% of retention time	0.31	0.35	0.37	0.38	0.38	<1
RSD% of peak area	0.19	0.33	0.12	0.20	0.22	<1

Table 3. The results of linearity and sensitivity studies for CIT, PXN, DXN, FXN, and SRN

	CIT	PXN	DXN	FXN	SRN
Linear range (µg/ml)	2-50	2-50	2-50	2-50	2-50
Slope	16012	8751.1	72645	19021	35801
Intercept	3960.4	615.1	-10788	- 1247.7	-4361.8
SE of slope	52.4	17.1	146.5	38.1	95.6
SE of intercept	1425.4	465.7	3983.8	1035.3	2600.2
Correlation coefficient (*r*)	0.9998	0.9999	0.9999	0.9999	0.9999
LOD (µg/ml)	0.29	0.17	0.18	0.18	0.24
LOQ (µg/ml)	0.89	0.53	0.55	0.54	0.73

*SE: standard error

Table 4. Validation results of intra- and inter-day precision and accuracy for CIT, PXN, DXN, FXN, and SRN

		Intra-day (n= 4)		Inter-day (n= 12)	
Analyte	**Concentration level (µg/ml)**	**Accuracy (%)**	**RSD (%)**	**Accuracy (%)**	**RSD (%)**
CIT	15	100.3	0.32	101.6	2.33
	20	100.6	0.21	101.2	1.33
	25	102.1	0.05	102.9	1.44
PXN	15	102.8	0.17	102.7	0.26
	20	101.5	0.17	101.2	0.23
	25	100.4	0.09	100.2	0.33
DXN	15	98.7	0.06	97.9	1.05
	20	97.7	0.07	97.1	0.83
	25	101.2	0.02	100.6	0.65
FXN	15	100.3	0.21	100.1	0.21
	20	98.1	0.14	97.7	0.28
	25	100.5	0.14	100.2	0.32
SRN	15	100.8	0.07	100.6	0.22
	20	98.8	0.08	98.7	0.21
	25	100.3	0.06	100.1	0.25

Analyses of the Pharmaceutical Formulations

Commercially available formulations of PXN (Paxera 10® tablets) and DXN (Duloxx 30® capsules) were analyzed to prove the feasibility of the developed technique. Paxera tablets contain excipients such as lactose, dicalcium phosphate, croscarmellose sodium, starch, magnesium stearate, and colorants, while Duloxx capsules include sugar pellet, polysorbate 80, crospovidone, hypromellose 6 CPS, talc, hypromellose acetate succinate, and triethyl citrate. Formulations were prepared for analysis as described in the "Analysis of Commercial Formulations" section. Each sample was analyzed in triplicate, and the chromatograms are presented in Figure 3. No interfering compounds were found to be eluted from the column within the retention windows of the analytes. Additionally, the peak purity indices calculated via the DAD were greater than 0.999 for both PXN and DXN, indicating the absence of impurity.

The results of sample analysis by the developed method are summarized in Table 5. The obtained results were found to be satisfactory and in accordance with the manufacturer's declaration. The standard addition method was utilized to evaluate the method's accuracy in the presence of other matrix components, i.e., excipients. For this purpose, known amounts of standard solutions at 10 µg/ml level were added to pre-analysed samples. Obtained recoveries were higher than 96%, demonstrating the adequate accuracy of the HPLC method.

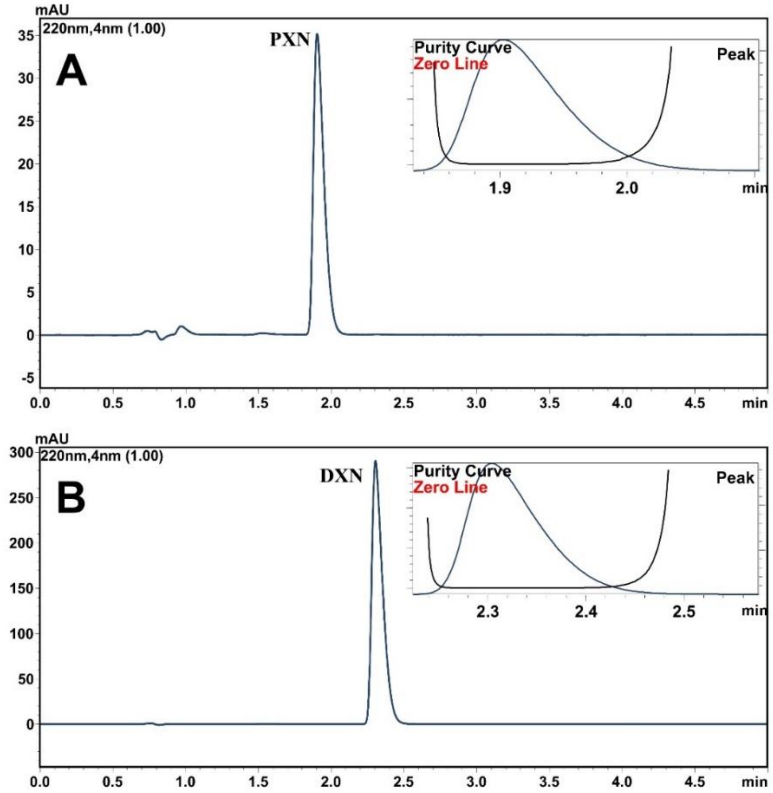

Figure 3. Representative chromatograms of Paxera® tablets (A) and Duloxx® capsules (B) at 220 nm (20 µg/ml)

Table 5. Assay results and mean recovery studies of PXN and DXN in pharmaceutical dosage forms

	Pharmaceutical formulations	
	PXN	**DXN**
Labeled claim (µg/ml)	20.00	20.0
Amount found (µg/ml)[a]	20.25	21.15
RSD (%)[a]	0.2	0.1
Bias (%)	1.25	5.7
Added (µg/ml)	10	10
Found (µg/ml)[a]	29.90	30.94
Recovery (%)	96.5	97.8
RSD% of recovery[a]	0.1	0.2
Bias (%)	-3.5	-2.2

[a] Mean of three experiments

Conclusion

A novel and reliable HPLC-DAD method was optimized for the quantification of DXN, FXN, CIT, PXN, and SRN in pharmaceutical formulations. DoE was employed for the first time to optimize the chromatographic conditions of selected analytes. Significant chromatographic parameters were determined by the preliminary trial and error experiments. Optimum levels of mobile phase pH, acetonitrile ratio, and flow rate were determined by BBD with desirability function considering the *Rs* of two peak pairs, elution of the first analyte, i.e. CIT, as well as the peak symmetry. Under optimum conditions, five antidepressants were separated under isocratic conditions within 3.5 min. The proposed technique was effectively utilized to determine PXN and DXN in commercial formulations. Acceptable

accuracy and precision were obtained by the real sample analyses. In addition, no interference from matrix components was observed. The proposed approach presents a quick, reliable, and uncomplicated substitute for QC or content uniformity testing of chosen antidepressants.

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AUTHOR CONTRIBUTIONS

Concept: S.Y.; Design: S.Y.; Control: S.Y.; Sources: S.Y.; Materials: S.Y.; Data Collection and/or Processing: S.Y., T.Ö.; Analysis and/or Interpretation: S.Y., T.Ö.; Literature Review: S.Y., T.Ö.; Manuscript Writing: S.Y.; Critical Review: S.Y., T.Ö.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

STUDY OF THE MINERAL ELEMENT CONTENT OF RED OAK (*QUERCUS RUBRA* L.) IN COMPARISON WITH SOIL

KIRMIZI MEŞENİN (QUERCUS RUBRA L.) MİNERAL ELEMENT İÇERİĞİNİN TOPRAK İLE KARŞILAŞTIRILMASI

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ABSTRACT

Objective: *The purpose of the present work was to to determine the content of mineral elements in annual shoots with leaves and in the fruits of wild individuals of red oak (Quercus rubra L.) from two different places of growth in comparison with their content in the soil under the plants.*
Material and Method: *The annual shoots with leaves of red oak with soil samples under plants were collected in August 2022 and fruits collected in September 2022 in Ukraine in the mixed forests near Tynne village (Rivne Oblast) and near Lisnyky village (Kyiv Oblast). The research was carried out by the X-ray fluorescence method on the "ElvaX-med" energy dispersive spectrometer.*
Result and Discussion: *As a result of the study, both types of raw materials of Quercus rubra (annual shoots with leaves and fruits) revealed the presence of 4 macro- (S, Cl, K, Ca), 8 micro- (Mn, Fe, Cu, Zn, Br, Rb, Sr, Pb) and 4 ultramicroelements (Cr, Co, Ni, Zr). It was determined that red oak plants are concentrators of potassium and sulfur from the soil (which is indicated by the high content of these macroelements both in the soil under the plants and in all studied raw materials). A high content of calcium in the raw material of red oak from both locations was noted, and this content is apparently characteristic of the plant itself, regardless of the soil on which it grows.*
Keywords: *Calcium, macroelements, microelements, potassium, red oak, sulfur, Quercus rubra, ultramicroelements, X-ray fluorescence method*

ÖZ

Amaç: *Bu çalışmanın amacı, kırmızı meşe (Quercus rubra L.) yabani bitkilerinin yıllık sürgünlerinde ve meyvelerinde mineral element içeriğini belirlemek ve bu içeriği bitkilerin altındaki toprakla karşılaştırmak olarak belirlenmiştir.*
Gereç ve Yöntem: *Kırmızı meşenin yıllık sürgünleri ile bitkilerin altındaki toprak örnekleri 2022 Ağustos'unda Ukrayna'da Tynne köyü (Rivne Oblast) ve Lisnyky köyü (Kyiv Oblast) yakınlarındaki karışık ormanlarda toplanmıştır. Araştırma, "ElvaX-med" enerji dispersif spektrometre üzerinde X-ışını floresans yöntemiyle gerçekleştirilmiştir.*
Sonuç ve Tartışma: *Çalışmanın sonucunda, Quercus rubra'nın (yıllık sürgünler ve meyveler) her iki tip ham maddesinde 4 makro- (S, Cl, K, Ca), 8 mikro- (Mn, Fe, Cu, Zn, Br, Rb, Sr, Pb) ve 4 ultramikroelementin (Cr, Co, Ni, Zr) varlığı ortaya çıkmıştır. Kırmızı meşe bitkilerinin topraktan*

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potasyum ve kükürtü yoğunlaştırdığı belirlenmiştir (bitkilerin altındaki toprakta ve incelenen tüm ham maddelerde bu makroelementlerin yüksek içeriği ile gösterilmektedir). Her iki bölgeden gelen kırmızı meşe ham maddesinde yüksek kalsiyum içeriği belirlenmiş ve bu içeriğin bitkinin kendine özgü olduğu, büyüdüğü topraktan bağımsız olduğu görülmüştür.
Anahtar Kelimeler: *Kalsiyum, makroelementler, mikroelementler, potasyum, kırmızı meşe, kükürt, Quercus rubra, ultramikroelementler, X-ışını floresans metodu*

INTRODUCTION

Mineral elements are compounds necessary for human metabolism. They are involved in the construction of organs, tissues, cells and their components, the maintenance of ionic balance in cells, the regulation of the activity of many enzymes and are part of hormones, vitamins, pigments and often determine their chemical and biological activity [1].

Medicinal plants, which are able to accumulate a significant amount of necessary mineral elements, can be used for the prevention and comprehensive treatment of many diseases that arise due to the imbalance of micro- and macroelements in the human body [2,3].

It should be noted that the content of mineral substances in plants can vary depending on the composition of the soil, climatic conditions and other factors [4], that's why it is advisable to study the elemental composition of medicinal plants in combination with the study of the elemental composition of the soil.

Red oak (*Quercus rubra* L., Fagaceae) is an invasive plant brought to Europe from North America [5], which has acclimatized well in the territory of Ukraine, is resistant to diseases and actively invades new territories, displacing the official medicinal species, common oak, from natural habitats, therefore, its raw material base is constantly growing. The raw material of red oak is used in traditional medicine for colds and viral diseases, to increase immunity and as an astringent, and is of scientific interest for pharmacognostic study with the aim of expanding the spectrum of use of *Quercus* L. species in official medicine, as well as the further development of new medicines based on it [6].

MATERIAL AND METHOD

Sample Collection

The annual shoots with leaves of red oak with soil samples under plants were collected in August 2022 and fruits of red oak collected in September 2022 in Ukraine in the mixed forests near Tynne village (Rivne Oblast) and near Lisnyky village (Kyiv Oblast).

Analytic Equipment

The study of the qualitative composition and quantitative content of mineral elements was carried out by the X-ray fluorescence method on the energy dispersive spectrometer "ElvaX-med" (Elvatech Ltd., Ukraine) in the Scientific and Technical centre "Viria Ltd." (Kyiv, Ukraine).

Method for the Assay of Mineral Elements

The method includes the following steps: to 50 mg of raw materials crushed to a powdery state, a binding organic compound without metal admixture was added. The mixture was dried, and a tablet with a diameter of 10 mm, a thickness of no more than 2 mm and a weight of 50 mg was made from this mass. The resulting tablet was analyzed on the device for 10 minutes. The fluorescence spectrum consists of a number of analytical lines. Each line corresponds to the energy of fluorescent radiation characteristic of the atoms of this element. Since the energy dispersive measurement method is used in the analyzer, the lines of all atomic elements in the sample are present in the resulting spectrum. The energy range is from 1 to 40 KeV. This corresponds to the range determination of elements from Na to U. The intensity of the spectral lines depends on the concentrations of the determined elements.

The composition of clean filter paper (State Standard GOST 12026-76) was previously measured and its spectrum was taken as background. The spectrum of the difference between the spectra of the working sample and the background spectrum was used for the calculation.

The mass fraction of the element was calculated according to the calibration characteristics of the analyzer. Calibration of the analyzer for determiniung the mass fraction of an individual element was carried out using standard solutions of metal ions according to State Standard Samples of Solutions of Ukraine (1.0 mg/dm^3; State Standard Samples of Ukraine DSZU 022.86-98), which are used for calibration, attestation and verification of analytical devices: photocolorimeters, spectrophotometers, atomic absorption spectrophotometers, etc.

The sensitivity threshold of the method, when evaporating 50 ml of water, for most elements is 0.01 μg/l (10-5 ppm) [7].

The result was taken as the average arithmetic value of five consecutive measurements, the statistical processing of the obtained results was carried out using the Student's test to determine the standard deviation at the significance level of 95% according to the monograph of the State Pharmacopoeia of Ukraine (SPhU) "5.3.N.1. Statistical analysis of the results of a chemical experiment".

RESULT AND DISCUSSION

According to the results of the investigation in samples of red oak raw materials collected in a mixed forest near the village of Lisnyky village (Kyiv Oblast) 10 mineral elements in fruits and 11 mineral elements in annual shoots with leaves were identified. 20 mineral elements were found in the soil under the plant. In samples of *Q. rubra* raw materials collected in a mixed forest near Tynne village (Rivne Oblast) 16 mineral elements were identified in fruits and 11 mineral elements in annual shoots with leaves. 20 mineral elements were found in the soil samples collected under investigated plants from two locations.

Spectrograms of the content of mineral elements are presented in Figure 1–6 and the results are in Table 1-2 and Figure 7.

Table 1. The content of mineral elements in the raw material of *Q. rubra* in comparison with the soil (Lisnyky, Kyiv Oblast), μg/g

Element \ Tested sample			Fruits	Annual shoots with leaves	Soil under the plant
Mineral substances	Macro	S	1187.41±39.18 * 3.3**	1691.32±67.65 z3.9	4846.20±193.84 2.8
		Cl	-	-	444.91±19.57 4.3
		K	3174.00±136.48 4.2	2320.66±95.14 4.0	3678.74±172.90 4.6
		Ca	788.32±30.74 3.8	2155.11±103.44 4.7	795.78±29.44 3.6
	Micro (trace)	Mn	8.21±0.40 4.8	315.15±13.55 4.2	919.44±28.50 3.0
		Fe	25.62±1.02 3.9	71.46±2.35 3.2	6139.06±251.70 4.0
		Cu	2.51±0.11 4.3	-	6.12±0.24 3.9
		Zn	4.95±0.14 2.8	15.94± 0.62 3.8	24.23±1.13 4.6
		Br	-	-	4.87±0.18 3.6
		Rb	16.16±0.64 3.9	2.81±0.08 2.8	18.91±0.92 4.8
		Sr	1.87±0.09 4.8	5.42±0.22 4.0	14.40±0.56 3.8
		Pb	-	-	13.21±0.64 4,8

Table 1 (*continue*). The content of mineral elements in the raw material of *Q. rubra* in comparison with the soil (Lisnyky, Kyiv Oblast), μg/g

Element \ Tested sample			Fruits	Annual shoots with leaves	Soil under the plant
	Ultramicro	Ti	-	-	1488.40±43.16 2,8
		Cr	-	2.85±0.11 3.8	28.95± 0.89 3.0
		Co	-	4.56±0.20 4.3	290.34±8.71 2.9
		Ni	4.49±0.20 4.4	3.09±0.10 3.2	30.93±1.54 4.9
		Zr	-	-	158.44±4.75 2.9
		Sn	-	-	214.82±7.30 3.4
		W	-	-	29.11±1.07 3.6
		Ba	-	-	865.05±40.65 4.6

Note: "-" – chemical element wasn't detected;
The obtained results are presented in the form: $\frac{\overline{X} \pm \sigma\overline{X}}{cv}$,where:
* $\overline{X}$ – arithmetic mean; $\sigma\overline{X}$ (MSE) – the mean squared error;
** cv – coefficient of variation;
$p < 0.05$

Table 2. The content of mineral elements in the raw material of *Q. rubra* in comparison with the soil under investigated plants (Tynne, Rivne Oblast), μg/g

Element \ Tested sample			Fruits	Annual shoots with leaves	Soil under the plant
Mineral substances	Macro	S	1390.89±69.54 * 4.9**	4132.47± 198,35 4.7	5709.82±235.15 4.1
		Cl	76.27±2.36 3.1	-	1056.94±52.85 5.0
		K	3475.87±142.51 4.1	2498.99±97.46 3.8	5724.41±234.70 4.0
		Ca	590.50±27.75 4.6	3063.76± 107.23 3.4	6088.35±280.06 4.5
	Micro (trace)	Mn	3.38±0.14 4.1	41.88± 2.09 4.9	326.92±11.44 3.4
		Fe	13.87±0.44 3.1	40.80± 1.67 4.0	5632.21±259.08 4.6
		Cu	2.67±0.10 3.7	1.46± 0.04 2.7	10.20±0.38 3.7
		Zn	6.50±0.2 3.0	12.04± 0.38 3.1	37.09±1.07 2.8
		Br	0.36±0.016 4.4	-	6.07± 0.22 3.6
		Rb	24.01±1.05 4.3	5.69± 0.21 3.6	8.61± 0.35 4.0
		Sr	2.28±0.10 4.3	6.90± 0.31 4.4	15.57±0.45 2.8
		Pb	0.13±0.004 3.0	-	14.58±0.65 4.4

Table 2 (*continue*). The content of mineral elements in the raw material of *Q. rubra* in comparison with the soil under investigated plants (Tynne, Rivne Oblast), μg/g

Element \ Tested sample			Fruits	Annual shoots with leaves	Soil under the plant
	Ultramicro	**Ti**	-	-	1228.09± 40.52 3.2
		Cr	0.97±0.036 3.7	1.65± 0.04 2.4	43.49± 1.91 4.39
		Co	0.38±0.015 3.9	-	221.88±7.98 3.5
		Ni	0.29±0.009 3.1	1.92± 0.07 3.6	49.08±1.57 3.1
		Zr	0.10±0.002 2.0	-	64.28±1.99 3.0
		Sn	-	-	223.35±10.05 4.4
		Y	-	-	7.22±0.34 4.7
		Ba	-	-	1220.81±40.28 3.2

Note: The explanations of symbols in Table 1

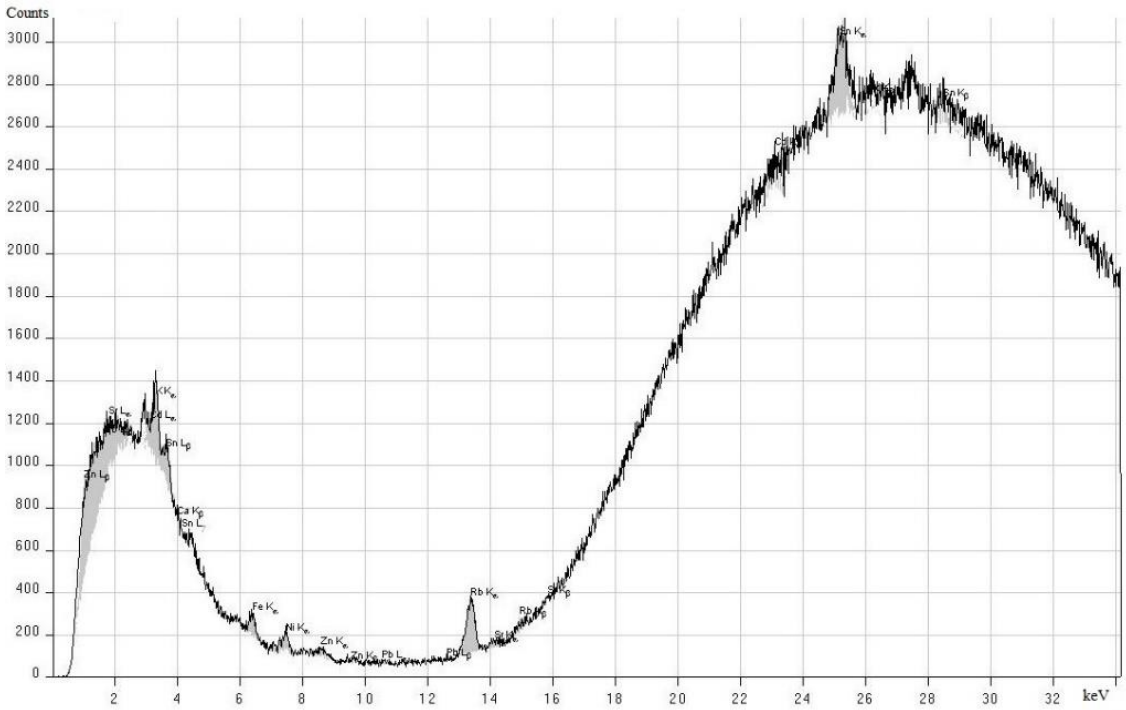

Figure 1. Spectrogram of the content of mineral elements in *Q. rubra* fruits (Lisnyky, Kyiv Oblast)

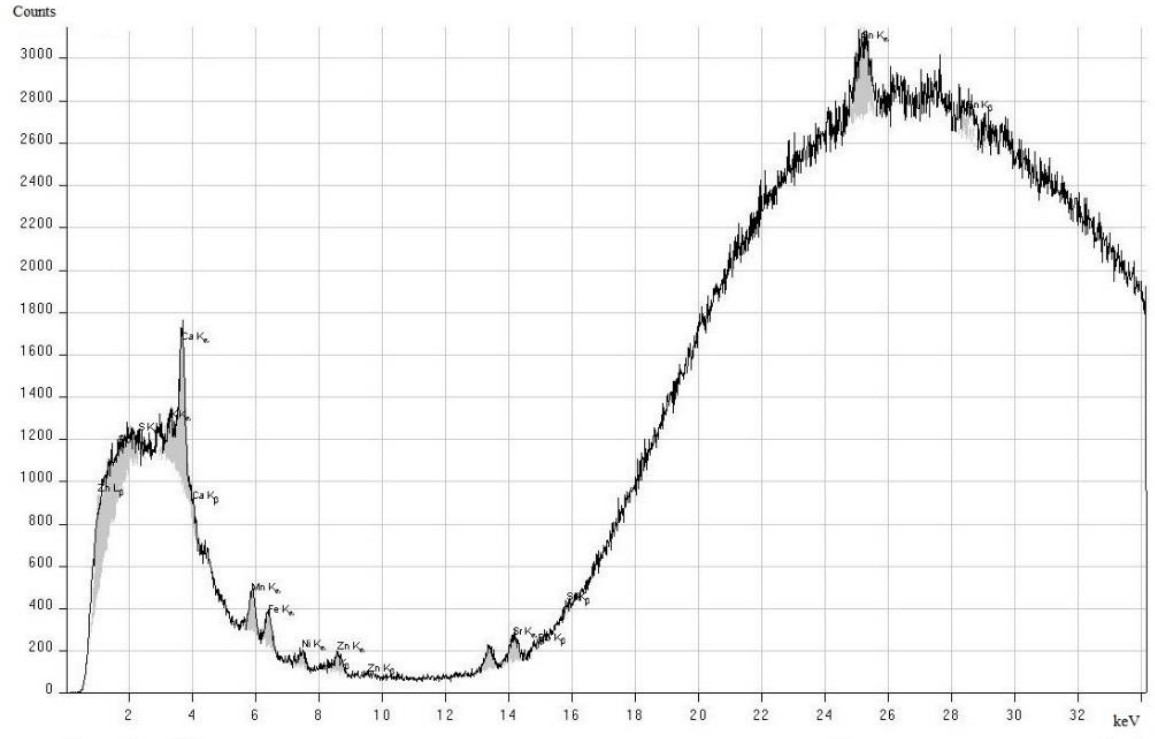

Figure 2. Spectrogram of the content of mineral elements in *Q. rubra* annual shoots with leaves (Lisnyky, Kyiv Oblast)

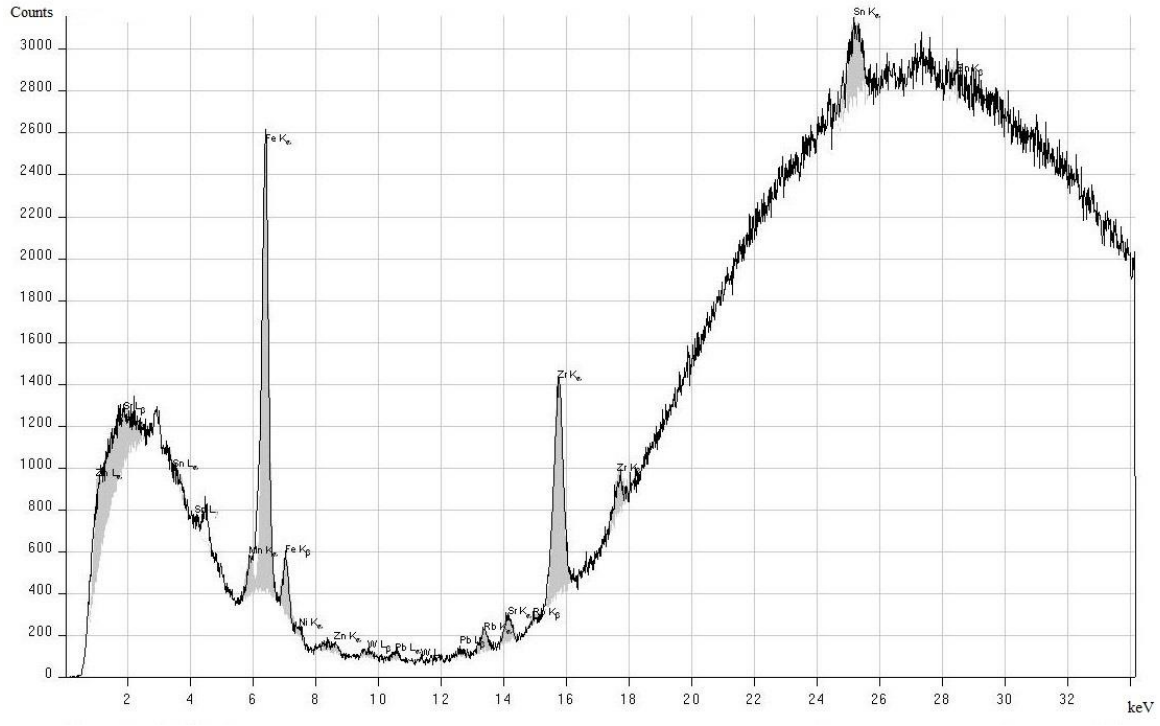

Figure 3. Spectrogram of the content of mineral elements in the soil under *Q. rubra* plants (Lisnyky, Kyiv Oblast)

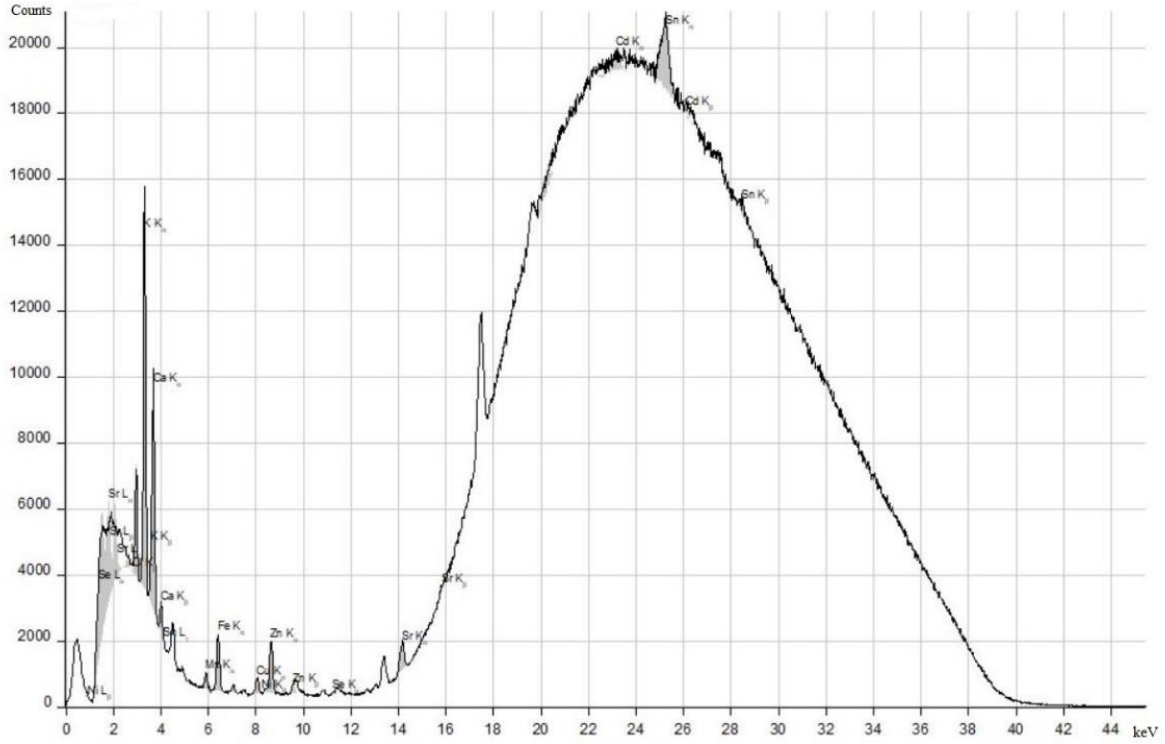

Figure 4. Spectrogram of the content of mineral elements in *Q. rubra* fruits (Tynne, Rivne Oblast)

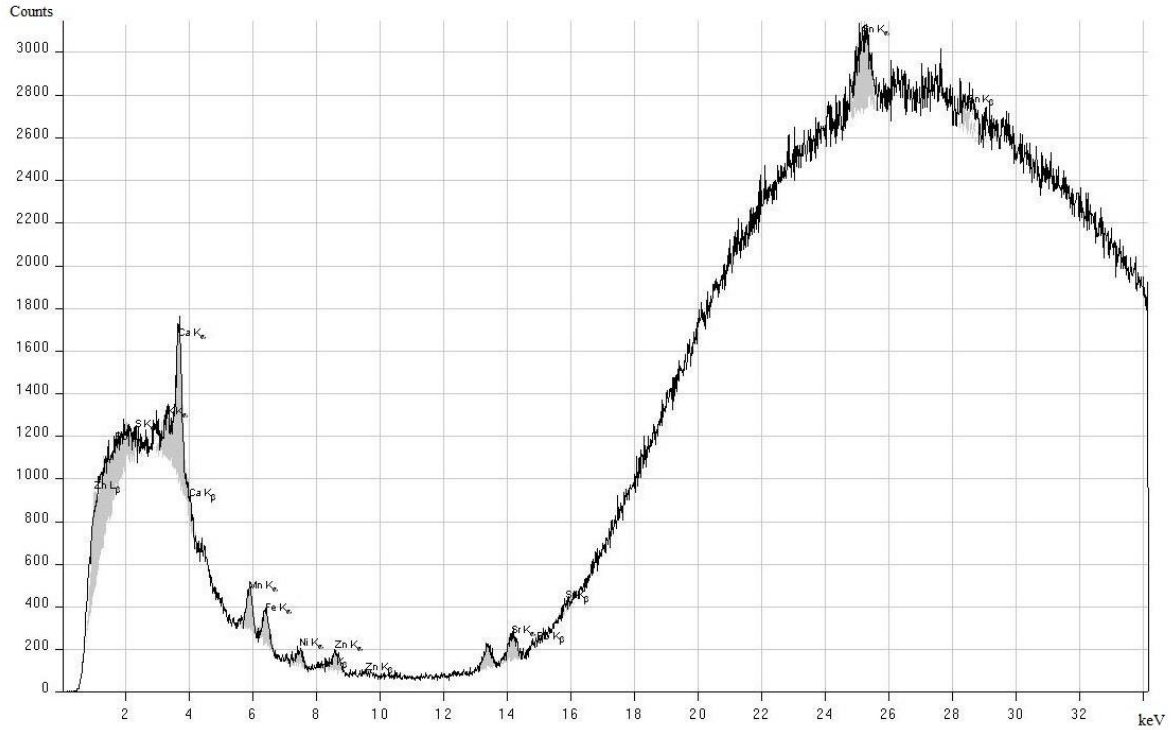

Figure 5. Spectrogram of the content of mineral elements in *Q. rubra* annual shoots with leaves (Tynne, Rivne Oblast)

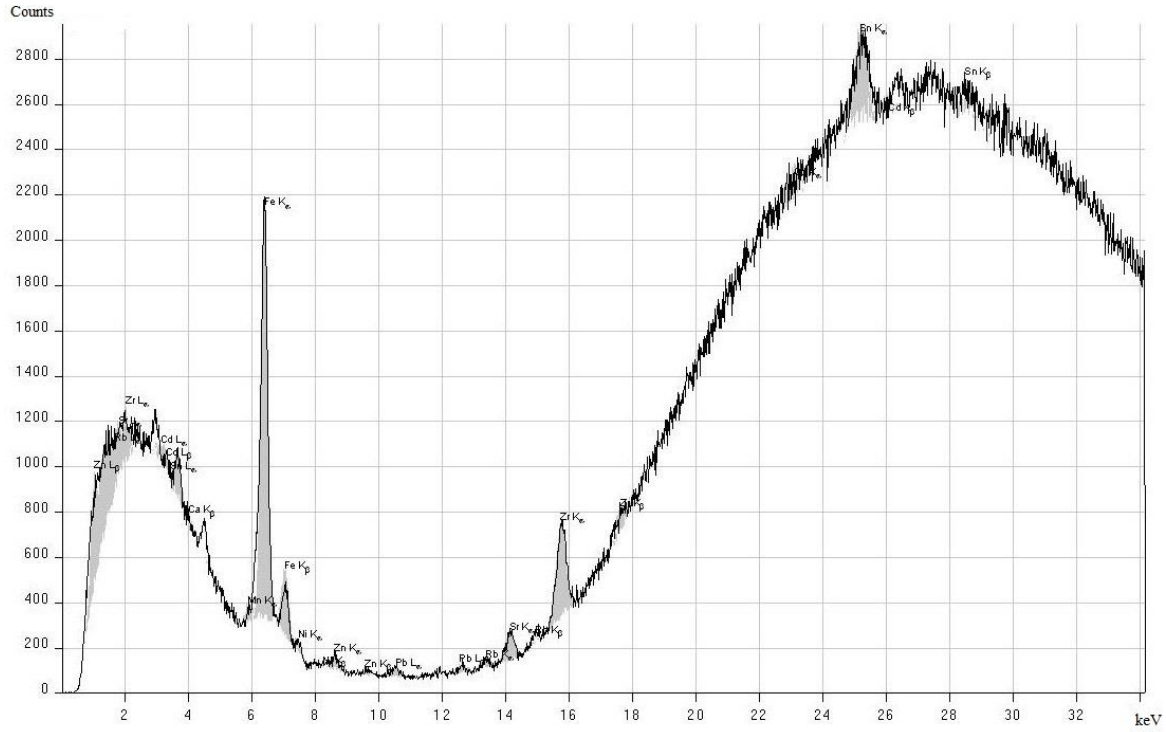

Figure 6. Spectrogram of the content of mineral elements in the soil under *Q. rubra* plants (Tynne, Rivne Oblast)

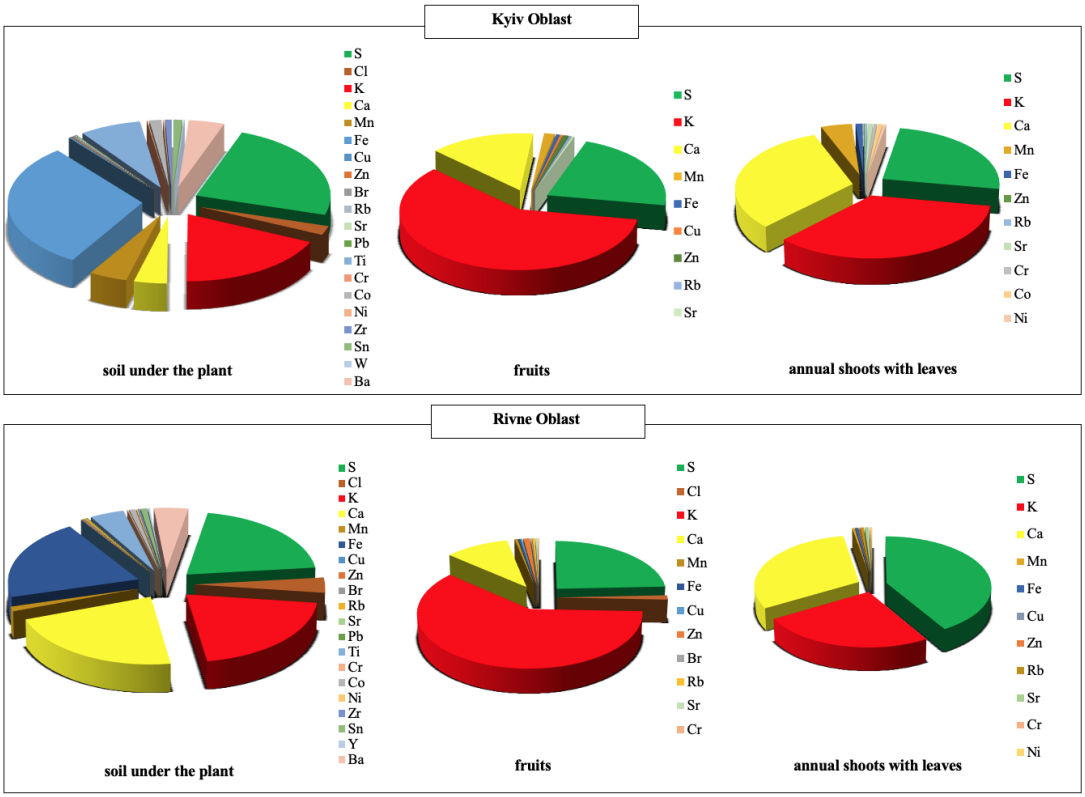

Figure 7. Comparative diagrams of the content of mineral elements in the soil under the plants and in the raw materials of *Q. rubra* collected in Kyiv and Rivne Oblasts

Among the macronutrients in the raw material of annual shoots with leaves, potassium and calcium dominate, the content of sulfur is somewhat lower; the macroelement composition is dominated by manganese, the content of ferrum is significantly lower (by 4.4 times) and zinc is even lower (by 19.7 times), strontium and rubidium are present in small amounts; cobalt, nickel and chromium were found among ultramicroelements.

Potassium dominates among macronutrients in fruits, the content of which is 4 times greater than calcium, and 2.6 times greater than sulfur; the microelement composition is dominated by ferrum, the content of rubidium is somewhat lower, and manganese, zinc, copper, and strontium are present in small amounts; nickel was found among the ultramicroelements.

The high content of potassium in the soil (3678.74 μg/g) correlates with the high content in fruits (3174.00 μg/g) and annual shoots with leaves (2320.66 μg/g); the same regularity can be noted for sulfur. At the same time, the significant content of calcium in annual shoots with leaves is apparently characteristic of the plant itself, determined genetically, since the calcium content in the soil under the red oak plants growing in Kyiv Oblast is not high enough.

The fact that a fairly high content of iron in the soil does not lead to a significant accumulation of this trace element in the raw material of red oak is noteworthy. The same applies to titanium, barium, and stanium, which are not detected at all in red oak raw materials, despite their significant content in the soil.

At the same time, the low content of some macroelements in the soil (Cu, Br) can be associated with the insignificant content or the complete absence of these elements in the raw materials.

In general, the total content of mineral elements (μg/g) was the highest in annual shoots with leaves – 6588.37, of which: 6167.09 – macroelements, 410.78 – microelements, and 10.5 – ultramicroelements; it is somewhat smaller in fruits – 5213.54, of which: 5149.73 – macroelements, 59.32 – microelements and 4.49 – ultramicroelements.

For all research objects, the following series of element accumulation can be identified according to the decrease in their content: for fruits – macroelements: K > S > Ca; macroelements: Fe > Rb > Mn > Zn > Cu > Sr; for annual shoots with leaves – macroelements: K > Ca > S, microelements: Mn > Fe > Zn > Sr.

In the mineral composition of red oak annua shoots with leaves collected in Kyiv Oblast, potassium is 35%, sulfur – 26%, calcium –33%, manganese – 5%, and 1% of the total amount is ferrum, the content of other mineral elements is lower than 1% (Figure 7).

In the mineral composition of fruits collected in Kyiv Oblast, the content of potassium is 61%, sulfur – 23%, calcium – 15%, and the content of other mineral elements is less than 1% (Figure 7).

As can be seen from the data in Table 2, the raw material of *Q. rubra* collected in Rivne Oblast is characterized by a high content of sulfur, potassium and calcium. Among microelements, annual shoots with leaves are dominated by manganese and ferrum, and in fruits – rubidium, the content of which is 1.4 times higher than in raw materials collected in Kyiv Oblast. Chromium, cobalt, nickel, and zirconium are among the ultramicroelements in the fruits (chromium and nickel are also present in annual shoots with leaves).

Among the macronutrients in the fruits, potassium dominates, the content of sulfur is less (by 2.5 times) and calcium is much less (by 5.8 times); among microelements, rubidium significantly predominates, the content of ferrum is somewhat lower, other microelements are present in red oak fruits in small quantities; the content of ultramicroelements (Cr, Co, Ni, Zr) in fruits is less than one μg/g.

Among the macroelements in the raw material of annual shoots with leaves, a high content of sulfur and calcium is observed, the content of potassium is slightly lower; the microelement composition is characterized by a high content of manganese and iron, the content of which is 3 times greater than the content of other trace elements present in this raw material; the ultra-microelement composition is represented by chromium and nickel.

The high content of sulfur in the soil (5709.82 μg/g) correlates with the high content in annual shoots with leaves (4132.47 μg/g). Among the macronutrients in fruits, potassium prevails (3475.87 μg/g), the content of which in the soil is also significant (5724.41 μg/g). The content of ultramicroelements in the soil significantly exceeds their content in raw materials. Titanium and barium, which are present in significant quantities in the soil, are completely absent in the red oak raw materials

collected in Rivne Oblast.

The total content of mineral elements (μg/g) in the raw material of red oak collected in Rivne Oblast was the highest in annual shoots with leaves – 9807.56, of which: 9695.22 – the content of macroelements, 108.77 – microelements, and 3.57 – ultramicroelements; it is somewhat smaller in fruits – 5588.47, of which 5533.53 – the content of macroelements, 53.2 – microelements, and 1.74 – ultramicroelements.

Based on the results of the analysis, a series of accumulation of elements was identified for all objects according to the decrease in their content: for fruits – macroelements: K > S > Ca > Cl; microelements: Rb > Fe> Zn > Mn > Cu > Sr > Br > Pb; for annual shoots with leaves – macroelements: S > Ca > K, microelements: Mn > Fe > Zn > Sr > Rb > Cu.

According to the results of the study of the content of mineral elements in the fruits and annual shoots with leaves of *Q. rubra* wild plants collected from different places of growth in comparison with their content in the soil under investigated plants (Tables 1–2), different regularities of the accumulation of mineral elements in raw materials of red oak in Kyiv and Rivne Oblasts.

The raw material collected in Rivne and Kyiv Oblasts is characterized by a high content of K, S, Ca, Mn, and Fe. In general, the composition of mineral elements in the studied raw material of red oak from the both investigated locations is similar, some differences (for example, the presence of bromine, cobalt and zirconium in the fruits of red oak growing in Rivne Oblast) are obviously related to the peculiarities of the mineral composition of the soil where studied plants grow.

The composition of the soil under investigated plants in both Rivne and Kyiv Oblast is slightly different, in particular, the total quantitative content of mineral elements in the soil in Kyiv Oblast is 9765.63 μg/g, and the soil under the plant in Rivne Oblast – 18579.52 μg/g, which also affects the mineral composition of red oak plants growing on this soil. In particular, based on the obtained results, it can be assumed that red oak plants are concentrators of potassium and sulfur from the soil (this is indicated by the high content of these macroelements both in the soil under the plants and in all studied raw materials).

A high content of calcium in the raw material of red oak from both locations was noted, and this content is apparently characteristic of the plant itself, regardless of the soil on which it grows. This is supported by the significant quantitative content of calcium in annual shoots with leaves – 2155.11 μg/g in red oak plants from Kyiv Oblast, which grew on soil with a relatively low Ca content – 795.78 μg/g.

The content of heavy metals was within the limits of the requirements put forward by the SPhU for plant raw materials [8].

The identified differences in the accumulation of macro- and microelements by various organs of the studied plant can be explained by the unequal degree of assimilation of certain elements, as well as the ecological conditions of the place of growth [9], to confirm the influence of the soil on the accumulation of mineral elements, additional studies of raw materials collected from places of growth with different soil composition are required.

It should be noted that the mineral elements present in all samples of the studied raw materials have a significant physiological role for the human body [10]. Deficiency of one of the minerals can cause a violation of the metabolism of the human body. For example, Zn and Cu stimulate immune activation, participate in the processes of hematopoiesis and wound healing [11,12], Se is an irreplaceable microelement with antioxidant properties, necessary for the optimal functioning of the immune system and the thyroid gland [13], Ca participates in formation of bone tissue, is a part of cellular structures, it is a mandatory component of the system of maintaining the acid-alkaline balance of the body's internal environment [14]. Sulfur has an anti-allergic effect, participates in the production of collagen, is necessary for the brain, blood vessels and liver, is able to reduce pain in muscles and joints [15], is part of the active centers of the molecules of a number of enzymes in the form of SH functional groups, which participate in many enzymatic reactions, in particular, in the creation and stabilization of the native three-dimensional structure of proteins, and in some cases they act directly as catalytic centers of enzymes, they are part of various coenzymes, including coenzyme A [16]. The fruits of plants collected in both investigated places of growth contain a significant amount of potassium, which is necessary for the normal functioning of the cardiovascular system, stabilization of the water-salt balance, and maintenance of normal blood pressure [17].

In our previous work devoted to the study of polyphenolic compounds in raw materials of red oak [18], it was identified that the leaves and annual shoots of *Q. rubra* are distinguished by the content of rutin, chlorogenic and sinapic acids.

A high content of such mineral elements as sulfur, potassium, calcium is determined. They take part in various metabolic processes related to the transport of electrons (sulfur in the electron transport chain takes one of the unpaired oxygen electrons into a free orbital, participates in fixing and transporting methyl groups) with the formation of the transmembrane potential and the spread of the potential change across the cell membrane by exchange with sodium ions along the concentration gradient (biological role of potassium), with the functioning of cell membranes, the work of the nuclear apparatus of the cell (calcium inhibits the release of histamine, thus reducing the manifestations of allergic reactions, pain syndrome and inflammatory processes, is a blood coagulation factor, participates in the formation of the immune response, etc.) [19], may make an additional contribution to the revealing of potential types of pharmacological action characteristic of the polyphenols found in the raw material: P-vitamin, anti-inflammatory, analgesic, anti-allergic, antibacterial, as well as help lower blood pressure and improve heart function [20].

According to literature data the raw materials (bark, fruits, leaves) of a number of species of the genus *Quercus* contain a significant amount of mineral elements. In particular, in the species *Q. brantii* Lindl., *Q. infectoria* Oliv., *Q. cerris* L., *Q. coccifera* L., *Q. libani* Oliv. and *Q. suber* L., among the macroelements there are Ca, P, Mg, K and Na, and the microelement composition is represented by Fe, Zn, Cu and Mn [21,22].

In the most studied raw material of the official medicinal species of the genus *Quercus* – the bark of the common oak (Q. *robur* L.) – the mineral composition is represented by such macroelements as K, Ca, Mn and Fe; microelements – Mg, Cu, Zn, Sr, Pb and B [23].

Our results of determining the quantitative content of mineral elements in the raw material of red oak generally agree with the data of other authors regarding the mineral composition of some species of the oak genus [21,22,24].

Thus, according to the results of Ozkan et al. [25], obtained for a number of *Quercus* species growing in Turkey, the majority macronutrient for all the studied species (Q. *brantii*, *Q. infectoria, Q.cerris, Q. coccifera*, *Q. libani* and *Q. suber*) is potassium, the content of which significantly exceeds the corresponding content of other mineral elements. At the same time, the quantitative content of potassium in the species studied by the author varies from 10930 to 11910 μg/g; the calcium content is somewhat lower (from 8320 to 8670 μg/g). The microelement composition of the species of the oak genus studied by Kalamac et al. [22] in terms of composition and quantitative content is also similar to *Q. rubra* (in particular, a high content of manganese and ferrum is noted).

In general, the content of mineral elements in other studied species of the oak genus collected from places of growth in Turkey was 3.4-4.7 times higher than the corresponding content we identified for the raw material of red oak growing in Kyiv and Rivne Oblasts of Ukraine. This may be related both to the peculiarities of the species *Q. rubra* itself, and to the mineral composition of soils in Turkey, which, unfortunately, were not investigated in the relevant works of Turkish authors on the study of the mineral composition of species of the oak genus [22].

Basically, comparing the data available in the literature on the mineral composition of different species of the oak genus with the results of our research, we can conclude that the mineral composition of the species of the genus *Quercus* is quite similar, although certain differences are noted, in particular, for red oak is characterized by a high content of sulfur in the raw material, which is not found in other species of the oak genus.

Conclusion

Quantitative content of macro-, micro- and ultramicroelements of two types of raw materials (annual shoots with leaves and fruits) of *Q. rubra* from two different places of growth (Kyiv region, Rivne region) was determined in comparison with their content in the soil collected under the plants.

The presence of 16 mineral elements was found in the raw material of wild red oak from natural habitats in Rivne Oblast: in the fruits – 4 macro- (S, Cl, K, Ca), 8 – micro- (Mn, Fe, Cu, Zn, Br, Rb, Sr, Pb) and 4 – ultramicroelements (Cr, Co, Ni, Zr); in annual shoots with leaves: 3 – macro- (S, K, Ca), 6

– micro- (Mn, Fe, Cu, Zn, Rb, Sr) and 2 – ultramicroelements (Cr, Ni). The presence of 12 mineral elements was found in the raw material of wild red oak from natural habitats in Kyiv Oblast: in the fruits – 3 macro- (S, K, Ca), 6 – micro- (Mn, Fe, Cu, Zn, Rb, Sr) and 1 – ultramicroelement (Ni); in annual shoots with leaves: 3 – macro- (S, K, Ca), 5 – micro- (Mn, Fe, Zn, Rb, Sr) and 3 – ultramicroelements (Cr, Co, Ni).

The raw material collected in both locations in Ukraine is characterized by a high content of K, S, Ca, Mn and Fe. In general, the composition of mineral elements in the studied raw material of red oak is similar, some differences (for example, the presence of bromine, cobalt and zirconium in the fruits of red oak growing in Rivne Oblast) are obviously related to the peculiarities of the mineral composition of the soil the studied plants grow.

Based on the obtained results, it can be assumed that red oak plants are concentrators of potassium and sulfur from the soil (this is indicated by the high content of these macroelements both in the soil under the plants and in all the studied raw materials). A high content of calcium in the raw material of red oak from both locations was noted, and this content is apparently characteristic of the plant itself, regardless of the soil on which it grows. The obtained results show that the raw material of red oak is rich in vital mineral elements.

Thus, the results of the study of the mineral composition of different types of *Q. rubra* raw materials followed by a conclusion about the prospects of using annual shoots with leaves and fruits of red oak for the development of medicines with different directions of action (anti-inflammatory, analgesic, anti-allergic, antioxidant, etc.) and dietary supplements.

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AUTHOR CONTRIBUTIONS

Concept: O.K., T.O., I.H.; Design: O.K., T.O., H.S.; Control: O.K., I.H.; Sources: T.O., I.H.; Materials: O.K., T.O., I.H., H.S., O.S, M.K.; Data Collection and/or Processing: O.K., T.O., I.H., M.K.; Analysis and/or Interpretation: O.K., T.O.; Literature Review: T.O., I.H.; Manuscript Writing: O.K., T.O., I.H. O.S, M.K., H.S.; Critical Review: O.K.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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DERLEME MAKALE / REVIEW ARTICLE

ARAROT (*MARANTA ARUNDINACEA* L.) RİZOMLARININ FİTOKİMYASAL, TIBBİ VE BESİNSEL ÖZELLİKLERİ VE ÇEŞİTLİ KULLANIMLARI

PHYTOCHEMICAL, MEDICINAL, AND NUTRITIONAL PROPERTIES AND VARIOUS USAGE OF ARROWROOT (MARANTA ARUNDINACEA L.) RHIZOMES

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ÖZ

Amaç: *Maranta arundinacea L. (Ararot) (Marantaceae) tropiklerde yetişen otsu, çok yıllık bir bitkidir. Yüksek nişasta içerikli yenilebilir silindirik rizomlara sahiptir. Rizomlar halk tıbbında yatıştırıcı, kızarıklık giderici, anti-inflamatuvar ve antiseptik olarak kullanılmaktadır. Bu çalışmada günümüzde ekonomik değeri olan ve çeşitli endüstrilerde uygulama potansiyeli bulunan ararotun botanik özellikleri, yayılışı ve geleneksel kullanımı araştırılmıştır. Dahası yenilebilir rizomlarının kimyasal içeriği ve rizomlardan elde edilen ekstre, un, nişasta ve tozun biyolojik aktiviteleri ve rizomların çeşitli kullanım alanlarına ait bilgiler derlenmiştir.*

Sonuç ve Tartışma: *Rizomlar alkaloit, karbonhidrat, kardiyak glikozitler, protein, amino asit, terpen, saponin, flavonoit, reçine, tanen, zamk, lignin, antrakinon, sterol, lif ve mineral içerir. Rizom ve rizomdan elde edilen ürünlerin antioksidan, immünostimulan, anti-ülserojenik, antidiyareik, anti-inflamatuvar, antimikrobiyal, antidispeptik, antihipertansif, hipokolesterolemik, hipoglisemik, antikanser ve hepatoprotektif aktiviteleri bildirilmiştir. Ararot unu kolayca sindirilebildiğinden çocuklar için hazırlanan ekmek ve bisküvilerde ve ayrıca kurabiye ve unlu mamullerde kullanılır. Ararot nişastası; çorba, tatlılar, puding, sos, kurabiye, şekerleme, bisküvi, kek ve jöle yapımında kullanılır. Ararot nişastası glüten içermediğinden özel bisküvi ve fırın ürünlerinin hazırlanmasında kullanılır. Ararot tozu ve nişastasının kozmetik endüstrisinde kullanımı vardır. Ararot nişastasının eczacılık, hijyen ürünleri, çevre yönetimi, tarım, biyofilm, biyomedikal mühendisliği ve biyoyakıt üretimi gibi alanlarda uygulamaları mevcuttur. Ararot lifi kâğıt mendil, ince kâğıt, karton, ambalaj kâğıdı ve çanta gibi yırtılmaya dirençli kâğıt yapımına uygundur.*

Anahtar Kelimeler: *Maranta arundinacea, ararot, ararot nişastası, biyolojik aktivite, kimyasal içerik*

ABSTRACT

Objective: *Maranta arundinacea L. (arrowroot) (Marantaceae) which grows in the tropics is a perennial herbaceous plant. It has edible cylindrical rhizomes with high starch content. Rhizomes are used in folk medicine as a sedative, anti-redness, anti-inflammatory and antiseptic. In this study, the botanical properties, distribution and traditional use of arrowroot which has economic value today and has application potential in various industries, were investigated. Moreover, information on the chemical content of edible rhizomes, the biological activities of extracts, flour, starch and powder obtained from rhizomes and various usage areas of rhizomes were compiled.*

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Result and Discussion: *Rhizomes contain alkaloids, carbohydrates, cardiac glycosides, protein, amino acids, terpenes, saponins, flavonoids, resins, tannins, gums, lignin, anthraquinones, sterols, fibers and minerals. Antioxidant, immunostimulant, anti-ulcerogenic, antidiarrheal, anti-inflammatory, antimicrobial, antidyspeptic, antihypertensive, hypocholesterolemic, hypoglycemic, anticancer and hepatoprotective activities of rhizome and rhizome-derived products have been reported. Arrowroot flour is easily digestible, it is used in breads and biscuits prepared for children, as well as cookies and baked goods. Arrowroot starch is used in making soups, desserts, puddings, sauces, cookies, candies, biscuits, cakes and jelly. Arrowroot starch does not contain gluten, therefore; it is used in the preparation of special biscuits and bakery products. The use of arrowroot powder and starch is common in the cosmetic industry. Arrowroot starch has applications in fields such as pharmacy, hygiene products, environmental management, agriculture, biofilm, biomedical engineering and biofuel production. Arrowroot fiber is suitable for making tear-resistant paper such as tissue paper, cardboard, wrapping paper and bags.*
Keywords: *Arrowroot, arrowroot starch, biological activity, chemical content, Maranta arundinacea*

GİRİŞ

Maranta arundinacea L. (Ararot, Batı Hint Ararotu) türü tropiklerde yetişen otsu, çok yıllık bir bitkidir [1-8]. Yenilebilir türleri içeren 29 cins ve 627 türden oluşan [9] Marantaceae familyası üyesidir ve ekonomik açıdan önemlidir [9,10]. Yüksek nişasta içerikli yenilebilir silindirik rizomlara sahiptir [2-5,8,11,12]. Rizomlu, un haline getirilen ve yenilebilen birçok tür [*Canna edulis* (L.), *Canna indica* (L.), *Tacca leontopetaloides* (L.), *Puerariae lobata* (Wild.), *Puerariae flos* (DC.) ve *Puerariae radix* (DC.)] [13] için ararot ismi kullanıldığından bazı karışıklıklar olsa da bu türler arasında genelde ararot olarak bilinen *M. arundinacea* türüdür [13,14]. Cinse adını veren "maranta" kelimesi ilk olarak İtalyan asıllı doktor ve botanikçi Bartolomeu Maranti (1500-1571) tarafından ortaya atılmıştır [13,15]. Kırmızımsı çiçek saplarından dolayı da "arundinacea" kelimesi eklenerek tür adı oluşturulmuştur [15]. Sinonimlerinin fazla olması [*Marantu cunnacori folia* (Plumier, 1703), *Maranta silvatica* (Rosc., 1807), *Maranta sylvatica* (Rosc. ex Sm, 1812), *Maranta indica* (Tussac, 1813), *Maranta ramosissima* (Wall., 1832), *Maranta protracta* (Miq., 1844), *Maranta tessellata* var. *kegeljanii* (E. Morren, 1875), *Maranta arundinacea* var. *variegatum* (N.E.Br., 1986), *Phrynium variegatum* (N.E.Br., 1886), *Maranta arundinacea* var. *variegata* (Ridl., 1891), *Maranta arundinacea* var. *divaricata* (Roscoe, 1917), *Maranta arundinacea* var. *sylvestris* (Matuda, 1951)] sorunlara neden olmuştur [13].

Bu derleme çalışmada günümüzde büyük ekonomik değere ve çeşitli endüstrilerde (farmasötik, gıda, kozmetik, boya, kâğıt endüstrisi gibi) uygulama potansiyeline sahip olan; ararot olarak bilinen *M. arundinacea* türünün botanik özellikleri, yayılışı, geleneksel kullanımı, rizomların kimyasal içeriği ile birlikte rizom ve rizomlardan elde edilen ürünlerin (un, nişasta ve toz) biyolojik etkileri ve çeşitli kullanım alanlarına ilişkin bilgiler derlenmiştir.

Botanik Özellikler

M. arundinacea; ince, küçük gövdeli [5,16], yaklaşık 1-1.5 m yüksekliğe kadar boylanabilen, oval-mızrak şeklinde ve 2-10 inç ebatlarında çok sayıda yaprağa sahip bir türdür [14,16,17]. Yaprak damarları ana damara paralel yerleşmiş olup sapları yaprağın gündüz ışığa yönelimini, gecede kapanmasını sağlayan bir yapıya sahiptir. Bu nedenle bitki bazı ülkelerde "dua bitkisi" olarak bilinir [15]. Dikimden yaklaşık 90 gün sonra uzun pedikül boyunca çiftler halinde küçük, beyaz-krem çiçekler görülür [7] ve ikiz salkımlar halinde büyürler [16,18]. Çiçekler 3 ayda gelişir ancak tohum nadiren oluşur [14]. Bitki 2.5-3 cm genişliğinde ve 20-40 cm uzunluğunda beyaz etli silindirik rizomlara sahiptir. Rizomlar; iki-üç veya tek bir demet halinde bulunur [5,8,14,16,17], değişken şekilli, toprak altında dikey veya yatay olarak büyür [13] ve uzun lifli köklere sahiptir [5,8].

Ararot rizomlarının mikroskobik incelemesinde epidermal hücrelerin altında bir dizi parankimatik hücre olduğu görülür. Bu parankimatik hücreler tamamen merkeze doğru oval şekilli nişasta granülleri ile doludur [15,18]. Toz rizom incelemesinde ksilem demetleri, nişasta taneleri, kalsiyum oksalat kristalleri görülür [18].

İsimlendirme

Ararotun popüler isimleri, "yemeklerin yemeği" anlamına gelen aruak, arauque veya aruá-aru terimlerinden köken alır. Brezilya'nın kolonizasyonu sırasında yerli terim araruta olarak Portekiz diline girmiştir. Brezilya'nın etnik-kültürel çeşitliliği içerisinde ararot için başka kelimelerde kullanılmıştır (araruta-comum, ararutinha, araruta-caxulta, araruta-palmeira, araruta-gigante, araruta ramosa, agutiguepe, agutigu-pé ve embiri gibi) [13]. Diğer Latin Amerika ülkelerinde tür arruz, envers blanc ve dictame (Antiller); ararot-Barbados (Barbados); sagú ve bribrí (Kosta Rika); araroetoe ve arraroet (Curaçao); amaranta ve pitisilén (Porto Riko); mouchasse (Santa Lusia); marantha (Kolombiya); guapo, guate, guate gallina, salú, bordocillo, caramaco, maranta ve yuquilla (Venezuela) [13]; Bermuda ararot (Bermuda) [13,16] ve ararute (Karayipler) [5] gibi çeşitli isimlerle bilinir. Amerika'da olduğu gibi bitki Asya kıtasında da popüler olup terminolojisinde büyük farklılıklar vardır [aloro, araru, aroro ve uraro (Filipinler); cauallo (Hindistan); belanda [13] ve ubi bemban (Malezya) [19]; hoangting (Vietnam); kuzuukon (Japonya) [13,15]; hulankeeriya, aerukka (Sri Lanka) [16,20]; sitalpati, muktapati, arrowroot (Bangladeş) [9]; adalut (Myanmar) [7]; garut [16,21], ubi garut [19], marantale [13] (Endonezya)]. İsimlendirmede aynı ülke içinde de bölgesel farklılıklar görülmüştür. Bitki Endonezya'nın çeşitli bölgelerinde sago banban (Batak Karo), sago nare (Minangkabau), sago andrawa (Nias), sagu (Palembang), Patat (Sunda), arut/jelarut/irut/larut/ararot (Doğu Java), labia walanta (Gorontalo) ve hudasula (Ternate) yerel adları ile de bilinir [5]. İngilizcedeki terminoloji rizomun şeklinden kaynaklanmaktadır (Amerikan kolonileşmesi döneminde rizomun oka benzetildiği varsayılmaktadır) [13,17]. Kök olarak sınıflandırılmış ve "arrowroot" denilmiştir [13]. Arrowroot kelimesinin ilk kez 1696 yılında kullanıldığı bildirilmiştir [15]. Hala pfeilwurz (Almanya), pijlwortel (Hollanda) ve arruruz (Fransa) gibi çeşitleri kullanımlarını da bulmak mümkündür [13,15]. Diğer isimler arasında Batı Hint ararotu ve itaat bitkisi de yer alır [16].

Yayılış

Bitki tropikal Amerika'nın yerlisidir [3,7,11,18,22]. Meksika, Orta Amerika [4,5,10], Latin Amerika [13], Batı Hint Adaları ve Güney Amerika'ya [5,10] özgüdür. On yedinci ve on sekizinci yüzyıllarda Asya'nın çoğuna yayılmıştır [4], Afrika'da da görülür [13]. Birçok sıcak ülkede yetiştirilir ve Jamaika, Bahamalar, Bermuda, Hollanda, Çin, Mauritius, Ekvator Ginesi, Gabon, Florida, Kamboçya [5], Endonezya, Hindistan, Sri Lanka ve Filipinlerde doğal kabul edilir [3,5,8,11,17,19]. Batı Hint Adaları'nda yaygın olarak yetiştirilirken [2,6,17,18], Avustralya [3,11,17,18], Güneydoğu Asya, Güney Afrika ve Amerika Birleşik Devletleri dâhil olmak üzere dünyanın birçok tropikal bölgesinde yetişmektedir [18]. Dünyadaki ararot talebinin yaklaşık %95'i St. Vincent (Batı Hint Adaları) tarafından karşılanmaktadır [8].

Tropikal veya subtropikal iklim koşullarında 1000 m'ye kadar olan rakımlarda yetişir. Yıllık 1000-2000 mm yağışa ihtiyaç duyar [13]. 20-30°C sıcaklıklarda iyi gelişir [13-15]. Birçok böcek ve patojen saldırısına karşı dayanıklıdır [11]. İyi drene edilmiş, kumlu veya tınlı, hafif asidik toprakta ve kısmi gölgede en iyi şekilde gelişir [4,13]. Yumru kalitesini ve özelliklerini düşürmeden gölgeye (ağaç altı) ve marjinal araziye uyum sağlayabilir [4,19,23]. Ekimden 10-12 ay sonra hasada hazır hale gelir. Her kök yaklaşık 30-50 g ağırlıktadır [7]. Hasatta rizomlar köklerden ayrılır ve küçük, büyük ve tohumluk olarak sınıflandırılır. Büyük rizomlar nişasta eldesi için küçük rizomlar hayvan yemi olarak ve tohumluk rizomlar da yeniden dikim için ayrılır [13]. Ayrıca hasat sırasında tarlada kalan küçük rizomlar aynı alanda 5-7 yıl ürün verir. Rizomların oda koşullarında depolama süresi çok kısıtlıdır, çeşidine göre en fazla 7 gün depolanabilir fakat iyi havalandırmalı depolarda bu süre 6 aya kadar uzatılabilir [15].

Geleneksel Kullanım

Türün geleneksel gıda hazırlamada ve geleneksel tıpta kullanımı vardır [10,18]. Eski zamanlardan beri çeşitli tıbbi uygulamaları ile bilinen önemli bir bitkidir [7,13,20]. Tarihte Karayipler bölgesinde ve Amazon ormanlarında bulunan Caraíba, Caiapós, Guaranis, Pataxós ve Nhambiquaras etnik grupları türün evcilleştirilmesini ve yetiştirilmesini sağlamıştır [13]. Arkeolojik kazı çalışmaları yetiştiriciliğinin 7000 yıl öncesine uzandığını belgelemiştir [15]. Tarihi seramik kalıntılarda bulunan nişasta granülleri

Kolomb öncesi insanların besinlerinde ararot nişastası kullandıklarını göstermiştir [13]. Zehirli okların panzehri olarak kullanılan bitki Mayalar ve Orta Amerika'nın diğer sakinleri tarafından büyük saygı görmüştür [16,18]. Mayalar çiçek hastalığında görülen yaraların tedavisinde ararot lapası ve ararottan hazırlanan bir tür içecek kullanmıştır [15]. Kızılderililer için önemli tıbbı bir bitki olup yılan zehrine karşı panzehir olarak kullanılmıştır [7,13]. Eski Hindistan'da hekimlerin ararotu tedavide kullandığı bildirilmiştir. Halk tıbbında yatıştırıcı, kızarıklık giderici, anti-inflamatuvar ve antiseptik [15] olarak ve ayrıca ishal, dizanteri ve kolit [24] tedavisinde kullanımları rapor edilmiştir. Geleneksel olarak ishal tedavisinde rizomlar kullanılır [11]. Rizomlar suda veya sütte kaynatılıp tatlandırıldığında ishal için iyi bilinen geleneksel bir ilaçtır [25,26]. Sütte pişirilen rizom tozu iritabl bağırsak sendromunda ve ülseratif kolitte tahrişi hafifletmek ve ülserlerin iyileşmesini kolaylaştırmak için şekerle birlikte verilir [24]. Maritus'ta rizom tozu çocuklarda ishal tedavisinde kullanılır [16]. Ararot yatıştırıcı özellikleri nedeniyle geleneksel tıpta popülerdir [1]. Eski zamanlardan beri yaraların tedavisinde kullanılan hafif ve doğal emilen bir toz olarak ün kazanmıştır [17]. Brezilya'nın Pará eyaletindeki Amazon'un Marudá kıyısında yer alan bir topluluk bitkiyi tüberküloz ve uyuşukluk için indikatör olarak kullanmıştır [16]. Halen Brezilya ve Asya geleneksel tıbbında yapraklar ve rizomlar infüzyon şeklinde çay olarak tüketilir [13]. Günümüzde mideyi yatıştırmak [18], idrar yolu ile ilgili hastalıkların tedavisi ve ishali hafifletmek için kullanılmaktadır [4,18]. Bazı ülkelerde bağırsak iltihaplarının tedavisinde tüketildiği görülmüştür [15]. Kırsal kesimin %80'inde bitkisel veya doğal tedavi olarak ararot kullanılmaktadır [26]. Ayurveda tıbbında Tugaksheeri olarak bilinir ve çeşitli Ayurvedik formülasyonların hazırlanmasında yaygın olarak kullanılan önemli bir bitkidir. Düşük glisemik indeksi nedeniyle sindirimi kolay ve hastaların beslenme taleplerini karşılamak için besleyici yoğun nişastalı bir rizoma sahip olduğundan Samhithas ve Nighantus'a göre, madhura rasa (tadı tatlı), sheetha veerya (soğuk etki), guru (ağır) snigdha (tatsız) guna özellikleri vardır. Balya (güç sağlar), paushtikam (besleyici), dhatuvridhikara (besleyici dhatus) olup kshaya (tüberküloz), swasa (nefes darlığı), kasa (öksürük), daha (yanma hissi), raktha dosha (kan hastalıkları), kamala (sarılık), pandu (anemi) ve mutrakruchra (disüri) tedavisinde kullanılır [24].

Türkiye'de Ararot ile İlgili Kayıtlar

"Osmanlı Son Döneminde İlginç Bir Tıbbi Süreli Yayın: Âfiyet Gazetesi (Afiéte La Santé) ve Dizini" isimli bir çalışmada gazetenin sene 1, sayı 21, 9 Nisan 1330 Çarşamba/22 Nisan 1914 tarihli sayısında "Ararot nedir? Çocuklu validelere yeni bir gıda" başlığıyla bir bölüm yer almıştır [27]. "Bir Osmanlı Hekimi Besim Ömer ve Çocuk Beslenmesi" isimli bir çalışmada 19. yüzyılda yetişen dönemin hekimlerinden Besim Ömer Paşa "7. ayda çocuğa verilebilecek gıdalar arasında Amerika'da yetişen kökten çıkarılan besleyici bir nişasta olan ararot ile yapılmış sulu bulamacı önermiştir. Bünyesi hassas ve ishale yatkın olanlara iyi geleceğini ve iki tatlı kaşığı ararot ve et suyundan yapılan bulamacın besleyici olacağını vurgulamıştır. Sütten kesilen çocuklara öğlen veya uykudan önce 100 g süt, 5 g un (buğday, arpa, ararot), 6 g şekerden oluşan bir bulamaç vermeyi önermiştir [28]. "19. yüzyılda İngiltere'den Osmanlı Devletine Seyahat Etmenin Altın Kuralları" isimli bir çalışmada ise seyyahlar için Osmanlı Devleti'ne gerçekleştirilecek seyahatlerde gerekli olan temel malzemeler arasında ararotta sayılmakta ve "Ararot seyyahlar için taşınabilirliği ve kullanışlılığı açısından en faydalı yiyecektir. Beş dakika içerisinde hazırlanabilir." denmektedir [29].

"Çocuk sağlığının Atatürk dönemi siyasetindeki ve meclis gündemindeki yeri" isimli bir çalışmada 1921 yılında meclis tutanaklarında (TBMMZC, D.1, C.9, 4.4.1337 (1921): 356) çocuk gelişimi ve beslenmesinde önemli rol oynadığı düşünülen çocuk maması yapımında kullanılan ararot unundan bahsedilmektedir [30]. 1925-1926 tarihlerine ait Türkiye Cumhuriyeti Devlet Salnamesi esas alınarak hazırlanmış "Türkiye Cumhuriyeti Maliye ve Ticaret Vekâleti Salnamesinin Transkripsiyon ve Değerlendirmesi (1925-1926)" isimli bir tez çalışmasında ise Fransa ve Romanya'dan Türkiye'ye ararot ithal edildiği belgelenmiştir [31].

Kimyasal İçerik

Ararot rizomlarının kimyasal içeriği ile ilgili birçok çalışma literatürde yer almakla birlikte; içeriğin kullanılan ekstraksiyon yöntemi, bitkinin orijini, yaşı, çevresel faktörler gibi etkenlere bağlı olarak değiştiği bildirilmiştir [8]. Literatür taramalarına göre ararot rizomlarının farklı polaritedeki (petroleteri, kloroform, etilasetat, metanol, su ve etanol) ekstreleri alkaloitler, karbonhidratlar, kardiyak

glikozitler, proteinler, amino asitler, terpenler, saponinler [3,10,12,13,18,24,32], flavonoitler [10,13,16,18], reçineler [33], tanenler [16], zamk [10,16,18], lignin, antrakinonlar [34] ve steroller [3,10,13,18] içerir. Nishaa ve arkadaşları (2013) ararot rizomlarının petol eteri, kloroform, etil asetat ve etanol ekstrelerini incelemiş ve etanol ekstresinde taranan bileşik gruplarının tamamının bulunduğunu bildirmiştir (Tablo 1). Ayrıca GC-MS analizi ile rizomların etanol ekstresinde 49 farklı bileşiğin varlığı tespit edilmiştir [3]. Yapılan bir başka çalışmada farklı yaşlardaki ararot rizomlarının (6, 9, 12 ay) etanol ekstrelerinin toplam fenol, toplam flavonoit ve toplam tanen içerikleri sırasıyla: 0.021-0.171 mg GAE/g kuru ağırlık; 0.023-0.379 mg QE/g kuru ağırlık; 1.086-4.746 mg tanen/g kuru ağırlık olarak bildirilmiştir [12].

Tablo 1. Ararot rizomlarının fitokimyasal içeriği [3]

Fitokimyasal içerik	Petroleteri ekstresi	Kloroform ekstresi	Etilasetat ekstresi	Etanol ekstresi
Flavonoit	-	-	+	+
Alkaloit	-	-	-	+
Tanen	-	-	+	+
Glikozitler	+	+	+	+
Steroit	+	+	+	+
Fenolik bileşikler	-	-	+	+
Kardiyak glikozitler	+	+	+	+
Saponin	+	+	+	+
Karbonhidrat	+	+	+	+
Protein	-	-	-	+

+: Var; -: Yok

Rizomlar mükemmel bir protein kaynağıdır [12,35]. Ararot tatlı patates, patates, cassava, muz ve benzeri diğer tropikal gıda kaynaklarına kıyasla daha fazla protein içerir [5]. Amerika Birleşik Devletleri Tarım Bakanlığına bağlı Tarımsal Araştırmalar Servisi 2018 yılında ararot rizomu ve ararot unu ile ilgili araştırma sonuçlarını yayımlamıştır (Tablo 2) [36,37]. Ararot ununda bulunan amino asitler (triptofan, treonin, izolösin, lösin, lizin, metionin, sistein, fenilalanin, tirozin, valin, arjinin, histidin, alanin, aspartik asit, glutamik asit, glisin, prolin ve serin) ve miktarları da bu araştırmada yer almıştır (Tablo 3) [36]. Rizomlar potasyum, demir, manganez, bakır [7,35,36,37], kalsiyum [7,13,36,37], klor [7,36,37], fosfor, magnezyum ve çinko [13,35-37] gibi mineralleri ve niasin, tiamin, piridoksin, pantotenik asit ve riboflavin gibi B grubu vitaminleri içerir [7,37].

Tablo 2. Ararot rizomu ve ararot ununun içeriği [36,37]

İçerik	Birim	Ararot unu (100 g)	Ararot rizomu (100 g)
Su	g	11.4	80.8
Enerji	kkal	357	65
Enerji	kJ	1490	271
Protein	g	0.3	4.24
Toplam lipit	g	0.1	0.2
Kül	g	0.08	1.42
Karbonhidrat	g	88.2	13.4
Lif, toplam diyet lifi	g	3.4	1.3
Ca	mg	40	6
Fe	mg	0.33	2.22
Mg	mg	3	25
P	mg	5	98
K	mg	11	454
Na	mg	2	26
Zn	mg	0.07	0.63

Tablo 2 (*devamı*). Ararot rizomu ve ararot ununun içeriği [36,37]

İçerik	Birim	Ararot unu (100 g)	Ararot rizomu (100 g)
Cu	mg	0.04	0.121
Mn	mg	0.47	0.174
Vitamin A (RAE)	µg	0	1
Vitamin A (IU)	IU	0	19
Vitamin B_1 (tiamin)	mg	0.001	0.143
Vitamin B_2 (riboflavin)	mg	0.0	0.059
Vitamin B_3 (niasin)	mg	0.0	1.693
Pantotenik asit	mg	0.13	0.292
Vitamin B_6 (piridoksin)	mg	0.005	0.266
Vitamin B_9 (folat, toplam)	µg	7	338
Vitamin B_{12} (kobalamin)	µg	0.0	0.0
Vitamin C (toplam askorbik asit)	mg	0.0	1.9
Vitamin D (D_2+D_3)	µg	0.0	0.0
Vitamin D (D_2+D_3)	IU	0	0
Yağ asidi, toplam doymuş	g	0.019 • 14:0→0.001 • 16:0→0.017 • 18:0→0.001	0.039 • 14:0→0.002 • 16:0→0.035 • 18:0→0.002
Yağ asidi, toplam tekli doymamış	g	0.002 • 16:1→0 • 18:1→0.002	0.004 • 16:1→0 • 18:1→0.004
Yağ asidi, toplam çoklu doymamış	g	0.045 • 18:2→0.036 • 18:3→0.009	0.0092 • 18:2→0.074 • 18:3→0.018
Kolesterol	mg	0	0

Tablo 3. Ararot ununda bulunan amino asitler ve miktarları [36]

Amino asitler	Miktar (g/100 g)	Amino asitler	Miktar (g/100 g)
Triptofan	0.004	Valin	0.014
Treonin	0.012	Arjinin	0.012
İzolösin	0.01	Histidin	0.004
Lösin	0.019	Alanin	0.014
Lizin	0.013	Aspartik asit	0.047
Metionin	0.006	Glutamik asit	0.05
Sistein	0.006	Glisin	0.014
Fenilalanin	0.012	Prolin	0.009
Tirozin	0.009	Serin	0.013

Ararot rizomlarının nem (%6-79.88), kül (%0.31-4.14), protein (%0.00-12), karbonhidrat (%7.2-98.65), enerji (65-357 kkal), lipit (%0.12-1.79), ham lif (%0.17-23.25) ve toplam nişasta (%6.4-30) içerikleri birçok çalışmada bildirilmiştir [1,4-8,10,14,16,18,21,23,24,32,38,39] (Tablo 4). Ararot yüksek kaliteli zengin karbonhidrat kaynağıdır [5,13,15,18,23,32]. Karbonhidrat bileşiminin çoğu nişastadır [2,3,7,14,19,40]. Histokimyasal çalışmalar rizomların çok sayıda nişasta taneciği içerdiğini göstermiştir [18]. Rizomlar 14 aylık yaş, taze durumda iken en yüksek nişasta içeriğine sahiptir [41] ve her yaşta nişasta ve lif bakımından zengindir [8,12]. Yüksek diyet lifi içeriğini [12] çözünür ve çözünmeyen lifler oluşturur [14,16,32] (sırasıyla: %2.37 ve %12.49) [16]. Rizomların selüloz, albümin, şeker [15] ve glikoz içerdiği de bildirilmiştir [7].

Tablo 4. Ararot rizomlarının fizikokimyasal özellikleri

Parametreler	Miktar[a]	Kaynaklar
Nem (%)	6-79.88	[7,8,10,12,18,24,36,37]
Kül (%)	0.31-4.14	[7,8,10,12,18,24,36,37]
Protein (%)	0.00-12	[7,8,10,12,21,36,37]
Karbonhidrat (%)	7.2-98.65	[7,8,10,12,21,23,36,37]
Enerji (kkal)	65-357	[21,36,37]
Lipit (%)	0.12-1.79	[7,8,36,37]
Ham lif (%)	0.17-23.25	[7,8,16,24,39]
Toplam nişasta (%)	6.4-30	[1,2,4-6,8,10,23,38]

[a]: en düşük ve en yüksek değerler

Nişastalar

Nişasta bileşimi (amiloz/amilopektin oranı) biyolojik kaynağa göre değişen, farklı yapı ve işlevlere sahip iki tür glikoz polimeri olan amiloz ve amilopektinden oluşan, bitkisel enerji rezervi bir polisakkarittir [8,14,42]. Amilopektin oldukça dallanmış α-1-6 bağlantısıyla bağlanan çok sayıda kısa (1-4)-α-D-glukan zincirinden yapılmış bir moleküldür [8,14] ve suda daha az çözünür [42]. Amiloz, α-1-4 bağıyla bağlanan glikoz birimlerinden oluşan doğrusal bir moleküldür [8,14]. Nişastanın iki ana yapısal bileşeni olan amiloz ve amilopektinin en yaygın bileşimi % 80-90 amilopektin ve % 10-20 amilozdur [20]. Amiloz ve amilopektinin moleküler ağırlığı, şekli ve bileşimi doğaları üzerinde büyük etkiye sahiptir [8,14]. Nişastanın retrogradasyon (moleküler yeniden düzenleme), viskozite ve jel stabilitesi gibi fizikokimyasal özellikleri bu iki molekül arasındaki farklardan etkilenir [8,14,20]. Amiloz amorf iken granül içindeki amilopektin yarı kristal bir yapıya sahiptir. Nişastanın yarı kristal taneli yapısı ekonomik rekabet edebilirliğine katkıda bulunan ana faktörlerden biridir. Granül boyutu nişasta kalitesini belirleyen önemli bir özelliktir. Nişastanın şişme gücü, çözünürlüğü ve sindirilebilirliği özellikle granüllerin boyutundan ve biçiminden etkilenir. Daha küçük granüller daha büyük olanlardan daha kötü şişme özelliklerine sahiptir, bu da daha yavaş jelatinleşmelerine neden olur. Nişasta granüllerinin boyutu ve şekli; jelatinleşme sıcaklığını, bağlı nem miktarını, nişasta hamurunun viskozitesini, nişasta fraksiyonlarının oranını, iyot örneğinin rengini ve diğer fizikokimyasal özellikleri etkiler. Son araştırmalar, nişasta granül boyutunun hem gıda hem de endüstriyel uygulamalarda önemli bir faktör olduğunu göstermiştir [20]. Nişasta düşük maliyeti, bolluğu, yenilenebilir olması ve çok çeşitli ham maddelerde bulunması nedeniyle dikkat çekici olup [14,20,22,43] selülozdan sonra ikinci en büyük biyokütle kaynağıdır [20]. Mısır, cassava [42,44-46], buğday, pirinç [42,45,46], patates, ararot ve sago [45,46] ticari sektörde kullanılan başlıca nişasta kaynaklarıdır. Nişasta; gıda, tekstil ve kâğıt endüstrilerinde yaygın olarak kullanılmaktadır [6,10,45]. Diğer uygulamaları ise eczane, hijyen ürünleri, çevre yönetimi, tarım, biyomedikal mühendisliği, biyoyakıt üretimi [6,10], inşaat, petrol, ilaç, kozmetik ve kimya [45] endüstrileri gibi alanlarda rapor edilmiştir. Ticari nişastalar gıda katkı maddeleri olarak gıda endüstrisi için oldukça değerli olup [45] koyulaştırıcı, dengeleyici ve emülgatör olarak kullanılır [20,44,45]. Ayrıca nişastanın kimyasal modifikasyonunu gerektiren yağ ikame maddeleri olarak kullanılır [45]. Nişastadan unlu mamuller, soslar, çorbalar, şekerlemeler, dondurmalar, şeker şurupları, atıştırmalıklar ve bebek mamaları üretilir [20]. Yenilebilir filmlerin ve kaplamaların üretiminde en sık kullanılan biyopolimerler proteinler, polisakkaritler, lipitler ve bunların kombinasyonlarıdır [42]. Yenilebilir film ve kaplama üretimi için kullanılan polisakkaritler arasında nişasta kolay işlenmesi, bolluğu, biyolojik olarak parçalanabilirliği, sürekli bir matris oluşturma yeteneği [14,22,42], şeffaflığı, iyi gaz bariyeri özelliği, yüksek kullanılabilirliği ve düşük üretim maliyeti nedeniyle iyi bir hammaddedir [6,47] ve biyouyumluluğu, biyolojik bozunabilirliği ve toksik olmaması gibi özellikleri nedeniyle en çok çalışılan doğal biyopolimerdir [22,48].

Ararot Nişastası (Amylum Marantae)

Ararot Nişastası Eldesi

Ararot rizomları yüksek nişasta içeriğine sahip olduğundan nişasta ekstraksiyonu ekonomik açıdan ilgi çekicidir [22]. Ararot nişastası eldesinde izlenen basamaklar diğer kökler ve yumrular için

izlenen basamaklarla aynıdır. Nişasta eldesi bir dizi işlemi içerir [8,14]. Bunlar: rizomların taşınması, depolanması, yıkama, parçalama/ezme, ekstraksiyon, saflaştırma, kurutma, paketleme ve stoklamadır. Yıkanan rizomlar ezilir, rendelenir ve parçalanır [13]. Rendeleme/öğütme ile parçalanma, bitki hücre duvarlarını açtığı ve bitki içindeki nişasta granüllerini ortaya çıkardığı için en önemli basamaktır [8,14]. Rende sürtünme ve kesme yoluyla boyut küçültür ve parçalanma verimliliğini arttırır [8]. Ararot çiftçileri iki nişasta çıkartma yöntemi kullanır. Biri yerel halk arasında "ilod" olarak bilinen geleneksel işlemle, ikincisi ise motorla çalışan bir öğütücü sistemi kullanmayı içerir [8,14]. Sonrasında nişasta, liflerden ayırmak için suda ters akıma tabi tutulur; elde edilen sulu ekstre saflaştırılır; istenmeyen maddeler ve çözünür şeker uzaklaştırılır. İçinde nişasta bulunan kısım sudan ayrılır konsantre halde nişasta dekantasyonu önlemek için özel bir hojenizasyon tankına aktarılır, süzülür [13]. Belirli bir sıcaklık ve nem değerinde kurutulur [8,13-15], son ürün siloya alınır, burada soğutulur ve paketlenene kadar saklanır [13].

Ararot Nişastasının Kimyasal Özellikleri

Ararot nişastasının teknik-fonksiyonel özellikleri çeşitli çalışmalarda araştırılmış ve jelatinleşme sıcaklığı: 65-75°C, maksimum viskozite: 0.40-498.00, viskozite bozunması: 10.00-133.00, retrogradasyon eğilimi: 36.00-189.00, nihai viskozite: 94.00-669.00, şeffaflık (%): 16.00-18.50 aralıklarında bulunmuştur [13]. Ararot nişastası ve diğer ticari nişastalar (patates, buğday, şeker palmiyesi, pirinç, sago ve cassava nişastaları) nem, amiloz ve kül içeriği bakımından karşılaştırılmış ve sırasıyla nem içeriği: %15.24; 18-19; 13; 15; 12-13; 10-20 ve 13, amiloz içeriği: %35.20; 20-25; 26-27; 37.60; 26-28; 24-27 ve 17, kül içeriği: %0.33; 0.4; 0.2; 0.2; 0.1; 0.2 ve 0.2 olarak bildirilmiştir [8]. Literatür taramalarında farklı orijinlerden elde edilen ararot nişastası örneklerinin içerik bakımından incelendiği (nem: %8.10-15.34, kül: %0.18-1.50, yağ: %0.20-1.0, lif: %0.06-4.60, protein: %0.0-0.65, toplam nişasta: %81.60-99.32, amiloz: %15.21-42.01 ve amilopektin: %57.99-84.79) ve renk parametrelerinin değerlendirildiği görülmüştür (Tablo 5). Sindirilebilirlik ve jel oluşturma yeteneği gibi özellikleri [20,49] ve yüksek amiloz içeriği (%15.21-42.01) [13] nedeniyle mısır (%28-33), cassava (%16-19), buğday (%30-32) ve patates (%18-20) nişastaları ile rekabet edebilecek durumdadır [49].

Tablo 5. Ararot nişasta tozu karakterizasyonu

Bileşikler	**Ararot nişasta tozu***	**Kaynaklar**
Nem (%)	8.10-15.34	[3,6,8,13,40,43]
Kül (%)	0.18-1.50	[3,6,8,13,40,43]
Yağ (%)	0.20-1.0	[3,6,13,40,43]
Lif (%)	0.06-4.60	[6,13,23,43]
Protein (%)	0.0-0.65	[3,13,40,43]
Toplam nişasta (%)	81.60-99.32	[6,13,43]
Amiloz (%)	15.21-42.01	[3,5,6,8,13,20,38,40,42,43,49]
Amilopektin (%)	57.99-84.79	[5,13,43]
Renk parametreleri		
L*	75.52	[6]
a*	0.83	[6]
b*	6.00	[6]
ΔE*	18.84	[6]

L: aydınlık değeri; a: kırmızı ve yeşilliği; b: sarı ve mavilik; *: en düşük ve en yüksek değerler

Ararot nişastası %2.12 Tip 2 dirençli nişasta içerir [23]; düşük protein, yağ, kül ve lif [2,6] (%8.7 çözünmeyen diyet lifi ve 0.5 çözünür diyet lifi [3,40]) bileşimine sahiptir. Yapılan araştırmalar cassava, patates, muz ve kanna'dan izole edilen diğer nişastalara benzer kimyasal bileşime, şekle, parçacık boyutuna ve termal geçiş sıcaklıklarına sahip olduğunu göstermiştir [2,4,6]. Muz, tatlı patates, zencefil ve jak meyvesinden elde edilen nişastalara kıyasla daha yüksek şişme gücüne sahiptir. Ararot nişastasının sergilediği yüksek şişme gücü büyük granül boyutu ve yüksek amiloz içeriği ile açıklanır. Ararot nişastasının 80°C'deki çözünürlüğü muz, kanna ve jak nişastaları için bildirilenlerden daha yüksektir [6]. Ararot nişastası mükemmel sindirilebilirlik, nispeten düşük sıcaklıklarda jelatinleşme

kapasitesi [22] ve iyi özelliklere sahip filmler geliştirmek için gerekli olan yüksek amiloz içeriği gibi özel fizikokimyasal özellikler [8,20,22] ve kana uyum sağlama, çok yönlü, zehirsiz, ekolojik ve biyo-birikimli olma gibi birçok avantaja sahiptir [8,14] ve süspansiyonları psödoplastik davranışa gösterir [2].

Ararot Nişastasının Morfolojik Özellikleri

Ararot nişasta granülleri beyaz renkli olup yuvarlak ve çok köşeli şekillere sahiptir [38]. Sri Lanka'nın 5 farklı bölgesinde yetişen ararot rizomlarından elde edilen nişasta granüllerinin elektron ve ışık mikroskobu ile yapılan morfolojik incelemelerinde oval, düzensiz küresel ve küresel şekiller baskın bulunmuştur. Oval şekilli granüllerin ortalama yüzdesi %48.46-59.34 aralığında iken granüllerin uzunluğu 42.91-45.86 µm ve eni 30.81-32.32 µm aralığında rapor edilmiştir [20]. Nişasta granüllerinin SEM incelemelerinde 7-16 µm boyutunda pürüzsüz ve küresel bir yapıya sahip oldukları; asit hidrolizi sonrasında şeklin düzensizleştiği ve genişlediği, 11-22 µm boyutlarına ulaştığı fakat yapısal değişiklik olmadığı belirlenmiştir [50]. 9-42 µm arasında değişen dairesel ve oval nişasta granüllerinin elektron mikroskobu kullanılarak görüntülendiği bir çalışmada granüllerin Tip A (büyük ve oval, çap ≥15 µm) ve Tip B (küçük ve küresel, çap <15 µm) olarak sınıflandırıldığı görülmüştür [13]. Başka bir çalışmada boyutların 10-16 µm arasında değiştiği ve şekillerin yuvarlak, oval ve çokgen olduğu bildirilmiştir [51]. Asit hidrolizi sonrası nişasta granüllerinin morfolojisinde önemli bir sapma olmamıştır. Son dönemde yapılan SEM analizleri sonucu nişasta granüllerinin elipsoitten ovale kadar düzensiz ve dairesel geometriler sergilediği, granüllerin yüzeyinin pürüzsüz ve çatlak izi olmadığı gözlenmiştir [8]. Başka bir çalışma ise nişasta granüllerinin 0.74-0.99 dairesellik; 0.39-0.96 yuvarlaklık ve 1.05-2.54 eliptik değerlerine sahip olduğunu göstermiştir, bu küresel ve eliptik şekillerin varlığını doğrulayan bir sonuçtur [6].

Ararot Rizomlarının Biyolojik Etkileri ve Çeşitli Alanlarda Kullanımları

Biyolojik Etkileri

Rizom ve rizomdan elde edilen ürünlerin antioksidan, immünostimulan, anti-ülserojenik [9,16,17,24], antidiyareik [5,9,17,35], anti-inflamatuvar, antimikrobiyal [13-17] (antibakteriyal [9,16,52], antifungal [26], vibriosidal [9,24]), antidispeptik, antihipertansif, hipokolesterolemik, hipoglisemik [6,13], antikanser [32] ve hepatoprotektif [24] aktiviteleri rapor edilmiştir. Literatürde rizomların metanol ekstresi *in vitro* antioksidan ve antibakteriyal; etanol ekstresi *in vitro* antioksidan ve anti-inflamatuvar ve *in vivo* antioksidan ve prebiyotik; rizom infüzyonu hipoglisemik; rizomdan elde edilen nişasta antidispeptik ve *in vivo* anti-ülseratif; rizomlar hipoglisemik ve rizom tozu antidiyareik, immünomodülatör ve *in vivo* prebiyotik etkili bulunmuştur. Rizom tozu aracılı selenyum nanopartiküller anti-inflamatuvar, rizom ununun şeker ekstresi prebiyotik, rizomdan elde edilen kurabiyeler (30 g/gün) immünomodülatör, rizom analog pirinç hipoglisemik ve antihipertansif, çıtır rizom gevreği (20 g/gün) antropometri, rizomun bütirillenmiş nişastası hipokolesterolemik, rizom çıtır gevrekleri (21 g/gün) antropometri ve rizom gevreği (20 g/gün) antihipertansif etkili bulunmuştur [16].

Antimikrobiyal ve Prebiyotik Etki

Genç rizomların antimikrobiyal aktiviteye sahip olduğu bilinmektedir [12]. Rizomların ve rizomlardan elde edilen ürünlerin antimikrobiyal etkinliği birçok mikrobiyal suşa (*Staphylococcus aureus* [16,52], *Staphylococcus epidermidis* [16], *Escherichia coli* [16,53], *Clostridium perfringens* [53], *Streptococcus mutans*, *Salmonella* sp., *Bacillus cereus* [16], *Lactobacillus* sp. [16,53,54] (*L. acidophilus* [13,55], *L. plantarum* [34], *L. casei* [16] (rhamnosus ve shirota suşları)), *Bifidobacterium bifidum, Bifidobacterium longum* [16]) karşı test edilmiş ve etkili bulunmuştur. Rizomların Tinea Pedis'i tedavi etme etkisi antifungal aktivitesine bağlanmıştır [26]. Yapılan çeşitli araştırmalarda ararot rizom ekstresinden hazırlanan gümüş nanopartiküllerin *in vitro* olarak *S. aureus* ve *E. coli*'nin [16]; ararottan hazırlanan selenyum nanoparteküllerin *S. mutans*'ın [16] ve rizomların metanol ekstresinin metisiline dirençli *S. aureus*'un büyümesini inhibe ettiği bildirilmiştir [52]. Ararot unu içeren diyet ile beslenen farelerde yapılan *in vivo* çalışmada *C. perfringens* ve *E. coli* popülasyonlarının kontrol grubuna göre fark önemsiz olmakla birlikte daha düşük olduğu bulunmuştur [53]. Ararot unu veya nişastası ilaveli

(%2.5 ve %5) yoğurtların kontrole göre daha yüksek bakteri inhibe etme potansiyeli olduğu gözlenmiştir. Ararot rizomu, *Oroxylum indicum* Vent türünün kabukları ve *Commelina benghalensis* L. türünün tamamını içeren bitkisel bir ilacın *S. epidermidis*'te biyofilm oluşumunu engellediği bildirilmiştir [16].

Probiyotikler bağırsakta sağlıklı mikroflorayı sürdürerek konakçıya fayda (anti-kanserojen aktivite, gıdaların iyileştirilmiş besin değeri, serum kolesterol seviyelerinin ve laktoz intoleransının azalması, bağışıklık sisteminin iyileştirilmesi ve normal insan bağırsak mikroflorasının dengede tutulması gibi) sağlayan canlı mikroorganizmalardır. Bununla birlikte pH, metabolitler ve çözünmüş oksijen gibi çeşitli faktörler probiyotik organizmaların büyümesini ve hayatta kalmasını sınırlar. Probiyotik organizmanın normal insan mikroflorasının bir sakini olması ve üst gastrointestinal sistemden geçişte hayatta kalması gerekir. Bunun için organizma düşük mide pH'ına, safra tuzlarına, sindirim sırasında üretilen enzimlere ve metabolitlere dirençli olmalıdır. Prebiyotikler, probiyotiklerin hayatta kalmasını ve aktivitesini artıran seçici olarak fermente edilmiş bileşenlerdir [55]. Ararotta bulunan biyoaktif maddelerin potansiyel prebiyotik kaynağı olduğunu gösteren çalışmalar vardır [3,10, 13,16,39,40]. Bazı araştırmalar sindirilemeyen oligosakkaritlerin gastrointestinal kanaldaki normal mikroflorayı arttırabilen prebiyotikler olduğunu ve böylece mikroflora tarafından oligosakkarit fermantasyonuna yol açabileceğini bildirilmiştir. Bu; kısa zincirli yağ asitlerini arttırır, intraluminal pH'ı düşürür ve bağırsak geçirgenliğindeki artışı inhibe eder, sonuç olarak patojenik bakterilerin bağırsak epitel bariyerinden geçmesini önler [56]. Ararotun fermente ürünlerden daha yüksek biyokütle elde etmek ve canlılıklarını korumak için kullanılabilen frukto-oligosakkaritler [16,55] ve zengin prebiyotik görevi gören çözünür diyet lifi içerdiği bilinmektedir [55]. Toz edilmiş rizom içeren yemle beslenen farelerde rafinoz, laktuloz ve stakioz varlığı gösterilmiştir. Bu sakkaritler, besinlerin emilimine yardımcı olan ve gastrointestinal sistemin kimyasal, fiziksel ve mikrobiyolojik özelliklerini iyileştiren prebiyotik etkili bileşiklerdir. Bu etki kısmen laktobasiller gibi faydalı mikroorganizmaların çoğalmasını uyararak meydana gelir [13]. Bu durum çok sayıda probiyotik mikroorganizmanın (*L. casei* (rhamnosus ve shirota suşları), *Lactobacillus* G3, G1 ve F1 suşları, *B. bifidum* ve *B. longum* vb.) büyümeyi desteklemek için ararotta bulunan şekeri kullanması ile açıklanır. *Lactobacillus* G3 ve *B. bifidum*'un artan büyümesi, bu probiyotiklerin *Salmonella* sp., *B. cereus* ve *E. coli* gibi patojenik bakterilerle savaşmasına ve patojenik bakteri popülasyonlarının azalmasına neden olur. Bir çalışmada ararot rizom ekstresi sıçanlarda *L. casei* rhamnosus suşu ile karşılaştırıldığında *E. coli, Salmonella* sp. ve *B. cereus*'un büyümesini sırasıyla 3.2, 1.5-3.9 ve 1.4-3.5 log CFU/ml azaltmıştır. Fakat ekstre uygulaması bittiğinde *E. coli* miktarının arttığı laktik asit bakteri miktarının azaldığı gözlenmiştir [16]. Başka bir çalışmada ise ararot tozunun *in vitro* olarak *Lactobacillus* sp. popülasyonlarını belirgin şekilde arttırdığı ve *E. coli, Bifidobacteria* sp. ve *C. perfringens* sayılarını azalttığı bildirilmiştir [26]. Ararotun suda çözünen karbonhidratlarının ekstraksiyonu ve probiyotik *L. acidophilus* üzerindeki prebiyotik etkisi araştırılmış ve sonuçlar ararot karbonhidratlarının hem damıtılmış su (9.34) hem de yağsız süt (9.01) ortamında kontrole (6.71) kıyasla *L. acidophilus*'un CFU'sunu önemli ölçüde ($p<0.05$) artırdığını göstermiştir [55]. Böylece suyla ekstre edilen ararot karbonhidratlarının, probiyotiklerin canlılığını koruyarak önemli prebiyotik etki gösterdiği ortaya konmuştur [13,55]. Ararot karbonhidrat ekstresinin yoğurtlardaki probiyotiklerin hayatta kalmasını arttırdığı da bildirilmiştir [55]. Ararot ekstresi eklenmiş yoğurt, ekstre eklenmemiş kontrole göre daha uzun süre ve daha yüksek *Lactobacillus* sp. popülasyonu içermiştir [54]. Ararot unu ile beslenen farelerin bağırsaklarındaki *Lactobacillus* sp. gibi yararlı bakteri popülasyonu standart diyetle beslenen kontrol gruplarına göre önemli ölçüde daha yüksek bulunmuştur [53]. Endonezya'da yapılan bir çalışmada iki tür laktik asit bakterisi (*L. casei* ve *L. plantarum*) kullanılarak taze ararot rizomu içeren yoğurt üretimi hedeflenmiştir. Elde edilen ürünün DPPH değeri %59.30-86.62, pH değeri 4.29-4.81, toplam laktik asit değeri %0.87-0.95 ve toplam laktik asit bakteri yoğunluğu 7.5×107-7.6×109 CFU/ml aralığında olup bu değerler Endonezya Ulusal Standardı 2981:2009 standart probiyotik içeriğine de uygundur [34].

Gastrointestinal Sistem Üzerine Etki

Anti-diyareik Etki

Rizomların ve rizomdan elde edilen ürünlerin (toz, un, nişasta) ishali ve ağrıyı tedavi ettiği

bilinmektedir [3,5,16,18,25,26,53]. İshal başta olmak üzere mide ve bağırsak rahatsızlıklarında geniş kullanımları vardır [57]. Rizomların tüketilmesi gastrointestinal sistemdeki probiyotik bakteri sayısını artırır ve gastrointestinal epitel hücrelerinin sağlığı üzerinde olumlu etki yapar [53]. Besleyici olmasının yanı sıra "mukus zarları üzerinde yatıştırıcı ve yumuşatıcı etkisi" olduğu kayıtlıdır [25,26]. Genç rizomlar mukusu bağırsak duvarından çıkarabilir, ishali tedavi edebilir, mide hazımsızlığı ve mide ekşimesi semptomlarını hafifletebilir [12]. Sıçanlarda ve salamura karideslerde 200 ve 400 mg/kg dozlarında metanol ekstresi anti-diyareik aktivite göstermiş ancak hafif sitotoksik etkide kaydedilmiştir [5]. İshal önleyici olarak etkinliğine yönelik bazı kanıtlar fareler üzerinde yapılan ve kolera toksininin neden olduğu net su salgısını azalttığını ortaya koyan laboratuvar çalışmalarına dayanır [25,26]. Rizomlardan elde edilen ararot ununun çok sayıda yararı bildirilmiş olup en iyi bilinenleri sindirim sistemi ile ilgili olanlardır ve genellikle mide ağrısını hafifletmek ve ishali tedavi etmek için kullanılır [35]. Yapılan bir pilot çalışmada ararot tozu (5 ml x 3) irritabl bağırsak sendromuna bağlı diyareye karşı 11 hastada test edilmiş ve hastalarda ishalin azaldığı ve kabızlık üzerinde uzun vadeli bir etkiye sahip olduğu bildirilmiştir [16,25,26]. Karın ağrısı hafiflemiştir. Ancak 1 hastada kötüleşen dispepsi, bir hastada ciddi kabızlık ve iki hastada orta dereceli kabızlık görülmüştür [16,25]. Ararot nişastası vücudun tahriş olmuş veya iltihaplı iç dokularını yatıştıran ve koruyan yatıştırıcı özelliklere sahiptir, bu nedenle bağırsak şikâyetlerinde verilir [38] ve ishal tedavisinde etkilidir [3]. Ararottan üretilen dirençli nişastanın glikozu daha düşük molaliteye sahiptir, bu sodyum emilimine ve ardından suyun gelmesine yol açarak daha küçük dışkı hacmine neden olur. Ararot nişastası çinko içerir ve çinko tüketimi sağlıklı bir gastrointestinal sistem ile ilişkilidir. İshalli olan çocuklara semptomları azaltmak için çinko tüketmeleri önerilmektedir [16]. Yapılan araştırmalarda ararot ampullerinin peptik ülseri tedavi etmede kullanılabileceği bildirilmiştir [5]. Dahası ararot lif bakımından zengindir, lif intrakolonik basıncı düşürür ve divertiküler hastalıkta yararlı bir rol oynar [7].

Anti-ülseratif Etki

Ararot rizomları glisemik indeksi düşük gıda kategorisine girdiğinden anti-ülser etkilidir [5] (Glisemik indeks aralığı: 14-32) [5,16,39]. Ararot nişastası mide ülserinin tedavisi ve gastrointestinal sistemin korunması gibi potansiyel tıbbi özelliklere sahiptir. Bu nişastanın ülser indüksiyonundan sonra sıçan midelerini iyileştirdiği bildirilmiştir [58].Yapılan bir araştırmada 30 gün boyunca günde 4 g/gün (x3) ararot nişastası alımıyla birlikte kızarmış ve baharatlı yiyeceklerden uzak durmak, uyku düzenlemesi ve stresten sakınmak, amlapita hastalarında semptomları önemli ölçüde azaltmış ve hastalarda belirgin kilo artışı gözlenmiştir [59]. Pilor ligasyondan önce 7 gün boyunca suda 1.100 mg/kg vücut ağırlığı ararot nişastası verildiğinde pilor ligasyonuna bağlı ülserasyonu olan sıçanlarda mide sıvısında %56.81 azalma, mide asiditesinde %48.44 azalma, mide pH'ında %27.14 artış, peptik aktivitede %45.53 azalma, toplam karbonhidratta %56.44 artış ve neredeyse normal gastrik mukoza histolojisi gözlenmiş olup ararot nişastasının gastrik ülserasyonu önleme etkisi olduğu bildirilmiştir. Bu sırada; vücut ağırlığı, toplam protein ve ülser indeksi gibi veriler kontrol ile karşılaştırıldığında bulunan farkın önemsiz olduğu da bildirilmiştir [60]. Ararotun anti-ülseratif etkisinin dirençli nişasta içeriğinden kaynaklandığı öngörülmekte olup nişasta, mide mukozasının kalınlığını arttırmış ve pro-inflamatuvar sitokinleri (IL-6, IL-12, tümör nekroz faktörü-α (TNF- α) ve IFN-ɤ gibi) inhibe etmiştir. Yapılan araştırmalarda dirençli nişastanın bağırsaktaki normal bakteri florasını veya probiyotiklerin sayısını arttırdığı ve böylece probiyotik bakterilerin *Helicobacter pylori* gelişimini engelleyerek gastrit oluşma sıklığını azaltabileceği bildirilmiştir [16].

Antioksidan ve Anti-inflamatuvar Etki

Ararot bitkisinin ve rizomlarının antioksidan [5,11,13,15,16,18,41] ve anti-inflamatuvar [12,16,32,61] aktiviteleri yapılan çeşitli çalışmalarda rapor edilmiştir. Literatüre göre taze rizom ekstresinin antioksidan aktivitesi (1.78 µg/ml) taze yaprak ekstresinin antioksidan aktivitesinden (0.27 µg/ml) daha yüksektir [5]. Rizomun metanol ekstresi alkaloitler, karbonhidratlar, kardiyak glikozitler, proteinler, amino asitler, terpenler, saponinler, flavonlar, flavanonlar, tanenler ve zamk [3,16,18]; kloroform ve petrol eteri ekstreleri kardiyak glikozitler, steroller ve saponinler; sulu ekstresi karbonhidratlar, kardiyak glikozitler, fenolik bileşikler [16,18], alkaloitler, terpenler, saponinler [10,16] ve reçine [16,33] içerir. Ararot rizomunda bulunan biyoaktif bileşikler hidrojen peroksit (H_2O_2), nitrik

oksit (NO), 2,2-difenil-1-pikrilhidrazil (DPPH) [13,32] ve 2,2'-azinobis-3-etilbenzotiyozolin-6-sülfonik aside (ABTS) [16,32] karşı antioksidan aktivitelidir. Serbest radikaller tarafından üretilen oksidasyon ararot nişastası tarafından önlenir ve vücut hücreleri uzun vadede diyabet, hipertansiyon, kalp ve damar hastalıkları ve çeşitli kanserler gibi hastalıklara yol açan reaktif oksijen türlerine (ROS) karşı korunur [41]. Yani ararotta bulunan antioksidan etkili bileşikler ROS'un neden olduğu hasarı önleyebilir ve anti-inflamatuvar ajan olarak kullanılabilir [32]. Rizomların etanol ekstresinin antioksidan aktivitesinin araştırıldığı bir çalışmada DPPH, ABTS, H_2O_2 ve NO radikallerine karşı IC_{50} değerleri sırasıyla 293.4, 297.4, 336.1 ve 258.7 µg/ml olarak bildirilmiştir. İndirgeme gücü ve FRAP değerleri ise numunenin artan konsantrasyonları birlikte artmıştır. Antioksidan etki BHA ile benzerdir [11,16]. Başka bir çalışma, rizomların metanol ekstresinin *in vitro* olarak DPPH serbest radikallerine karşı C vitamini ile karşılaştırıldığında ve ABTS serbest radikallerine karşı Trolox ile karşılaştırıldığında IC_{50} değeri C vitamini ve Trolox'dan yüksek olmasına rağmen antioksidan özelliklere sahip olduğunu göstermiştir [16]. Sıçanlarda etanolün neden olduğu karaciğer hasarında oksidatif stres biyo-belirteci MDA ve hepatik hücre hasarı biyo-belirteçleri SGOT ve SGPT'ın kandaki konsantrasyonları negatif kontrol fareleriyle karşılaştırıldığında daha düşük bulunmuştur. Bu, rizom ekstrelerinin oksidatif stresi azaltma ve böylece hepatik hücrelere verilen zararı en aza indirme kabiliyetinin bir göstergesidir [34,62]. Bununla birlikte; atıştırmalık olarak günde 20 mg ararot çıtır gevreği tüketen Tip 2 diyabetli 14 hasta üzerinde yapılan bir çalışmada süperoksit dismutaz antioksidan enzim konsantrasyonunda artış tespit edilememiştir. NO artışı inflamasyonu sınırlayabilir ve aterosklerozu önleyebilir, fakat bu çalışmada NO'da da herhangi bir değişiklik tespit edilememiştir [16,63]. Rizom tozundan elde edilen etanol ekstresi kırmızı kan hücrelerinin membran stabilizasyonundaki artışla kanıtlanmış olan anti-inflamatuvar özelliklere sahiptir [16]. Ararot rizomları ile uyarılan selenyum nanoparteküllerin diklofenak ile karşılaştırıldığında anti-inflamatuvar özelliklerinin olduğu gösterilmiştir [57]. Akut inflamasyonda biyo-belirteç proteinlerden biri olan C-reaktif protein (CRP) konsantrasyonu inflamasyonda veya enfeksiyon alanlarında 1000 kata kadar artabilir [16]. Yapılan bir çalışmada ortalama 1.500 kkal diyet tüketen ve öğünler arasında ararot, tatlı patates, cassava ve kabak içeren bir atıştırmalığı dört hafta boyunca günde 32 g kadar tüketen Tip 2 diyabet hastalarında CRP konsantrasyonunda önemli bir düşüş görülmüştür [61].

Diyet ve Beslenme Üzerine Etki

Ararot rizomlarının diyet ve beslenme üzerine olumlu etkilerinin olduğu bilinmektedir [1,12,13,26,35,40,57]. Rizomlar hazımsızlık tedavisinde kullanılır [13,40]. Genç rizomlar kilo kaybını destekler [12]. Olgun rizomlarda beta-karoten, niasin ve tiamin ile birlikte nişastalı karbonhidratlar bulunur, böylece soyulup pişirildiğinde iyi sindirilebilir ve çok besleyici bir gıda haline gelir [23]. Rizomlar sindirim sistemine iyi gelen karbonhidrat ve lif (çözünür lif ve çözünmeyen lif) bakımından zengin olduğundan ararot nişastasının ekonomik ve sağlık değeri yüksektir [5]. Ararot nişastasının lif formu diğer nişasta türlerine göre daha kısadır, bu nedenle nişasta %84.35 sindirilebilirlik ile kolayca sindirilebilir [23]. Çözünür lif gıda hacmini arttırır, gıdanın kalınlaşmasına yol açar ve mide boşalmasını engelleyerek kişinin tok hissetmesini sağlar. Bu tokluk kişinin yemek istememesine veya yemek alımını sınırlamasına neden olur. Çözünür lif karbonhidrat ve yağ emilimini engeller [32]. Ararot yağ emiliminin azaltan dirençli nişasta içerir [32], bu nişastanın emilimi zordur ve kolonda fermantasyona uğrayarak kısa zincirli yağ asitlerini arttırır [16]. Bu durum dolaylı olarak kalori alımını azaltır [32]. Dirençli nişasta tüketimi azalmış vücut yağı ve anti-obezite ile ilişkilendirilir [16,58]. Dirençli nişasta tüketmenin diğer faydaları arasında safra taşlarını önleme, kilo verme ve kalsiyum, magnezyum, çinko, demir ve bakır gibi minerallerin emilimini artırma sayılabilir. Ararot tüketimi bağırsak tarafından emilen kısa zincirli yağ asitlerinin üretimini artırarak sodyum gibi elektrolitlerin emilimini uyarır ve sıvıların emilimine yardımcı olur [16]. Ararot; gembili (GI: 90), kimpul (GI: 95), ganyong (GI: 105) ve tatlı patates (GI: 179) gibi diğer rizomlu bitkilere kıyasla [23] daha düşük glisemik indekse (GI aralık: 14-32) sahiptir [5,16,39]. Ararottan elde edilen unlu ürünler düşük glisemik indeksli olduğundan sindirimi kolaydır [5,35]. Modifiye ararot nişastasından yapılan kurabiyelerin glisemik indeksi 31, buğdaydan yapılan kurabiyelerin glisemik indeksi 44'tür. Modifiye ararot nişastasından yapılan kurabiyeler buğdaydan yapılan kurabiyelere göre daha yüksek amiloz, toplam diyet lifi ve dirençli nişasta içermektedir [23]. Ararot nişastası yüksek sindirilebilirliği nedeniyle nekahet döneminde olan veya

organik zayıflığı olan çocuklar [8], bebekler [16,18,23,57], yaşlılar ve sindirim sorunu olan bireylerde [1,20,48] besleyici gıda olarak kullanılır. Ararot düşük glisemik indeksi nedeniyle otizm ve down sendromlu çocuklar için gıda olarak kullanılabilirken diyabet ve kalp hastalığı gibi dejeneratif hastalıkları önlemek için de kullanılabilir [23]. Ararot yumuşaktır, bu da onu özellikle mide bulantısı hisseden insanlarda nötr diyetler için uygun hale getirir [10,40]. Mükemmel bir protein kaynağı olan ararot [35]; buğday, yulaf, çavdar ve arpanın aksine [45] glüten içermez [5,15,35,45], bu da glüten intoleransı olanlar için glüten içeren ürünlerin yerini alma potansiyeline sahip olduğu anlamına gelir [20,45]. Ararot unu glütene duyarlı kişiler için dost olmanın yanı sıra glikoz ve lipitleri normal sınırlar içinde tutmaya da yardımcı olabilir. Bu nedenle özellikle glikoz ve lipit profillerini yönetmekte güçlük çeken kişilerde fonksiyonel gıda olarak kullanılabilir [5]. Ararotun tüm bu olumlu özelliklerine rağmen (kolay jelleşme kapasitesi, dirençli nişasta içermesi, glüten içermemesi, tıbbi özellikleri ve yüksek sindirilebilirliği gibi) insan diyetine girmesi için daha fazla bilimsel çalışmaya ihtiyaç vardır [26].

Hipokolesterolemik Etki

Ararotun hipolipidemik etkisi çeşitli çalışmalarda bildirilmiştir [13,16,18,61,63]. Ararotta bulunan fitokimyasallar antioksidan etkili olup ksantin oksidaz enzimlerini inhibe edebilir, bu da LDL oksidasyonunu yavaşlatır ve aterosklerozu önler [13,16]. Ararot rizomu düşük glisemik indeksi nedeniyle anti-kolesterol özellik gösterir [5]. Rizomlar safra asidine bağlanabilen çözünür lif içerdiğinden kolesterol seviyelerini düşürebilir. Safra asidi bağlanması, asit veya safra tuzunun enterohepatik dolaşıma yeniden emilimini önler; bu da büyük miktarda safra asidinin dışkı yoluyla atılmasına neden olarak vücuda giren kolesterolü azaltır. Lif tüketimi ayrıca glikoz emilimini engeller böylece insülin seviyelerinin düşmesine neden olur. Azalan insülin seviyeleri, kolesterol sentezlemek için 3-hidroksi-3-metilglutaril koenzim A redüktaz enzimini inhibe eder. Sindirilmemiş çözünür lif kolona geçer burada bakteriler tarafından asetat, propiyonat ve bütirat gibi kısa zincirli yağ asitlerini oluşturmak için fermente edilir. Propiyonat ve asetat emilerek hepatik portal vene girer, böylece hepatik hücreler tarafından kolesterol sentezi inhibe edilir [16]. %100 buğday unundan yapılan kek ve AIN-93 standart sıçan diyeti uygulanan kontrol grupları ile karşılaştırıldığında 28 gün boyunca %100 bütirillenmiş ararot nişastasından yapılan kekle beslenen sıçanlarda toplam kolesterol, LDL kolesterol ve trigliserit seviyelerinde önemli bir düşüş ve HDL kolesterol seviyesinde bir artış gözlenmiştir [16]. Bir ay boyunca günlük 1.500 kkal diyetle kombine atıştırmalık olarak ararot cipsi (20 mg/gün) tüketen 14 Tip 2 diyabet hastasında cips içermeyen diyetle beslenenlere göre önemli kilo kaybı; vücut kitle indeksinde düşüş; toplam kolesterol düzeyinde önemsiz bir azalma ve trigliserit seviyesinde dikkate değer artış görülmüştür [63]. Başka bir çalışmada Tip 2 diyabet hastalarına verilen ararot ve *Dioscorea esculenta* (Lour.) Burkill karışımı atıştırmalığın HDL olmayan kolesterol seviyelerini ve aterojenik indeksi düşürdüğü bildirilmiştir [61]. Bir diğer çalışmada ise *Lactobacillus fermentum* ve *Lacticaseibacillus casei* ile fermente edilen 12 saat inkübasyon sonunda hazırlanan ararot rizomu içeren yoğurdun sıçanlarda toplam kolesterol seviyesini düşürebileceği gösterilmiştir. Ayrıca ararotun *L. fermentum* ve *L. plantarum* ile fermantasyonu toplam kolesterol düzeylerini düşürmüştür. *L. fermentum* içeren yoğurdun kolesterol düşürücü etkisinin simvastatin etkinliğinden daha yüksek olduğu da yapılan araştırmalarda bildirilmiştir [34].

Hipoglisemik Etki

Glisemik indeks 55'in altındaysa bu düşük kabul edilir. Ararot rizomları düşük glisemik indeksleri nedeniyle diyabet hastalarına önerilmektedir. Glisemik indeksi düşük besinler glikoz emiliminin düşük olduğunu gösterir, böylece kan şekerini düşürür. Ayrıca ararot yavaş sindirimi indükleyen (120 dakikadan uzun) ve mide enzimlerine dirençli olan dirençli nişasta içerir [16]. Düşük glisemik indeksi ararotu diyabet tedavisi için yararlı doğal bir gıda ikamesi haline getirmiş ve birçok hastanın kan şekeri diyetinde ev ilaçlarının bir parçası olarak önerilmesine neden olmuştur [26]. Beslenme üzerine olumlu etkileri diyabet hastaları için modifiye edilmiş gıda üretiminde kullanımının önünü açmıştır [39]. Ararot unu ile hazırlanmış taze erişteler düşük glisemik indeksi nedeniyle saf buğday unundan hazırlanmış erişteler yerine tavsiye edilir. Ararot unundan yapılan bisküviler buğday unundan yapılan bisküvilere kıyasla daha düşük glisemik indekse sahiptir. %70 ararot ve %30 kırmızı fasulyeden yapılan atıştırmalığın glisemik indeksi 25 olarak hesaplanmıştır. %20 pedada unu ve %80

ararot unundan yapılan bisküvilerin glisemik indeksi tatlı patates, patates ve cassava nişastasından daha düşük bulunmuştur [16]. %15 ararot unu, %15 *Setaria italica* (L.) P.Beauv., %30 kırmızı barbunya, %18 margarin, %10 maltitol ve %12 yumurta sarısı içeren düşük glisemik indeksli (36.7) atıştırmalıklar diyabet hastaları için önerilmiştir [39]. Streptozotosin ile indüklenmiş diyabetik sıçanlara 28 gün boyunca uygulanan 120 mg/kg vücut ağırlığı ararot rizom infüzyonu, kontrol gruplarına kıyasla önemli ölçüde daha düşük kan glikoz seviyeleri ve önemli ölçüde daha yüksek insülin seviyeleri göstermiş; diyabetik fare modelleri de sağlıklı kontrollerle karşılaştırıldığında hiçbir farklılık gözlenmemiştir. Alloksan monohidrat ile indüklenmiş farelere 20 hafta boyunca ararottan elde edilmiş analog pirinç verildiğinde glikoz seviyesinde %18.97 oranında düşüş gözlenirken IR64 pirinci verildiğinde %39.18 artış gözlenmiştir [16]. 30 gün boyunca atıştırmalık olarak günde 20 mg çıtır ararot gevreği tüketen Tip 2 diyabetli 14 hasta incelenmiş ve kan şekeri seviyelerinde herhangi bir düşüş olmadığı bildirilmiştir [63]. Ararot, *D. esculenta*, cassava ve balkabağından yapılan lif açısından zengin bir atıştırmalık tüketen (4 hafta, 32 g/gün) ve günlük 1.500 kkal'lik kısıtlı bir diyet uygulanan diyabet hastalarında açlık kan şekeri seviyesinde veya glikoz bağlanmış hemoglobinde azalma olmadığının bildirilmesi gibi bazı zıt sonuçlara da ulaşılmıştır [61]. Fakat bu durumun bildirilmeyen ve yalnızca kalori alımı için sınırlandırılan diğer yiyeceklerden kaynaklandığı düşünülmüştür [16].

İmmünomodülatör Etki

Yapılan bir çalışmada arorot rizomlarından elde edilen sulu ekstre immünostimulan etkisi için *in vitro* olarak hayvan hücre kültüründe ve *in vivo* olarak BALB/c farelerde ve IgM üretimim stimulan aktivitesi için insan hibridom HB4C5 hücrelerine ve fare splenositlerine karşı test edilmiştir. HB4C5 hücreleri tarafından IgM üretimini ve splenositler tarafından immünoglobulin (IgG, IgA ve IgM) üretimini *in vitro* olarak uyardığı gösterilmiştir. Ayrıca ekstre splenositlerin interferon γ üretimini önemli ölçüde arttırmıştır [16,40]. *İn vivo* çalışmalar ekstre içeren diyetle beslenen farelerde serum IgG, IgA ve IgM değerlerinin arttığını göstermiştir [13,16,40]. Böylece ararot ekstresinin *in vitro* ve *in vivo* olarak immünostimulan etkili olduğu gösterilmiştir [16,26,40]. Ararot ununun immünomodülatör etkili olduğu da bildirilmiş [39] fakat bir çalışmada atıştırmalık olarak ararot unundan yapılan kurabiyeleri yiyen çocukların dışkısındaki IgA konsantrasyonunda herhangi bir farklılık gözlenmemiştir. Ararottan elde edilen dirençli nişasta 4 hafta boyunca sıçanlara uygulanmış ve sonuçta artmış immünoglobulin A serumu ve mezenterik lenf düğümlerinde daha yüksek bir CD4T hücre popülasyonu gözlenmiştir. Ararotun immünomodülatör etkisi içerdiği antioksidan etkili fenolik, flavonoit, C ve E vitaminleri ve metabolizmayı destekleyen ve bağışıklığı geliştiren karbonhidrat, protein, mineral ve vitamin içeriğinden ileri gelmektedir. Prebiyotik etkili olan ararot kısa zincirli yağ asitlerinin fermantasyonunu arttırır. Bu yağ asitleri bağışıklık hücreleri için enerji kaynağı olarak işlev görebilir dahası nötrofiller, dendritik hücreler, makrofajlar ve T-lenfositler gibi bağışıklık hücrelerinin farklılaşmasını, onarılmasını ve aktivasyonunu düzenleyebilir [16].

Antihipertansif Etki

Ararot kan akışını yumuşattığından ve çeşitli sağlık koşullarında kan basıncını koruduğundan homeopatide enfeksiyonları tedavi etmek için doğal bir tedavi olarak kullanılmıştır. Ayrıca kan dolaşımını ve metabolizmayı iyileştirmeye yardımcı olan fosfor, sodyum, potasyum, magnezyum, demir, kalsiyum ve B vitamini içerir [26]. Ararot iyi bir potasyum kaynağıdır ve potasyum kalp atış hızını ve kan basıncını düzenlemeye yardımcı olan hücre ve vücut sıvılarının önemli bir bileşenidir [7]. Ararotun kilo kaybı potansiyeli anjiyotensinojeni serbest bırakabilen adipositlerin sayısında azalmaya neden olur. Atıştırmalık olarak ararot çıtır gevreği tüketen Tip 2 diyabet hastalarında yapılan bir araştırmada anjiyotensin II seviyesinde ve sistolik ve diyastolik kan basıncında anlamlı olmamakla birlikte bir düşüş gözlenmiştir. Dört hafta boyunca ararot rizomlarından yapılan yapay pirinçle beslenen hipertansif fareler üzerinde yapılan araştırmalar, kontrole kıyasla (IR36 pirinci verilen fareler) sistolik kan basıncının normale düştüğünü göstermiştir. IR36 pirinci ile karşılaştırıldığında yapay pirincin daha fazla fenol içerdiği bunun da endotelyal nitrik oksit sentaz oluşumunu engellediği ve böylece NO'yu artırdığı bildirilmiştir. NO'nun kan basıncını düşürmek için damar genişletici görevi gördüğü düşünülmüştür [16].

Antikanser Etki

Yapılan bir çalışmada, sıçanlarda dimetilbenz(a)antrasen'in indüklediği meme kanserine karşı diyetle alınan ararotun etkileri incelenmiştir. Çalışma sonuçları, ararot içeren beslenmenin sıçanlarda meme kanseri riskini azalttığını göstermiştir. Ararotun, anahtar otofaji proteinlerini toplayacak ana başlatıcı olan Beclin 1'i indükleyerek anti-proliferatif ajan görevi gördüğü tespit edilmiştir. Bu çalışma, ararotun meme kanserine karşı kemopreventif ajan olarak potansiyelini vurgulamıştır [32]. Ararotun zengin lif içeriğinin de kolon kanseri riskini azaltmada rol oynayacağı belirtilmiştir [7].

Diğer Tıbbi Etkiler

Genç rizomlar yara iyileştirici etkiye sahiptir [12]. Rizomlar demir (III) çözünürlüğünü arttırabilir, böylece demir eksikliği anemisi için alternatif bir tedavi olarak kullanılabilir [16]. Dolaşımı iyileştirerek konjestif kalp yetmezliği gibi kalp rahatsızlıklarını önler. B vitamini ve demir açısından zengin olduğundan bebek sağlığını desteklemek, fetal malformasyon ve anormallik olasılığını azaltmak amacıyla hamileler tarafından tüketilmesi tavsiye edilir [26]. Antibakteriyal, antifungal ve anti-inflamatuvar özelliklerinden dolayı üriner sistem hastalıkları [13,18,26], atlet ayağı tedavisi [26,57] ve diş hekimliğinde ağızla ilgili sorunlar, diş eti iltihabı ve ağız ağrısı tedavisinde ve şişliği azaltmada kullanılır; ağız ve diş eti mukoza zarlarını yatıştırabildiğinden ağızla ilgili hastalık belirtilerinin iyileşmesini destekler. Ararot tozu bilinen hiçbir yan etkisi olmaksızın diş beyazlatma özelliğine sahiptir. Gargara olarak kullanıldığında antibakteriyal özelliği nedeniyle çeşitli bakteri suşlarının etkinliğini baskılar [26]. Ararot nişastasının ilaç yapımında kullanılabileceği bildirilmiştir [14]. İlaç hazırlamada yaygın olarak kullanılan bir polimer olan sodyum karboksimetilselüloz ile karşılaştırıldığında yardımcı madde olarak kullanılabileceği görülmüştür [13]. Baryumlu yemeklerin ve tabletlerin hazırlanmasında önemli bir bileşendir [10,11].

Gıda Endüstrisinde Kullanım

Gıda endüstrisinde nişastanın çeşitli gıda türlerinde jelleştirici ve koyulaştırıcı olarak yaygın kullanımı yeni doğal nişasta kaynaklarının arayışına yol açmıştır [42]. Ararot iyi bir nişasta kaynağı olarak kabul edilir; fiziksel özellikleri ve karakterizasyonu gıda endüstrisinde çeşitli uygulamalara uygun olduğundan [45] hammadde ve ek malzeme olarak geliştirilme ve modifiye edilme potansiyeline sahiptir [16]. Ararot nişastası ile hazırlanan yiyecekler yüksek *in vitro* ve *in vivo* sindirilebilirlik aralığındadır (%30.07-95.7) [13,40]. Yüksek sindirilebilirlik hazırlanan gıdada nişasta retrogradasyonu ile ilişkilidir [13]. Retrogradasyon faktörü çiğneme ve yutma aşamasında gıda çekiciliği ile tanımlanır [13]; yumuşaklık, sertlik ve gevreklik hissi sağlar [13,45]. Ararot nişastası macun ve jellerdeki özel nitelikleri nedeniyle dikkat çekicidir. Yüksek amiloz içeriği daha iyi yapılandırılmış jeller ve macunlar sağlarken, yüksek amilopektin içeriği daha yüksek viskoelastikiyete sahip malzemeler sağlar. Ararot nişastası yüksek su tutma kapasitesine, yüksek lipit absorpsiyon indeksine, orta derecede jelatinleşme sıcaklığına ve orta derecede macun stabilitesine sahiptir [13]. Yapılan çalışmalarda cassava ve tatlı patates nişastalarına ararot nişastası eklenmesinin ticari gıda ürünlerinde jel stabilitesini geliştirdiği bildirilmiştir [5]. Beyaz, kokusuz ve tatsız olduğundan koyulaştırıcı madde olarak pişirmede kullanılır [5,18,57]. Suda kaynatıldığında şeffaf, kokusuz, tadı hoş bir jöle verir [9]. Berraklık ve şeffaflık özellikleri avantaj olarak tanımlanır [13]. Renk özellikleri tek tip renkli gıda ürünlerinde (dondurmalar, meyve suları ve şekerlemeler gibi) uygulanabilirliğini kolaylaştırır [6]. Bu şekilde gıda endüstrisinde çok sayıda uygulamada kullanılmakla birlikte çeşitli uygulamalar için potansiyel oluşturmaktadır [2,4,6,8,13,14].

Rizomlarda karbonhidrat miktarı yüksek olduğundan pirinç ve buğday gibi temel gıdalara alternatif olabilir [16]. Şekerleme ve bisküvi yapımında [3,10,11] ve bebek maması hazırlanmasında kullanılır [3,10-12,15]. Yüksek lif içeriği ve antioksidan etkili bileşiklerin varlığı ararot ununu fonksiyonel gıdaların gelişimi için önemli bir kaynak haline getirmiştir [16]. Kolayca sindirilebildiğinden genellikle çocuklar için hazırlanan ekmek ve bisküvilerde [35] ve ayrıca kurabiye ve unlu mamullerde kullanılır [15,39,58]. Ararot unu kurabiye, bisküvi, kek, tatlı ve benzeri gıdaların yapımında kullanılan diğer malzemelerle karıştırılabilir [21]; soya veya kabak ile birlikte bebekler için tamamlayıcı gıda olarak geliştirilebilir [16]. Bazı gıda müstahzarlarında koyulaştırıcı ve dengeleyici

olarak ve tedavi amaçlı kullanılır [8,19,20]; mutfaklarda çorba, sos, salata sosu ve et suyu sosu hazırlamada kullanılır [7]. Ararot nişastası; çorbalar, tatlılar, pudingler, soslar, kurabiyeler, şekerleme, bisküvi, kek, jöle, yulaf lapası yapımında, turta dolgusunda ve diğer unlu mamuller gibi birçok gıdada koyulaştırıcı olarak sıklıkla kullanılır [5,7-9,14,15,38,40,45]. Dondurmada kristalleşmeyi önleyici etkisi vardır [15]. Çocuklar ve diyet kısıtlamaları olanlar için kolayca sindirilebilir bir besindir. Ararot nişastasının glüten içermemesi onu unlu mamullerde buğday ununun yerine ideal hale getirir [38] ve özel bisküvi ve fırın ürünlerinin hazırlanmasında kullanılır [15]. Ararot nişastasının ekstrüzyonla pişirilmesi çok iyi genleşme, renk ve daha düşük sindirilebilirlikle sonuçlanan ürünler verir; bu ürünler atıştırmalık yiyecek olarak tüketime uygundur [38]. Ararot nişastası alerjen içermediğinden ve sindirimi yatıştırdığından bebekler için uygundur. Ararot bisküvileri ısırması zor olduğundan, boğulma tehlikesi olmayacağından ve sentetik veya plastik olmadığından bebeklere diş çıkarma sürecinde verilebilir [26]. Ararot nişastasından şeker elde etme amaçlı yapılan bir çalışmada ararot nişastası bazlı kristal şeker elde edilmiş, şekerin verimliliği ve kalitesinin kullanılan şeker üretim yöntemine bağlı olduğu bildirilmiştir [23]. Yapılan bir başka çalışmada, buğday-ararot ekmeği homojen ve görsel olarak kontrol ekmeklerine (buğday ekmeği) benzer bulunmuştur. %30 ararot nişastası ile üretilen ekmek, kontrol ekmeğine benzer bir iç sertlik ve hacim sergilemiştir [45]. Taze ekmek üretiminde daha fazla ararot unu ilavesinin ham lif ve karbonhidrat seviyelerini arttıracağı, ararot ununun taze ekmek gibi gıdaların üretiminde yaklaşık %10-30 oranında katkı maddesi ile karışım olarak kullanılabileceği ve ekmek yapımında yaygın olarak kullanılan buğday ununun yerine geçebileceği bildirilmiştir [21]. Bu nedenle buğday ununun yerini alma potansiyeli vardır [4,5,19]. Endonezya'da yapılan bir çalışmada ararot nişastası kullanımının buğday unu ithalatını yılda 3 milyon tondan fazla azaltacağı bildirilmiş olup geliştirilmesine ve yetiştirilmesine öncelik verilmiş [19] ve Endonezya hükümeti tarafından Endonezya'da geliştirilmesi/ekilmesi öncelikli gıda ürünlerinden biri olarak ilan edilmiştir [5,23].

Kozmetik Endüstrisinde Kullanım

Ararot tozu ve nişastası kozmetik endüstrisinde yaygın olarak kullanılır [17,26,51]. Birçok kozmetik preparatın içeriğinde yer alır. Cildi besleyen, yağlanmayı azaltan, iltihaplanmayı ve tahrişi önleyen özelliklere sahiptir. Cilde hafif, yumuşak, ipeksi bir görünüm ve serinleme, kuruma ve tazelenme hissi verir. Aydınlatıcı, matlaştırıcı ve cildi yatıştırıcı etkisi nedeniyle yağlı, akneli ve hassas cilt tiplerine uygun krem ve losyonların hazırlanmasında kullanılır [17]. Teri ve nemi emme kabiliyeti nedeniyle koku giderici vücut tozlarında [17] ve ayrıca yüz pudralarında yer alır [15]. Tüm cilt tipleri için uygundur ve özellikle hassas ciltlere karşı naziktir, bu nedenle bebek ve yaşlı cilt bakım ürünleri için idealdir. Yapılan bir çalışmada Carbopol 934 ve ararot tozu (1:5) ile hazırlanan kozmetik jelin akne tedavisi ve cilt gençleştirme amaçlı ideal bir jel olduğu bildirilmiştir [17]. Ararot nişastası derma farmasötik bileşime sahiptir. Glikozitlerin varlığı dolaşım problemlerini iyileştirme gücünü gösterir. Flavonoitlerin varlığı nedeniyle iyi bir antispazmodik, anti-kanserojen ve bağışıklık uyarıcı özelliklere sahiptir. Bu bileşim; onu iyi bir nem emici yapar, yatıştırıcı etkilidir ve derine nüfuz etmeye izin verdiği için cilt üzerinde kullanmak güvenlidir [26].

Diğer Endüstrilerde Kullanım

Nişasta; farmasötik, kozmetik [51], gıda, tekstil, kâğıt [2,15], yapıştırıcı ve sabun sanayiinde [15] yaygın olarak kullanılmaktadır. Ararot nişastasının eczacılık, hijyen ürünleri, çevre yönetimi, tarım, biyomedikal mühendisliği ve biyoyakıt üretimi gibi alanlarda uygulamaları mevcuttur [2]. Nişasta, biyolojik olarak parçalanamayan plastiğin yerini alabilecek olası yeşil kaynak olarak ortaya çıkmıştır [14]. Düşük amiloz içeriğine sahip numunelerin yapısal ve absorpsiyon özelliklerini iyileştirdiği ve bebek bezleri, yetişkin pedleri ve ayrıca klinik pansumanlar gibi süper emici materyaller üretmek için kullanılabileceği bildirmiştir [8]. Ararot nişastasının hidrojel üretimi için potansiyeli vardır. Günümüzde su tutma kapasitesi, geçirgenlik ve sızma gibi toprağın fiziksel özelliklerini iyileştirmek için özellikle yapısı zayıf ve kuraklıktan etkilenen topraklarda kullanılmaktadır. Hidrojel bitkilerde su ve besin kullanımının artması, uygun ortam yaratma ve daha iyi bitki büyümesi ve verimi için besleyici rizosferik mikro-ortam için potansiyel bir teknolojidir [14].

Biyopolimer ve biyokompozit endüstrilerinde kaynak olarak kullanılma potansiyeline sahiptir [4,46]. Yüksek amiloz içeriği nedeniyle ararot nişastası, özellikle mekanik dayanıklılık ve bariyer

özellikleri açısından iyi teknik özelliklere sahip filmler yapmak için kullanılabilir. Nişastadaki yüksek amiloz miktarı film çözünürlüğünü azaltır ve film bütünlüğünü geliştirir [8,14,42,48,49,58,64,65]. Böğürtlen posasının ararot nişastasına katılması ile yenilebilir filmlerin oluşturulması sağlanmıştır [14].

Ararot nişastasının yanı sıra atık rizom lifi de endüstride çeşitli alanlarda kullanılmaktadır. Nişasta eldesi sırasında büyük miktarda atık ararot lifi oluşur [8]. Ararot lifinin boyu kısa sert ağaç liflerine benzer ve çapı çok daha küçüktür [8,14,66]. Bu lifin yırtılma direnci yüksektir [14]. Bu nedenle kâğıt mendil, ince kâğıt, karton [14,8], ambalaj kâğıdı ve çanta gibi yırtılmaya dirençli kâğıt yapımına uygundur [8,14,66]. Bilgisayarlar için üretilen karbon içermeyen kâğıtların üretiminde kullanılır [15]. Ararot, biyolojik olarak parçalanabilirliği nedeniyle biyomateryal oluşturmak için doğal lif kaynağı olarak kullanılır [14,34,67]. Ararot rizomları hidrofiliktir. Ararot lifini polilaktik asit ile birleştirerek, lifin biyolojik olarak parçalanabilirliği uzatılabilir. Ambalaj malzemeleri, biyolojik malzemeler ve tarımsal kullanımlarda liften yararlanılabilir [14]. Biyomedikal malzemelerde yararlı uygulamaları vardır [34]. Tarım endüstrisinde, sindirimi ve üretimini iyileştirmek için sığır yemi olarak kullanılır [26,68]. Yeşil gübre olarak değerlendirilebilir [15]. Önceden jelatinleştirilmiş form, petrol sahası hizmetlerinde sondaj sıvılarını kalınlaştırmada kullanılır [26]. Kahverengi pembe yaprakları nedeniyle süs bitkisi olarak yetiştirilir [15].

SONUÇ VE TARTIŞMA

Bu derleme çalışmada, günümüzde büyük ekonomik değeri olan, çeşitli endüstrilerde uygulama potansiyeline sahip Ararot (*M. arundinacea*) türünün botanik özellikleri, yayılışı, geleneksel kullanımı, rizomların kimyasal içeriği ile birlikte rizom ve rizomlardan elde edilen ürünlerin (un, nişasta ve toz) biyolojik etkileri ve çok çeşitli kullanım alanlarına ilişkin bilgiler bilimsel verilere dayandırılarak incelenmiştir.

M. arundinacea türü yüksek nişasta içerikli yenilebilir silindirik rizomlara sahiptir [2-5,8,11,12]. Bitki tropikal Amerika'nın yerlisidir [3,7,11,18,22]. Meksika, Orta Amerika [4,5,10], Latin Amerika [13], Batı Hint Adaları ve Güney Amerika'ya [5,10] özgüdür. 17. ve 18. yüzyıllarda Asya'nın çoğuna yayılmıştır [4], Afrika'da da görülür [13]. Türün geleneksel gıda hazırlamada ve geleneksel tıpta kullanımı vardır [10,18]. Tarihi seramik kalıntılarda bulunan nişasta granülleri Kolomb öncesi insanların besinlerinde ararot nişastası kullandıklarını göstermiştir [13]. Rizomların halk tıbbında yatıştırıcı, kızarıklık giderici, anti-inflamatuvar ve antiseptik [15] olarak ve ayrıca ishal, dizanteri ve kolit [24] tedavisinde kullanıldığı bildirilmiştir.

Ararot rizomları alkaloitler, karbonhidratlar, kardiyak glikozitler, proteinler, amino asitler, terpenler, saponinler [3,10,12,13,18,24,32], flavonoitler [10,13,16,18], reçineler [33], tanenler [16], zamk [10,16,18], lignin, antrakinonlar [34] ve steroller [3,10,13,18] içerir. Rizomlar yüksek kaliteli karbonhidrat [5,13,15,18,23,32] ve diyet lifi içerir [12]. Karbonhidrat bileşiminin çoğu nişastadır [2,3,7,14,19,40]. Ayrıca iyi bir protein kaynağıdır [12,35]. Ararot unu triptofan, treonin, izolösin, lösin, lizin, metionin, sistein, fenilalanin, tirozin, valin, arjinin, histidin, alanin, aspartik asit, glutamik asit, glisin, prolin ve serin amino asitlerini içerir. Rizomlar potasyum, demir, manganez, bakır, kalsiyum, klor, fosfor, magnezyum, çinko [36,37] ve B grubu vitaminleri içerir [7,37].

Rizom ve rizomdan elde edilen ürünlerin antioksidan, immünostimulan, anti-ülserojenik, antidiyareik [9,17], anti-inflamatuvar, antimikrobiyal [16], antidispeptik, antihipertansif, hipokolesterolemik, hipoglisemik [6,13], antikanser [32] ve hepatoprotektif [24] aktiviteleri rapor edilmiştir.

Ararot nişastası mükemmel sindirilebilirlik, nispeten düşük sıcaklıklarda jelatinleşme kapasitesi [22] ve iyi özelliklere sahip filmler geliştirmek için gerekli olan yüksek amiloz içeriği gibi özel fizikokimyasal özellikler [8,20,22] ve kana uyum sağlama, çok yönlü, zehirsiz, ekolojik ve biyo-birikimli olma gibi birçok avantaja sahiptir [8,14]. Ararot nişastası fizikokimyasal özellikleri ile mısır, cassava, buğday ve patates nişastaları ile rekabet edebilecek durumdadır [49].

Ararot nişastası fiziksel özellikleri ve karakterizasyonu gıda endüstrisinde çeşitli uygulamalara uygun olduğundan [45] hammadde ve ek malzeme olarak geliştirilme ve modifiye edilme potansiyeline sahiptir [16]. Rizomlarda karbonhidrat miktarı yüksek olduğundan pirinç ve buğday gibi temel gıdalara alternatif olabilir [16]. Ararot ununun ekmek yapımında anılan buğday ununun yerine geçebilme

potansiyeli vardır [21]. Ararot nişastası; çorbalar, tatlılar, pudingler, soslar, kurabiyeler, şekerleme, bisküvi, kek, jöle, yulaf lapası yapımında, turta dolgusunda ve diğer unlu mamuller gibi birçok gıdada koyulaştırıcı olarak sıklıkla kullanılır [15,38]. Ararot nişastasının glüten içermemesi onu unlu mamullerde buğday ununun yerine ideal hale getirir [38] ve özel bisküvi ve fırın ürünlerinin hazırlanmasında kullanılır [15].

Ararot tozu ve nişastası kozmetik endüstrisinde yaygın olarak kullanılır [17,26,51]. Birçok kozmetik preparatın içeriğinde yer alır. Cildi besleyen, yağlanmayı azaltan, iltihaplanmayı ve tahrişi önleyen özelliklere sahiptir [17]. Ararot nişastasının eczacılık, hijyen ürünleri, çevre yönetimi, tarım, biyomedikal mühendisliği ve biyoyakıt üretimi gibi alanlarda uygulamaları mevcuttur [2]. Yüksek amiloz içeriği nedeniyle ararot nişastası, özellikle mekanik dayanıklılık ve bariyer özellikleri açısından iyi teknik özelliklere sahip filmler yapmak için kullanılabilir [8,14,42,48,49,58,64,65].

Ararot nişastası eldesi sırasında ortaya çıkan atık rizom lifi kâğıt mendil, ince kâğıt, karton [8,14], ambalaj kâğıdı ve çanta gibi yırtılmaya dirençli kâğıt yapımına uygundur [8,14,66]. Bilgisayarlar için üretilen karbon içermeyen kâğıtların üretiminde kullanılır [15]. Ararot, biyolojik olarak parçalanabilirliği nedeniyle biyomateryal oluşturmak için doğal lif kaynağı olarak kullanılır [14,34,67]. Tarım endüstrisinde, sindirimi ve üretimini iyileştirmek için sığır yemi olarak kullanılır [26,68]. Önceden jelatinleştirilmiş form, petrol sahası hizmetlerinde sondaj sıvılarını kalınlaştırmada kullanılır [26].

Ararot bitkisi yetiştiği bölgelerde ekonomik, tıbbi, kültürel ve ekolojik öneme sahiptir. Ararot rizomlarından elde edilen ürünler (un, toz, nişasta); gıda, ilaç, kozmetik, kağıt, ambalaj, hijyen, tarım, biyoyakıt, biyofilm vs. endüstrilerinde oldukça değerlidir. Fizikokimyasal özellikleri ve çok yönlü, zehirsiz ve biyo-birikimli olma gibi birçok avantaja sahip olan ararot nişastası ve ararot unu sağlıklı beslenme ve gıdalara alternatif olma özellikleriyle fazlasıyla gündelik hayatımızda yer almaktadır. Ararot rizomları gıda endüstrisindeki yeri, besin değeri, tıbbi özellikler, çeşitli endüstrilerde hammadde olma potansiyeli ve çok çeşitli diğer kullanımları ile her geçen gün dikkatleri daha çok çekmektedir. Bu derleme çalışma; gıda tüketiminde kişilerin bilinçli tercihlerini ve çevre dostu ürün kullanımını destekleyecektir. Tüm olumlu özelliklerine rağmen insan diyetine girmesi için daha fazla bilimsel çalışmaya ihtiyaç duyulmaktadır. Derlenen veriler ile ararot rizomları ve ondan elde edilen ürünlerin çok çeşitli alanlarda yeni araştırmalara konu olacağı ve popülaritesinin günden güne artacağı öngörülmektedir.

YAZAR KATKILARI

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ÇIKAR ÇATIŞMASI BEYANI

Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler.

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DERLEME MAKALE / REVIEW ARTICLE

ENFLAMATUAR BAĞIRSAK HASTALIĞI VE TIBBİ BİTKİLER: GÜNCEL BİR GÖZDEN GEÇİRME

ENFLAMMATORY BOWEL DISEASES AND MEDICINAL PLANTS: A CURRENT REVIEW

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ÖZ

Amaç: *Enflamatuar bağırsak hastalığı (EBH), ülserasyon, kanama, sıvı ve elektrolit kaybı ile karakterize, atak ve remisyon dönemlerinden oluşan gastrointestinal sistemin (GİS) kronik enflamasyonudur. Ülseratif kolit ve Chron, etiyolojisi ve patogenezi tam olarak belli olmayan EBH'nin majör klinik formlarıdır. Son yıllarda ülkemizde ve dünyada insidansı ve prevalansı gittikçe artan EBH, bireylerin yaşam kalitesini olumsuz etkilemektedir. Bu durum hastalığın tedavisini daha da önemli hale getirmektedir. Fakat bu tedavi yöntemleri hastalığın remisyon süresinin uzatılması ve progresyonunun önlenmesi için yetersiz kalabilmektedir. Bu nedenle hastalar esas tedavilerine ek olarak farklı tamamlayıcı tedavi arayışlarına yönelmektedir. Bu yöntemler arasında hastaların en sık başvurduğu tamamlayıcı tedavi, bitkisel ürünlerdir.*
Sonuç ve Tartışma: *Bitkisel ürünlerin kullanımı, hastalık üzerinde olumlu etkiler yapabildiği gibi olumsuz etkilere de yol açabilmektedir. Bu nedenle belli standartlara sahip ürünlerin uygun hastalıkta, uygun formda, uygun dozda ve hekim/eczacı kontrolünde kullanılması gerekmektedir. Literatürdeki çalışmalar değerlendirildiğinde; enflamatuar bağırsak hastalıklarında kullanılan tıbbi bitkilerin fazlalığına rağmen birçoğunun potansiyel etki mekanizmasının ve olumlu/olumsuz etkilerinin tam olarak ortaya konmadığı görülmektedir. Bu bitkilerin yanlış ve bilinçsizce kullanımı hastalığın seyrinin kötüleşmesine yol açabileceğinden enflamatuar bağırsak hastalıklarında kullanılan tıbbi bitkilerle ilgili daha fazla araştırmaya ihtiyaç duyulmaktadır.*
Anahtar Kelimeler: *Chron hastalığı, enflamatuar bağırsak hastalığı, tıbbi bitkiler, ülseratif kolit*

ABSTRACT

Objective: *Enflammatory bowel disease (EBD) is a chronic enflammation of the gastrointestinal tract (GIS) that consists of episodes of attacks and remissions characterized by ulceration, bleeding, fluid and electrolyte loss. Ulcerative colitis and Chron, are the major clinical forms of EBD, the etiology and pathogenesis of which are unclear. EBD, the incidence and prevalence of which has been increasing in our country and in the world in recent years, negatively affects the quality of life of people. This makes the treatment of the disease even more important. However, these treatment methods may be insufficient for prolonging the remission period of the disease and preventing its progression. For this reason, patients tend to seek different complementary therapies in addition to their medical treatments. Among these methods, the most frequently applied complementary therapy is herbal products.*
Result and Discussion: *The use of herbal products can have positive effects on the disease as well as cause negative effects. For this reason, products with certain standards should be used in the appropriate disease, in the appropriate form, in the appropriate dose and under the control of the*

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doctor / pharmacist. When the studies in the literature are evaluated; Despite the abundance of medicinal herbs used in anxiety and sleep disordersenflammatory bowel disease, the potential mechanism of action and positive/negative effects of many of them have not been fully revealed. Since the wrong and unconscious irrational use of these herbs may lead to worsening of the course of the disease, more researches are needed to examine examine medicinal herbs used in enflammatory bowel disease.
Keywords: *Chrons disease, enflammatory bowel disease, medicinal herbs, ulcerative colitis*

GİRİŞ

Son yıllarda gelişmiş ve gelişmekte olan ülkelerde enflamatuar bağırsak hastalığının (EBH) insidansının ve prevalansının arttığı görülmektedir. Tedavide kullanılan konvansiyonel yöntemler hastalığın remisyonunu sürdürmede ve progresyonu önlemede zaman zaman başarılı olamamakta ve yan etkiler görülebilmektedir. Bu yüzden pek çok hasta, esas tedavisinin yanı sıra tamamlayıcı tedavi yöntemlerine başvurmaktadır. Bunlar arasında en sık kullanılanı bitkisel ürünlerdir [1-9]. Bitkisel ürünler, farklı şekil ve formülasyonlarda sunulmaktadır. Bitkisel ürün pazarındaki bu çeşitlilik, medyanın etkisi, denetim ve yönetmelik eksiklikleri, aktar ve benzeri ortamlarda satış gibi pek çok sebepten ötürü ciddi sorunlar ortaya çıkabilmektedir. Ayrıca hastalarda ‘‘Bitkisel ise zararsızdır/Yan etkisi yoktur’’ algısı bu tip ürünlerin bilinçsizce kullanımına sebep olmaktadır. Bu ürünlerin tek başına yol açabileceği olumsuz etkilerin yanı sıra ilaçlarla, besinlerle ya da kendi aralarında etkileşime girebileceği de unutulmamalıdır. Tüm bu nedenlerle bitkisel ürünler hekim/eczacı önerisi ve kontrolünde, uygun süre, uygun doz ve uygun formülasyonlarda kullanılmalıdır. Bu şekilde kullanılan birçok bitkisel ürünün hastalığın remisyon süresini uzatıp yaşam kalitesini yükselttiğine dair sonuçları içeren farmakolojik ve klinik çalışmalar bulunmaktadır [9-12].

Tanım ve Sınıflandırma

EBH, ülserasyon, karın ağrısı, diyare, kanama, sıvı ve elektrolit kaybıyla sonuçlanan sitokinler, proteolitik enzimler ve serbest radikaller üreten nötrofiller ve makrofajların akışı ile karakterize, relaps (atak) ve remisyon (iyileşme) dönemlerinden oluşan gastrointestinal sistemin (GİS) kronik enflamasyonudur. Bağırsakta meydana gelen hem kronik hem de tekrarlayan enflamasyon durumu, bu enflamasyonun GİS’teki lokalizasyonu ve bağırsak duvarında meydana getirdiği histolojik değişiklikler ile ayırt edilebilen farklı klinik tablolar ortaya çıkmaktadır. Ülseratif kolit (ÜK) ve chron hastalığı (CH) EBH’nin majör klinik formlarıdır. Bu iki ana form haricinde EBH’nin yaklaşık %10’unu bu iki formdan birine dahil olmayan indetermine (ara) kolit oluşturmaktadır [1-7].

Epidemiyoloji

EBH’nin son yıllarda hem gelişmiş hem de gelişmekte olan ülkelerde giderek arttığı bilinmektedir. Görülme oranı kentsel ve soğuk iklim bölgelerinde daha yüksek, kırsal ve sıcak iklim bölgelerinde daha düşüktür. Erkeklerde ve kadınlarda benzer düzeylerde görüldüğü, genç erişkinlerde cinsiyet farketmeksizin bu düzeyin daha yüksek olduğu saptanmıştır. ÜK ve CH’de kadınlarda prevalansın en yüksek olduğu yaş aralığı değişmezken, erkeklerde prevalansın en yüksek olduğu dönem 45-54 yaş aralığıdır. Fakat EBH’nin insidansı ve prevalansı hakkında eksiklikler bulunmaktadır. Asya, Afrika ve Latin Amerika ülkelerinde yetersiz veri varken Japonya, Kore, Hong Kong ve Çin gibi yeterli veri bulunan gelişmiş ülkelerde özellikle 20-39 yaş arasındaki erkek bireylerde CH prevalansının daha yüksek olduğu ve her iki cinsiyette de artan bir eğilim olduğu bilinmektedir [12-17]. Ayrıca Kuzey Avrupa ve Kuzey Amerika ülkeleri de sırasıyla 12 ile 19/100.000/yıl ve 5 ile 29/100.000/yıl arasında değişen yüksek insidans verilerine sahiptir [18]. Dünyada saptanan en düşük insidans değerlerinin 0,69/100.000 kişi/yıl (ÜK) ve 0,09/100.000 kişi/yıl (CH) ile Sri Lanka’da olduğu bilinmektedir [19]. En yüksek insidans değerleri olarak 11.2/100.000/yıl (ÜK) ve 17.4/100.000/yıl (CH) ile Avusturalya ilk sıradadır [20]. Türkiye, gelişmekte olan bir ülke olarak “Doğuyla batı arasında geçiş” konumunda olduğundan hastalığın prevalansı doğu toplumlarındaki kadar düşük olmamakla birlikte Batı’daki kadar da yüksek değildir [21]. 2004-2007 yılları arasında Enflamatuar Bağırsak Hastalıkları Derneği (İBHAYD) tarafından yürütülen bir araştırmada; EBH insidansları 4.1/100.000/yıl (ÜK) ve 2.6/100.000/yıl (CH) olarak bildirilmiştir. İBHAYD’ın verilerine göre ülkemizde her iki klinik durum kadın bireylerde daha baskın olup, kadın-erkek oranı 1.27/100.000/yıl (ÜK) ve 1.24/100.000/yıl (CH) olarak bulunmuştur [22].

Etiyopatogenez

EBH'nin etiyopatogenezi karmaşıktır ve hala tam olarak anlaşılamamıştır. Fakat günümüzde, hastalığın her iki klinik formunun oluşmasındaki ana belirleyicilerin çevresel faktörler, genetik ve epigenetik faktörler, bağırsak mukozal bariyeri, immünolojik faktörler, anjiyogenez ve bunlar arasındaki etkileşim olduğu bilinmektedir [8,23-28].

Çevresel Faktörler

EBH'nin etiyopatogenezinde potansiyel etkisi kanıtlanmış çevresel faktörler; sigara kullanımı, beslenme, anne sütü, D vitamini, hijyen, uyku yoksunluğu, fiziksel hareketsizlik, psikolojik stres, apendektomi, antibiyotikler ve diğer ilaçlar olarak bildirilmiştir [23-28].

Genetik ve Epigenetik Faktörler

EBH'nin etiyolojisindeki önemli nedenlerden birinin genetik faktörler olduğu bilinmektedir. Ailesinde EBH öyküsü olan bireylerin hayatının ilerleyen yıllarında EBH riski daha yüksektir. EBH her yaşta gelişebileceği gibi en yüksek prevalansı erişkinlik dönemindedir. İkizlerde yapılan bir çalışmada; EBH için çevresel faktörlerin hastalığın oluşmasında %50'den daha az bir oranda etkisinin bulunduğu, genetik faktörlerin ise belirleyici ana faktör olduğu bildirilmiştir. Ayrıca ÜK ve CH gelişimi kıyaslandığında genetik faktörlerin CH üzerinde daha fazla etkisi olduğu bulunmuştur. Genetik faktörlerin EBH üzerindeki potansiyel etki mekanizmasının yapılan çalışmalarla aydınlatılmasının, hastalığın genetik arka planın incelenmesine, bu sayede daha doğru ve etkili tedavi yöntemlerine kapı aralayacağı düşünülmektedir [7,9,23,24].

Epigenetik değişiklikler, DNA'nın dizilişinde herhangi bir değişiklik olmaksızın yalnızca gen ekspresyonunun etkilendiği genetik-çevre etkileşimleri, hastalığın etiyolojisi ve patogenezi üzerinde merkezi bir rol oynamaktadır. Epigenetik değişikliklerin EBH üzerindeki potansiyel etkilerini belirlemek adına en çok çalışan yolağın DNA metilasyonu olduğu bilinmektedir. DNA metilasyonu yaşlanma ile ortaya çıkan bir süreç olup genetik değişiklikler ile bu süreç hızlanmakta ve EBH oluşumuna zemin hazırlamaktadır. DNA metilasyonunun yanı sıra epigenetik değişiklikler hakkında önemli bir yol haritası olan bir diğer parametre ise mikroRNA'lardır. Kolon biyopsilerinde mikroRNA'ların relaps/remisyon ÜK ve CH durumlarında profilleri incelenmiştir. Bunun sonucunda mikroRNA'ların EBH üzerinde rolü olabileceği düşünülmüştür. Yapılan epigenetik çalışmalar sonucunda bu biyobelirteçlerin hastalığın bir nedeni mi yoksa sonucu mu olduğu henüz netlik kazanmamıştır. Bu nedenle bu konu ile ilgili daha fazla çalışmaya ihtiyaç duyulmaktadır [7,24].

Bağırsak Mukozal Bariyeri

Bağırsak mukozal bariyeri; mekanik, kimyasal ve immün bariyer olmak üzere 3 kısımdan oluşur. Bu kısımlardan herhangi birinin hasar görmesi durumunda bağırsak mukozal bariyerinin fonksiyonu bozulabilmekte ve bunun sonucunda EBH'ye neden olabilecek enterik kaynaklı enfeksiyonlar ortaya çıkabilmektedir [24,29,30].

Mekanik bariyer, epitel hücreleri arasında hücre içi sinyal yollarını ve transkripsiyonunu düzenlemekte ve bu sayede bariyer fonksiyonunu kontrol etmektedir. Bariyer sıkı ve yapışkan bağlantıları içeren apikal bağlantı kompleksinden oluşmaktadır. Yapısındaki bu sıkı bağlantı, bağırsak mukozasını düzenleyerek EBH'nin oluşumunu indükleyebilecek antijenik maddelerin bağırsak mukozasına nüfuz etmesini önlemektedir. Bağırsaktaki epitel hücrelerini kaplayan mukus tabakası ve içeriğindeki müsinler kimyasal bariyeri oluşturmaktadır. Bu bariyer bağırsağı dış etkenlerden korumakta ve bağırsak ortamının dengesini sağlamaya yardımcı olmaktadır. Normal şartlarda bağırsak lümeninin yapısında "kommensal bakteriler" olarak adlandırılan yaklaşık 1000 mikroorganizma türü (gram-negatif bakteriler (Bacteroidetes vb.) ve gram-pozitif bakteriler (Firmicutes vb.), Proteobacteria (*Escherichia* ve *Helicobacter*) ve Actinobacteria gibi) bulunmaktadır. Ayrıca protistalar, mantarlar ve virüsler de bağırsak mikrobiyatasında bulunan gruplardandır. Potansiyel enfeksiyöz patojenler tarafından homeostazı bozulan bağırsak mikrobiyal bariyeri, bağırsak disbiyozu ile açıkça ilişkilidir [24,29-31].

İmmünolojik Faktörler

İmmünolojik faktörler, hastalığın patogenezinde önemli bir rol oynamaktadır. Özellikle adaptif bağışıklığın önemli bir bileşeni olan T hücreleri, vücudun bağışıklık tepkisini düzenlemektedir. T hücreleri periferik kanda çoğalmakta ve antijen ile uyarılıp farklılaşmaktadır. Bu sayede ana alt tipleri

olan T yardımcı hücrelerine (Th); Th1, Th2, Treg ve Th17'ye dönüşmektedirler. Yardımcı hücrelerin her birinin bağışıklık ile ilgili önemli fonksiyonları bulunmaktadır. Th1 yardımcı hücresi, İnterferon-Gama (Interferon gamma-IFN-γ) ile temas halinde olan T hücresinin etkileşimi ve bunun sonucunda farklılaşması ile oluşmaktadır. Th1 yardımcı hücreleri, patojenik ajanları vücuttan elimine etmekte ve IL-1, IL-2, IL-6, IL-8, IL-12 ve IFN-γ gibi proenflamatuar sitokinlerin salgılanmasında görev almaktadır. Th2 yardımcı hücreleri ise IL-4 hücrelerinin, sinyal dönüştürücü ve transkripsiyon aktivatörü 6'nın (STAT-6) salgılanmasını indükleyerek Th hücrelerinin yüzey reseptörlerinde farklılaşmasını teşvik etmektedir. Th2 yardımcı hücreleri alerjik reaksiyonlarda görev almakta, vücudu parazitlere karşı korumakta ve IL-4, IL-5, IL-9 ve IL-13 gibi antienflmatuar sitokinleri salgılamaktadır. Treg hücreleri, IL-10 ve dönüştürücü büyüme faktörü beta (Transforming growth factor beta-TGF-β) antienflamatuar sitokinler aracılığıyla Thl, Th2 ve Th17 başta olmak üzere diğer Th yardımcı hücrelerinin salımını inhibe etmekte ve doku onarımını desteklemektedir. Th17 hücreleri, Th hücrelerinin yüzeyinde bulunan IL-23 reseptörü üzerinde hareket ederek ve STAT-3'ü aktive ederek farklılaşmakta ve çoğalmaktadır. Bu aktivasyon süreci IL-17, IL-21 ve IL-22 gibi sitokinler varlığında gerçekleşmektedir [2,8,18,23,30].

Oksitadif Stres

GİS, oksitadif stresin ortaya çıkmasındaki temel etken olan reaktif oksijen türlerinin (ROS) önemli bir kaynağıdır. Vücuda alınan patojenler, bazı bağırsak epitel hücreleri, makrofajlar ve nötrofiller bağırsak epitelindeki koruyucu bariyere rağmen enflamatuar faktörlerin salınımının uyarılmasına, lipid peroksidasyonuna, DNA hasarına ve apoptozise yol açmaktadır. Bu durum başta süperoksit dismutaz ve indirgenmiş glutatyon olmak üzere enzimatik ve enzimatik olmayan antioksidan mekanizmaların bozulmasına neden olmaktadır. Bağırsak dokularındaki bu fizyolojik değişikler sonucunda kolonun enflamasyonu ve patolojik değişiklikler ortaya çıkmaktadır [23,31].

Anjiyogenez

Anjiyogenez, mevcut damarlardan köken alarak yeni kan damarlarının oluşma sürecidir. Normal şartlarda vücuttaki kan damarlarının büyümesi ve inhibisyonu dengededir. Fakat bu dengenin bozulması sonucunda birçok hastalık ortaya çıkabilmektedir. EBH'nin erken evrelerinde anjiyogenik inhibitörlerin etkisiyle proanjiyogenik faktörlerin serum seviyelerinde artışı görülebilmektedir. Bu durum "patolojik anjiyogeneze" neden olmaktadır. EBH'de enflamatuar yanıt ile anjiyogenez güçlü olarak ilişkilendirilen süreçlerdir [31].

Klinik Semptomlar

EBH'de intestinal (bağırsak kaynaklı) ve ekstraintestinal (bağırsak dışı) veya bu belirtilere eşlik eden otoimmün bozukluklar ortaya çıkabilmektedir. Bu klinik semptomlar hastalığın süresine ve tutulum yerine göre farklılık göstermekte olup, hastalığın şiddetine göre semptomların şiddeti değişkenlik göstermektedir. Diyare, abdominal ağrı, dışkıda kanama, iştahsızlık, kilo kaybı ve yorgunluk gibi intestinal bulguların yanı sıra ekstraintestinal bulgular da ortaya çıkabilmektedir. Her hastada farklı semptomlar ortaya çıkabileceği için bireyselleştirilmiş semptom takibi gerekmektedir. Bu süreçteki altın standart, semptomları yaşayan bireylerin ifadeleridir [13,32,33].

İntestinal Belirti ve Bulgular

CH, ağızdan anüse kadar gastrointestinal sistemin herhangi bir kısmında segmenter tarzda ilerleyebilme potansiyeline sahip olup, anatomik olarak çoğunlukla terminal ileum ve kolonu etkilemektedir. ÜK'de ise enflamasyon genellikle rektum, kolon ve çekum ile sınırlıdır. Mikroskobik olarak, CH transmural ve çoğunlukla süreksiz iken, ÜK sadece bağırsak mukozasını etkileyebilen sürekli bir düzendedir. ÜK hematokezi-mukuslu ya da irinli dışkı ile karakterizedir. CH'de hemoroid, fistül ve fissür gibi perianal hastalıklar ve kolon veya ince bağırsak ileusu yaygındır. ÜK'de kript epitellerinin yapımı bozulurken, hem ÜK hem de CH'de anal kriptoptit ve kript apseleri gözlenmektedir. Ayrıca her iki EBH formunda kronik bağırsak enflamasyonu ve relaps/remisyon dönemleri yaşanmaktadır [2,12,21,34].

Ekstraintestinal Belirti ve Bulgular

Ekstraintestinal semptomlar hastalık sürecinde ortaya çıkan bağırsak dışı semptomlardır. Bu

semptomların EBH varlığı veya tedavi süreci ile işkili olabileceği düşünülmektedir. EBH'li bireylerin yaklaşık %21-36'sında ekstraintestinal semptomlar görülebilmektedir. Bu belirti ve bulgular arasında en yaygın görülenleri; bulantı ve kusma, ateş, yorgunluk ve gece terlemeleri, anemi, artropati ve artralji, metabolik ve kemik sorunları (düşük kemik kütlesi ve osteoporoz), oküler semptomlar (üveit, kseroftalmi, blefarit ve episklerit), oral ve nazal hastalıklar (aftöz ülserleri ve nazal septal apse), deri hastalıkları (pyoderma gangrenosum ve eritema nodosum), hepatobiliyer sorunlar (primer sklerozan kolanjitis ve non-alkolik yağlı karaciğer hastalığı), pankreas hastalıkları (akut/kronik pankreatit), kardiyovasküler sorunlar (serebrovasküler/ iskemik kalp hastalığı ve mezenterik iskemi), solunum sistemi hastalıkları (kronik obstrüktif akciğer hastalığı) ve ürogenital sorunlardır (ürik asit ve kalsiyum oksalat taşları, glomerülonefrit, nefrotik sendrom, interstisyel nefrit) [5,13,21].

Ülseratif Kolit ve Chron Hastalığı

ÜK; rektal kanama, şiddetli ağrı ve diyare ile ilişkilendirilen, kolonun mukozal tabakasının bir kısmını ya da tamamını veya rektumu tutabilen bir hastalıktır. Ayrıca enflamasyon kriptit-kript apseleri ve mukoza-submukoza ile sınırlıdır. ÜK'li bireylerin yaklaşık %50'den fazlası demir eksikliği yaşamaktadır. Bununla birlikte hastalık ile ilgili semptomlar hafif veya şiddetli düzeyde olabilmektedir. ÜK, kolon ve rektumun iç astarında (kalın bağırsak) enflamasyona ve ülserasyona neden olur. ÜK'de cinsiyet baskınlığı bulunmamakta ve hastalığın başlangıç olasılığının en yüksek olduğu dönemin ise 30-40 yaşları arasında olduğu bilinmektedir. Belirtilerin şiddeti kolonun etkilenme veya enflamasyon düzeyine bağlıdır. ÜK'deki enflamasyon spesifik değildir ve rektumda (proktit), sol noktalı kolonda (sol taraflı kolit) veya tüm kolonda (uzantılı kolit veya pankolit) görülebilmektedir [12,15,34,35].

CH; kronik, transmural, segmental ve enflamatuar bir hastalıktır. GİS'in herhangi bir bölümünü etkileyebilmekle birlikte, genellikle terminal ileumda lokalize olduğu bilinmektedir. Relaps/remisyon dönemleri ve ülser, fistül, darlık ve bağırsak granülomlarının oluşumu ile karakterizedir. CH belirtileri genellikle sinsidir ve teşhisi güçleştirmektedir. Teşhisin gecikmiş olması bağırsak hasarının ve fibrozun artmasına neden olmaktadır. Klinik değerlendirmenin yanı sıra, kan ve dışkı bazlı biyobelirteçler de, EBH'yi enflamatuar olmayan diyareden ayırt etmek için kullanılmaktadır [12,15,34,35].

Teşhis

EBH'deki semptomların hastadan hastaya değişkenlik göstermesi hastalığın teşhisi ve ayrımını zorlaştırmaktadır. Bu nedenle hastalığın teşhisinde altın standart olarak kullanılan bir yöntem bulunmamaktadır. Fakat klinik, endoskopik, histolojik, radyolojik ve/veya biyokimyasal incelemelerin multidisipliner kombinasyonu hastalığın teşhisinde önemli yer tutmaktadır. EBH tanısından şüphenilen bireylerin tam kan sayımı, CRP düzeyleri, ayıntılı biyokimyasal testleri ve fekal tahlilleri değerlendirilmelidir. Bu değerlendirmelerin sonucunda akut faz proteinlerinde yükseklik, trombosit sayısında artış ve albumin düşüklüğü gözlemlendiği takdirde bu bulgular EBH açısından anlamlı görülmelidir. Gaitada gizli kan ve enflamatuar hücre varlığı da önemli EBH kriterlerindendir. Ayrıca tam idrar tahlilinde eritrosit ve oksalat kristalleri de görülebilmektedir. Son yıllarda en sık kullanılan önemli tanı kriterlerinden biri de Fekal kalprotektindir (FC). FC, EBH'de intestinal enflamasyona bağlı olarak yükselmektedir. Kalprotektinin EBH tanısı için %98 duyarlılığa ve %68 özgünlüğe sahip olduğu belirtilmiştir. Laboratuar bulgularını değerlendirdikten sonra hastalığı sınıflandırmak ve evrelemek için kesitsel görüntüleme, ileo-kolonoskopi, terminal ileum ve kolorektal örneklerin histopatolojisi, üst endoskopi, enteroskopi gibi farklı prensiplerdeki teşhis yöntemleri kullanılmaktadır [12,14].

Tedavi

EBH'nin etiyopatogenezi karmaşık olduğundan tedaviye yönelik mevcut tıbbi yaklaşımlar kısıtlıdır. Fakat günümüzde ilaç tedavisi (aminosalisilatlar, kortikosteroidler, immünomodülatörler, antibiyotikler), cerrahi tedavi (prokto-kolektomi, ileal kese anal anastomozu gibi), tıbbi beslenme tedavisi ve tamamlayıcı tedavi en sık kullanılan tedavi yaklaşımları olup EBH'nin geleneksel tedavisi için potansiyel olumlu etkileri olduğu kaçınılmazdır [1,12,18,23].

Enflamatuar Bağırsak Hastalığı ve Tamamlayıcı Tedavi

İnsanoğlu var olduğundan beri hastalıklar görülmeye başlamıştır. Bu hastalıklara doğadaki ürünlerle veya eski çağlardan beri kullanılan geleneksel yöntemlerle çare aranmaya çalışılmış olup bu sayede farklı tedavi yöntemleri doğmuştur. Bu tedavi yöntemleri, modern tıbba destek olmak amacıyla

birçok uygulamalara kapı aralamıştır. Dünyanın birçok yerinde ortaya çıkan bu uygulamalar ülkemizde de giderek yaygınlaşmaktadır. Son yıllarda tamamlayıcı tedavi, EBH'li bireylerde esas tedavilerine ek olarak cazip bir hale gelmiş olup bu uygulamalara karşı hastaların büyük bir ilgi ve merakı ortaya çıkmıştır. Fakat tamamlayıcı tedavide önemli bir paya sahip olan tıbbi bitkisel ürünlerin (bitkisel drog, bitkisel preparat, bitkisel tıbbi ürün, fitoterapötik ve fitofarmasötik) ve takviye edici gıdaların (nütrasötik, fonksiyonel gıda, bitkisel ilaç ve geleneksel bitkisel tıbbi ürün) diğer tedavi yöntemlerine kıyasla "daha etkili/daha yararlı/daha zararsız" olduğu düşüncesi gözden geçirilmesi gereken önemli bir konudur. Bu ürünlerin kullanımında hastalığın çeşidi ve süresi, yaş, cinsiyet gibi farklı parametreler göz önünde bulundurulmakla birlikte doğru ürün, uygun doz ve formülasyonda kullanılmalıdır [10,11,36].

Enflamatuar bağırsak hastalıklarında kullanılan tıbbi bitkiler Tablo 1'de belirtilmiştir.

Tablo 1. Enflamatuar bağırsak hastalıklarında kullanılan tıbbi bitkiler [37-70]

Bitki adı	**Yaygın adı**	**Familyası**	**Kullanılan Kısımları**	**Literatür**
Allium cepa L.	Soğan	Liliaceae	Folia, Bulbus	[37]
Allium sativum L.	Sarımsak	Liliaceae	Bulbus	[38]
Aloe barbadensis Miller	Aloe	Asphodelaceae	Gövde	[39,41,42,46]
Ananas comosus (L.) Merr.	Ananas	Bromeliaceae	Fructus	[46]
Andrographis paniculate (Burm. F.) Nees	Kreat/Yeşil chiretta	Acanthaceae	Folia	[39-42,46]
Artemisia absinthium L.	Pelin otu	Asteraceae	Flos, Folia	[38,40-43,46]
Bacopa monnieri L. Wettst.	Brahmi	Plantaginaceae	Herba	[44]
Borago officinalis L.	Hodan	Boraginaceae	Flos, Folia	[49]
Boswellia serrata Roxb.	Akgünlük	Burseraceae	Folia	[38-43,46]
Cannabis sativa L.	Hint keneviri	Cannabaceae	Semen	[38,41,42,46]
Camellia sinensis L.	Çay	Theaceae	Folia	[38,46]
Chrysanthemum indicum L.	Hint krizantemi	Asteraceae	Flos	[44]
Commiphora myrrha (Nees) Engl.	Mür	Burseraceae	Resina	[41,42]
Curcuma longa L.	Zerdeçal	Zingiberacae	Rhizoma	[38,39,41,46]
Ficus bengalensis L.	Banayan ağacı	Moraceae	Cortex	[44]
Garcinia cambogia (L.) Rob.	Hint hurması	Clusiaceae	Fructus	[44]
Gardenia jasminoides J. Ellis	Gardenya	Rubiaceae	Fructus	[44,46]
Glycine max L.	Soya fasulyesi	Fabaceae	Fructus	[50]
Glycyrrhiza glabra L.	Meyan	Fabaceae	Radix	[51]
Harpagophytum procumbens DC.	Şeytan pençesi	Pedaliaceae	Radix	[52]
Hypericum perforatum L.	Sarı kantaron	Hypericaceae	Herba	[53]
Linum usitatissimum L.	Keten	Linaceae	Semen	[54]
Matricaria spp.	Papatya	Asteraceae	Flos	[41]
Momordica charantia L.	Kudret narı	Cucurbitaceae	Fructus	[66-70]
Nigella sativa L.	Çörek otu	Ranunculaceae	Semen	[47,55]
Oenothera biennis	Eşek otu	Onagraceae	Semen	[41,42,46]
Physalis peruviana L.	Altın çilek	Solanaceae	Herba	[57]
Plantago psyllium L.	Karnıyarıkotu	Plantaginaceae	Fructus	[41,42]
Prunus mume (Siebold) Siebold & Zucc.	Çin eriği/ Japon kayısısı	Rosaceae	Fructus	[44]
Persea americana Mill.	Avokado	Lauraceae	Fructus	[58]
Ribes nigrum L.	Siyah frenk üzümü	Grossulariaceae	Fructus	[59]
Rosa L.	Gül	Rosaceae	Flos	[60]
Rosa canina L.	Kuşburnu	Rosaceae	Fructus	[61]
Rosmarinus officinalis L.	Biberiye	Lamiaceae	Herba	[62]

Tablo 1 (*devamı*). Enflamatuar bağırsak hastalıklarında kullanılan tıbbi bitkiler [37-70]

Bitki adı	Yaygın adı	Familyası	Kullanılan Kısımları	Literatür
Salvia spp.	Adaçayı	Lamiaceae	Folia	[55]
Silybum marianum (L.) Gaertn.	Deve dikeni/ Meryemana dikeni	Asteraceae	Fructus	[41]
Syzygium aromaticum (L.) Merr. & L.M. Perry	Karanfil	Myrtaceae	Flos	[63]
Triticum aestivum L.	Buğday	Poaceae	Frucuts	[42,42,46]
Uncaria tomentosa (Willd. Ex Schult.) DC.	Kedi pençesi	Rubiaceae	Fructus	[64]
Vaccinium myrtillus L.	Yaban mersini	Ericaceae	Fructus	[42]
Valeriana officinalis L.	Kediotu	Valerianaceae	Radix, Rhizoma	[65]
Withania somnifera L. (Dunal)	Ashwagandha/Gelinfeneri	Solanaceae	Radix	[44]
Zingiber officinale Roscoe	Zencefil	Zingiberaceae	Rhizoma	[44]

Tablo 1'de listelenmiş tıbbi bitkiler arasında son yıllarda EBH'li bireyler tarafından kullanımı giderek yaygınlaşan başlıcaları hakkındaki bilgiler aşağıda sunulmuştur.

***Momordica charantia* L.**

Momordica charantia L., Cucurbitaceae familyasına ait, Hindistan, Malaya, Çin, Tayland, Japonya, Singapur, Vietnam, Amazon, Doğu Afrika, Orta Doğu, Orta ve Güney Amerika gibi tropikal ve subtropikal bölgelerde yayılış gösteren ve genellikle meyvesi de dahil olmak üzere tüm kısımlarının acı bir tadı olduğu için "kudret narı, acı kabak, balsam armut, acı kavun, kugua veya karela" olarak bilinen bir bitkidir. "*Momordica*" ismi, bitkinin yapraklarının tırtıklı kenarlara sahip oluşundan gelmektedir. Bitkinin meyvesi ise dikdörtgenimsi şekilde ve silindir çıkıntıları olup küçük bir salatalığı andırmaktadır. Meyvenin dış kısmı olgunlaştığında zümrüt yeşil renginden turuncuya dönmekte, etli kısmı ise beyazdan kırmızılaşmaktadır. Bitkinin meyveleri olgunlaşma sürecinin her aşamasında kullanılabilmekte ve acı tadına rağmen dünyanın birçok yerinde gıda olarak tüketilebilmektedir. *M. charantia*'nın halk arasındaki popülerliğinin temel nedeni diğer sebze ve meyvelere kıyasla zengin içeriği olması ile açıklanmaktadır. Bitkinin tüm kısımları (tohum, kök, yaprak ve özellikle olgunlaşmamış meyve) önemli etkilere sahip olup eski zamanlardan beri tıbbi bitki olarak kullanılagelmektedir. *M. charantia*'nın antibakteriyel, antiviral, antitümör, immünomodülatör, antioksidan, antidiyabetik, antihelmentik, antimutajenik, hepatoprotektif, antienflamatuar ve antiülserojenik aktiviteleri içeren çeşitli tıbbi özellikleri olduğu bilinmektedir. *M. charantia*'nın Ayurveda Tıbbından başlayan geleneksel kullanımı, Türk halk hekimliğinde de olgun meyvelerinin zeytinyağında güneşte bekletilerek yumuşatılması sonucu elde edilen maseratın bal ile birleştirilerek mide ülserlerinin önlenmesi ve iyileştirilmesinde kullanılması ile devam etmektedir. Afrika halkında ise bitkininin yapraklarının kaynatılarak diyabet, kızamık, suçiçeği, uyuz, sıtma, sarılık, ülser ve yanıklarda kullanıldığı bilinmektedir. *M. charantia'*nın geleneksel tıpta bu geniş kullanımının yanı sıra son yıllarda EBH'de kullanımı giderek yaygınlaşmakta olup bu konuda yapılan çalışmalar hız kazanmıştır. Yapılan çalışmaların sonucunda bitkinin IL-1β, IL-6 ve TNF-α seviyelerini önemli ölçüde azalttığı ve IL-10 düzeylerini arttırdığı saptanmıştır. Bu antienflamatuar etki sayesinde EBH'li bireylerde hastalığın remisyon süresini uzattığı ve relaps dönemlerinin sıklığını azalttığı düşünülmektedir. *M. charantia'*nın, içeriğindeki triterpenoitler, saponinler, polipeptitler, flavonoitler, alkaloitler ve steroller sayesinde önemli farmakolojik etkilere sahip olduğu kanıtlansa da bazı olumsuz etkiler ortaya çıkardığı ve son yıllarda kullanımının sınırlandığı belirtilmiştir. Özellikle çocuklarda hipoglisemik komaya, kadınlarda intratuterin fetale neden olabileceği bu nedenle dikkatli kullanılması gerektiği bildirilmiştir. Bununla birlikte Glukoz-6-fosfat dehidrojenaz eksikliği olan bireylerin favizm riski ile karşı karşıya kalabileceği konusunda uyarılar bulunmaktadır. Ek olarak, *M. charantia*-ilaç etkileşimleri konusunda dikkatli olunması gerektiği ve yüksek dozlarda ve uzun süre kullanımının yan etkiler ortaya çıkarabildiği bildirilmiştir. Bu nedenle *M. charantia*'nın terapötik değerinin, etkinliğinin ve güvenirliğinin belirlenmesi adına daha geniş kapsamlı klinik çalışmalara ihtiyaç duyulmaktadır [66-70].

Nigella sativa L.

Halk arasında “Çörek otu, siyah kimyon veya siyah tohum” olarak bilinen *Nigella sativa* L., Ranunculaceae familyasına ait bir bitkidir. Doğu Akdeniz, Kuzey Afrika, Güney Hindistan ve Güneybatı Asya'da doğal yayılış göstermekte olup, Mısır, İran, Yunanistan, Suriye, Arnavutluk, Türkiye, Suudi Arabistan, Hindistan ve Pakistan’da kültürü yapılmaktadır. Mavimsi renkli çiçeklere, kapsül şeklindeki meyvelere ve küçük siyah renkli tohumlara sahip olup, 20–30 cm yüksekliğe kadar büyüyebilen, tek yıllık, otsu bir bitkidir. “Mucizevi bitki” (Arapça'da adlandırıldığı şekliyle “Habba sawda”) olarak da tanınan çörek otu, Müslümanlar arasında yaygın olarak kabul edilen bir inanca göre ölümü önlemek dışında tüm rahatsızlıkları tedavi etmek için kullanılan "cennetten gelen bitki" olarak kabul görmüş olup, Unani, Ayurveda ve Siddha gibi geleneksel tıpta da uzun süredir kullanım bulmaktadır. Kanıtlanmış etki ve kullanımlarına bakıldığında; tohumlarının antioksidan, antienflamatuar, immünomodülatör, nöroprotektif, antimikrobiyal, antihipertansif, kardiyoprotektif, antidiyabetik, gastroprotektif, nefroprotektif ve hepatoprotektif özellikler gösterdiği bilinmektedir. Bu etkileri içeriğindeki timokinon, timohidrokinon, timol, karvakrol ve alkoloitlere (nigellisimin ve nigellisimin-N-oksit, nigellidin, nigellisin) atfedilmektedir. *N. sativa*’nın EBH üzerindeki etkileri değerlendirildiğinde myeloperoksidaz, MDA, IL-1β, IL-6, IL-8, TNF-α, COX-2’nin serum düzeylerini düşürdüğü ve iNOS'un gen ekspresyonunu düzeylerini azalttığı bildirilmiştir. Yapısındaki timokinonun ise peroksizom proliferatör ile aktive edilen reseptör gamamodülatörünün ekspresyonunu artırdığı bildirilmiştir. Bununla birlikte NF-κB ve STAT yolu üzerinde düzenleyici olduğu bilindiğinden EBH üzerinde de potansiyel olumlu etkisi olabileceği düşünülmektedir. *N. sativa* veya içeriğindeki timokinon sınırlı toksisiteye sahip olsa da bu sonuçların büyük çoğunluğu preklinik çalışmalara dayanmaktadır. Bu nedenle farmakolojik ve toksikolojik profilleri belirlemek adına daha fazla klinik çalışmaya ihtiyaç duyulmaktadır [71-75].

Zingiber officinale Roscoe

Zingiber officinale Roscoe (Zencefil), Zingiberaceae familyasına ait bir bitkidir. Birçok ülkede rizomları baharat ve gıda takviyesi olarak kullanılmaktadır. Bu yaygın kullanımının yanı sıra tarih boyunca halk arasında sindirimi kolaylaştırmada, romatizmal hastalıklarda, diş ağrısı, bulantı, öksürük, iştahsızlık ve kanama bozukluklarında kullanılmış olup modern tıpta ise antioksidan, antienflamatuar ve antiülser gibi etkileri nedeniyle kabul görmektedir. Bilinen bu farmakolojik etkilerinden içeriğindeki fenolik bileşikler ve terpenlerin sorumlu olduğu bilinmektedir. EBH üzerindeki etkisini değerlendirmek amacıyla yapılan çalışmalarda, zencefilin IL-4, IL-6, IL-13, IL-1β, interferon-c, IL-17, IL-22, TNF-α, IFN-γ ve TGF-β sitokin ekspresyonunu, iltihaplanma şiddetini, enflamasyon boyutunu ve kript hasarını doza bağlı bir şekilde azalttığı ortaya konmuştur. Bununla birlikte glutatyon peroksidaz, malondialdehit (MDA) ve toplam antioksidan kapasite üzerinde etkisi olduğu ve oksitadif stresi de azalttığı bildirilmiştir. Nükleer faktör kappa B (NF-κB), Nod benzeri reseptör ailesi proteinleri, Toll benzeri reseptörler, STAT, mitojenle aktive edilmiş protein kinaz ve rapamisinin memelilerdeki hedef yollarını baskılayarak EBH’nin tedavisinde etkili olduğu sonucuna varılmıştır. Fakat EBH’nin tedavisinde ve bağırsak mikrobiyatasının iyileştirilmesinde zencefilin güvenliğini ve etkinliğini değerlendirmek, dozaj ve süreyi belirlemek için daha fazla klinik çalışmaya ihtiyaç duyulmaktadır. Literatürdeki çalışmalarda bu konu ile ilgili kısıtlı sayıdaki bilgiler değerlendirildiğinde, zencefilin uzun süre ve yüksek dozda kullanımının bulantı, pankreatit ve alerjik reaksiyonlara neden olabileceği bildirilmiştir [76-79].

Curcuma longa L.

Curcuma longa L., Zingiberaceae familyasına ait, “Zerdeçal” adıyla bilinmekte ve Hindistan, Güney Çin, Güneydoğu Asya, Papua Yeni Gine ve Kuzey Avustralya’da yayılış göstermektedir. "*Curcuma*" kelimesinin, sarı renk anlamına gelen Arapça "kurkum" kelimesinden köken aldığı bilinmektedir. *C. longa* sarı-beyaz çiçeklere, 10-15 cm sap uzunluğuna ve kahverengi-oval tohumlara sahip çok yıllık bir bitkidir. *C. longa,* antioksidan, antienflamatuar, antidiyabetik, hepatoprotektif ajan olarak dünyada pek çok farklı bölgede kullanılmaktadır. Nepal’de sarılık, bronşit, sıtma ve karaciğer hastalıklarının tedavisinde; Kolombiya'da dolaşımı uyarıcı, yara iyileştirici, bağışıklık sistemi güçlendirici olarak ayrıca hazımsızlık, diyabet, yüksek kolesterol ve böbrek enfeksiyonunda; Pakistan'da da yara iyileştirmede kullanılmaktadır. Hindistan’da ise özellikle rizomlarının baharat, gıda koruyucusu ve renklendirici olarak kullanılması nedeniyle "Hint safranı veya “Hindistan'ın Altın

Baharatı" olarak anılan *C. longa,* Ayurveda Tıbbının da önemli bir parçası olup Ayurveda Farmakopesinde yer almaktadır. Bunun yanı sıra pek çok ülkenin farmakopesinde de bulunmaktadır. *C. longa,* karbonhidratlar, lif, proteinler, lipitler, C vitamini, piridoksin, magnezyum, fosfor, potasyum, kalsiyum, terpenoitler, flavonoitler, fenolik bileşikler, organik asitler, antosiyanin ve tanenler içermektedir. Bu biyoaktif zenginlik *C. longa*'yı beslenme açısından zengin bir doğal gıda maddesi yapmaktadır [80-82].

Kurkumin, *C. longa*'nın renginden sorumlu, biyolojik olarak aktif fenolik bileşenlerinden biridir ve pek çok potansiyel olumlu etkileri bulunmaktadır. Çeşitli *in vitro* ve *in vivo* çalışmalarda antimikrobiyal, antioksidan ve immünomodülatör özelliklerle karakterize edilmiştir. Bunların yanı sıra EBH üzerinde olumlu etkileri olduğu yapılan çalışmalarla desteklenmiştir. Kurkumin, miyozin hafif zincir kinaz ekspresyonunu azaltarak, enflamasyonun neden olduğu değişmiş bağırsak bariyer fonksiyonunu iyileştirmektedir. ÜK'li bireylerde klinik ve endoskopik yanıtı iyileştirebileceği fakat CH'lilerde hastalığın nüksetmesini önlemede etkili olmadığı bildirilmiştir. Ayrıca bağırsak mukozasında kurkumin, ROS, süperoksit anyonları ve MDA düzeylerini azaltabilmektedir. Bununla birlikte *C. longa* ve kurkumin ile tedavinin IgE, IL-4, TGF-β, IL-17, IFN-γ ve Th1/Th2 oranını artırdığı, TNF-α üretimini azalttığı ve antienflamatuar etki gösteren STAT-3 yolağını inhibe ederek hücre proliferasyonunu inhibe ettiği saptanmıştır. Yapılan diğer çalışmalarda ise mevcut tedaviye ek olarak (sülfasalazin veya mesalamin ile birlikte) kurkumin kullanımının hiçbir yan etki olmadan remisyonu indükleme açısından olumlu sonuçlar gösterdiği, CRP konsantrasyonunu ve eritrosit sedimantasyon hızını önemli ölçüde azalttığı ve yaşam kalitesini iyileştirdiği ortaya konmuştur. Bu nedenle EBH tedavisinde etkin bir rol oynayabileceği düşünülmektedir. Fakat zerdeçalın ve kurkuminin EBH üzerindeki terapötik etkisini ve güvenliğini belirleyebilmek için örneklem büyüklüğünün artırıldığı daha fazla klinik çalışmaya ihtiyaç duyulmaktadır. Literatürdeki klinik çalışmalarda kurkuminin etkili ve güvenli olduğu, FDA (ABD Gıda ve İlaç İdaresi) tarafından "genellikle güvenli olarak kabul edilir" ibaresiyle onaylanmaktadır. Fakat yüksek dozda zerdeçal tüketiminin gebelikte uterus kasılmasını tetikleyebileceği ve demir emilimini engelleyebileceği; erkeklerde ise testosteron seviyesini azaltabileceği bildirilmiştir. Ayrıca preoperatif dönemden en az 2 hafta önce kullanımının sonlandırılması gerektiği, aksi takdirde kanın pıhtılaşmasını geciktirebileceği konusunda uyarılar bulunmaktadır. Safra kesesi ve kanama sorunları olan bireylerin tüketmemesi gerektiği de belirtilmektedir [80-82].

Cinnamomum zeylanicum J. Presl.

Cinnamomum zeylanicum J. Presl., Lauraceae familyasına ait, "Seylan tarçını, Gerçek tarçın" adlarıyla bilinen, Sri Lanka'ya ve Hindistan'ın güney bölgelerine özgü ve birkaç yüzyıldır dünya çapında farklı kültürler tarafından kullanılan yaygın bir baharattır. Baharat olarak kullanımının yanı sıra Ayurveda Tıbbında özellikle solunum, sindirim ve jinekolojik rahatsızlıklar için kullanılmakta olup kabuk, yaprak, çiçek, meyve ve kökler dahil olmak üzere bitkinin hemen hemen her kısmının önemli farmakolojik etkileri olduğu kanıtlanmıştır. Tarçının potansiyel yararlı etkileri sinamik asit, sinnamik aldehit, sinnamik alkol, kumarin ve öjenol gibi biyoaktif bileşiklerden kaynaklanmaktadır. EBH üzerindeki etkisi değerlendirildiğinde; yapısındaki sinnamik asitin enflamatuar sitokinlerin neden olduğu bağırsak bariyeri fonksiyonu hasarını iyileştirdiği, TNF-α, IL-6 ve IL-1β mRNA ekspresyonunu azaltarak antienflamatuar aktivite gösterdiği bildirilmiştir [83-85].

Boswellia serrata Roxb.

Boswellia serrata Roxb., Burseraceae familyasına ait Hindistan, Kuzey Afrika ve Orta Doğu'nun kurak dağlık bölgelerinde yetişen bir bitkidir. *B. serrata,* Ayurveda'daki en eski ve en değerli bitkilerden biridir. Ayurveda metinlerinde *B. serrata*'dan elde edilen reçinenin diyare, dizanteri, saçkıran, çıbanlar, ateş, ağız yaraları, boğaz ağrısı, öksürük, saç dökülmesi, sarılık, hemoroit, frengi ve adet düzensizliğinde yararlanıldığı bilinmektedir. Modern tıpta ise antiartritik, antienflamatuar, antihiperlipidemik, antiaterosklerotik, analjezik ve hepatoprotektif olarak kullanımı yapılan çalışmalarla kanıtlanmıştır. *B. serrata*'nın reçineli kısmının monoterpenler (α-tuyon); diterpenler (insensol, insensol oksit, izo-insensol oksit), diterpen alkol (serratol) gibi makrosiklik diterpenoitler; triterpenler; pentasiklik triterpenik asitler (bosvelik asitler) ve tetrasiklik triterpenik asitler açısından zengin bir biyoaktif içeriğe sahip olduğu bilinmektedir. İlk klinik çalışmalar *B. serrata*'nın EBH üzerinde etkili olabileceğini öne sürmüştür. Son yıllarda yapılan çalışmalar sonucunda EBH'li

bireylerde lökotrien sentezini inhibe ettiği ve proenflamatuar sitokinlerin seviyelerini azalttığı bilinmektedir. CH'li bireylerde yapılan bir çalışma, *B. serrata* ekstresinin hastalığın tedavisinde mesalamin kadar etkili olabileceğini ancak daha üstün olmadığını ortaya koymuştur. ÜK'li bireylerde ise bağırsak ağrısı, dışkıda belirgin ve gizli kan, bağırsak hareketleri ve kramplar, sulu dışkı gibi parametreleri önemli ölçüde iyileştirdiği belirlenmiştir. Mevcut sonuçlar doğrultusunda *B. serrata*'nın hem remisyonun sürdürülmesinde hem de hastalığın aktif döneminin yönetilmesinde etkisini değerlendirmek için gelecekteki araştırmaların önemi vurgulanmıştır. *B. serrata*'nın kullanım formu, dozu ve süresi ile ilgili tam olarak netlik bulunmamakta ve daha fazla klinik çalışmaya ihtiyaç duyulmaktadır [86-88].

***Linum usitatissimum* L.**

Linum usitatissimum L., yaygın bilinen adıyla "Keten tohumu" Linaceae familyasına ait Akdeniz ve Güneybatı Asya'da yetişen bir bitkidir. Keten bitkisinin drog olarak kullanılan kısımları tohumlarıdır. Keten tohumu içeriğindeki omega-3 yağ asitleri (özellikle alfa-linolenik asit), fitoöstrojenler ve çözünür lif nedeniyle "süper gıda" olarak bilinmekte olup antienflamatuar, antiproliferatif, antikarsinojenik, antidiyabetik, antihiperkolesterolomik, fungistatik, antihipertansif, immünosüpresif, antimalaryal, antioksidan ve antitrombotik etkileri bulunmaktadır. Keten tohumunun EBH üzerindeki etkisinin değerlendirildiği klinik ve *in vivo* çalışmalarda, INF-γ, TNF-α, IL-6, NF-κB ve bağırsak geçirgenliği üzerinde potansiyel etkileri olduğu bildirilmiştir. Bununla birlikte hastalığın şiddetini ve nötrofil infiltrasyonunu azaltmaktadır. Literatürdeki çalışmalar genel olarak değerlendirildiğinde; keten tohumu yağının ÜK'li ve CH'li bireylerde koruyucu potansiyel ve enflamatuar belirteçleri, hastalık şiddetini ve kan basıncını zayıflattığı gösterilmiştir. Ayrıca IL-2 ve IL-6 sitokinlerinin salınımını azalttığı, IL-10 sitokinlerinin salınımını arttırdığı, epitel bütünlüğünü, goblet hücre sayısını, mikrobiyal bariyeri ve enflamatuar infiltrasyonu desteklediği bulunmuştur. Sonuç olarak keten tohumu, yağı ve ekstresi EBH'de hastalık semptomlarını iyileştirmek ve tedaviyi desteklemek için bir seçenek olarak kullanılabilmektedir. Fakat hastalık üzerindeki etkinliğini tam olarak belirleyebilmek adına daha fazla klinik çalışmaya ihtiyaç duyulmaktadır [89-92].

***Allium sativum* L.**

Allium sativum L. (Sarımsak), Amaryllidaceae familyasına ait ve tüm dünyada geleneksel tıpta ve baharat olarak kullanılan otsu bir bitkidir. Eski Mısır ve Roma'da, yorgunluğu azaltmak için işçilere ve askerlere sarımsak verildiği, Antik Yunanistan'da bağırsak ve akciğer rahatsızlıklarını tedavi etmek için tüketildiği, Hindistan'da yüzyıllardır yaraları ve ülserleri yıkamak için antiseptik bir losyon olarak kullanıldığı, Çin ve Japonya'da ise sarımsaklı çayın uzun zamandır ateş, baş ağrısı, kolera ve dizanteri için önerildiği bilinmektedir. Günümüzde de sarımsağın antihipertansif, antibakteriyel, antiviral, antidiyabetik, kardiyoprotektif, hepatoprotektif ve hipolipidemik etkiler dahil olmak üzere çok çeşitli terapötik etkileri gözlenmiştir. Bu terapötik özelliklerinin fenolik bileşikler, saponinler, polisakkaritler ve ayrıca organosülfür bileşikleri dahil olmak üzere bir dizi aktif bileşik sayesinde ortaya çıktığı kanıtlanmıştır. Olumlu etkilerinin, dialil tiosülfonat (allisin), diallil sülfür, diallil disülfit, dialil trisülfit, E/Z-ajoen, S-alil-sistein ve S-alil-sistein sülfoksit (alliin) dahil olmak üzere organosülfür bileşikleriyle daha çok ilişkili olduğu bulunmuştur. Sarımsağın pek çok farmakolojik etkisinin yanı sıra son yıllarda yapılan çalışmalar sarımsak ekstresinin ÜK'nin önlenmesinde ve yönetiminde potansiyel bir rolü olduğunu düşündürmüştür. Taze sarımsaktan elde edilen ekstrenin yapılan *in vitro* çalışmalarda Th1 ve enflamatuar sitokinlerin üretimini azalttığı gösterilmiştir. Sarımsağın keskin kokusundan sorumlu molekül olan allisinin, insan SW620 ve HCT116 kolon kanseri hücrelerinde anti-apoptotik ve NF-κB aktivitesini modüle etme yeteneği ortaya çıkarılmıştır. Sarımsak ekstresinin bütün ve periferik kanın uyarılmış mononükleer hücrelerine uygulanmasının, monosit IL-12, TNF-α, IL-1α, IL-6, IL-8, IFN-γ ve IL-2'de önemli bir azalmaya ve antienflamatuar sitokinlerin üretimini engelleyen ve doku homeostazına katkıda bulunan IL-10 üretiminin düzenlenmesine yardımcı olduğu saptanmıştır. Sarımsak bileşiklerinin yan etkileri, yüksek dozlarda alındığında veya bireyin bu besine karşı bir hassasiyeti olduğunda ortaya çıkabilmektedir [93-95].

SONUÇ VE TARTIŞMA

Eski çağlardan beri tıbbi bitkiler, pek çok rahatsızlığın önlenmesi ve tedavisi amacıyla

kullanılagelmiştir. DSÖ, dünya nüfusunun yaklaşık %80'inin sağlık sorunlarını tedavi etmek amacıyla tıbbi bitkilere başvurduğunu bildirmektedir. Bununla birlikte pek çok gelişmiş ülkede reçete edilen ilaçların yaklaşık %25'i bitkisel kökenli etken maddeleri, standardize drogları ya da ekstreleri içermektedir. Özellikle 1990'lı yıllardan sonra, kullanımı dünyada giderek yaygınlaşan tıbbi ve aromatik bitkilerin yeni kullanım alanlarının bulunması ve doğal ürünlere olan talebin artması bu bitkilerin kullanımını cazip hale getirmektedir [9-11].

Türkiye coğrafi konumu, iklim, toprak ve bitki türü çeşitliliği, tarımsal potansiyeli ve geniş yüzölçümü sayesinde tıbbi ve aromatik bitkiler ticaretinde önde gelen ülkelerden biridir. Türkiye'nin bu önemi; gelişmiş ülkelerdeki bitkisel ilaç, bitki kimyasalları, gıda ve katkı maddeleri, kozmetik ve parfümeri sanayilerinin girdisini oluşturan pek çok ürüne kaynak oluşturan bitkilerin ülkemiz florasında bulunmasından kaynaklanmaktadır. Tıbbi bitkilerin ülkemizde tamamlayıcı tedavideki kullanımını giderek yaygınlaşmaktadır. Bu kullanımlardan biri de hem dünyada hem de ülkemizde insidansı ve prevalansı giderek artan EBH'dir [1-9]. EBH'nin en temel tedavisi, hemen hemen her hastalıkta olduğu gibi ilaç tedavisidir. Bunun yanı sıra cerrahi tedavi, tıbbi beslenme tedavisi ve tamamlayıcı tedaviler EBH'li bireylerde başvurulan diğer yöntemlerdir. EBH'nin tamamlayıcı tedavileri arasında tıbbi bitkiler önemli bir paya sahiptir. Hastaların birçoğu "daha zararsız/daha yararlı" olduğu düşüncesi ile tıbbi bitki/bitkisel ürün kullanımına yönelmektedir. Fakat tıbbi bitkiler ve bitkisel ürünler ancak doğru kaynak, doğru bitki ve drog, uygun doz ve süre, uygun form ve hekim/eczacı kontrolü halinde hastalık üzerinde potansiyel olumlu etkiler gösterebilmekte, aksi takdirde hastalığın seyri kötüleşebilmektedir [4-8]. İlgili literatür incelendiğinde; EBH'de kullanılan tıbbi bitkileri geniş bir derleme şeklinde ele alan çalışmaların ülkemiz literatüründe bulunmadığı görülmektedir. Bu derlemeyi yaparken temel amacımız; literatürde yer alan ve günümüzde bu amaçla kullanılan bitkileri biraraya toplamak ve bunlardan en yaygın kullanılanları hakkında genel bilgi vermektir. Bu nedenle çalışmamız EBH'de tıbbi bitkilerin kullanımı ile ilgili bir kaynak oluşturacaktır.

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Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler.

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REVIEW ARTICLE / DERLEME MAKALE

NANOPARTICLES FOR DUAL IMAGING: PET AND FLUORESCENCE IMAGING

İKİLİ GÖRÜNTÜLEMEDE NANOPARÇACIKLAR: PET VE FLORESANS GÖRÜNTÜLEME

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ABSTRACT

Objective: *Molecular imaging methods are gaining popularity in clinical and preclinical fields. There are many different imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT) and Near-infrared fluorescence (NIRF), and each has different advantages and disadvantages. Multimodal imaging methods, a combination of two or more molecular imaging modalities, have been developed to overcome the disadvantages of these molecular imaging methods. However, these imaging methods are conjugated with different vectors to improve the multimodal imaging methods used. In this field, drug delivery systems, peptides, proteins, antibodies and aptamers have been widely used for conjugation of multimodal imaging modalities to overcome some of the disadvantages that come from imaging modalities. In this review, PET and NIRF combination imaging modalities were explained and more specifically PET and NIRF nanoparticle dual imaging modalities with their pros and cons were investigated.*
Result and Discussion: *Dual imaging modalities overcome to limitations of single imaging modalities and provide a better understanding of biological, anatomical, and physiological processes. Multimodal imaging modalities offer higher sensitivity, resolution, and specificity with lower cost and toxicity although have several disadvantages.*
Keywords: *Dual imaging, fluorescence imaging, nanoparticles, PET, quantum-dots*

ÖZ

Amaç: *Moleküler görüntüleme yöntemleri klinik ve preklinik alanlarda popülerlik kazanmaktadır. Bilgisayarlı tomografi (BT), pozitron emisyon tomografisi (PET), tek foton emisyon tomografisi (SPECT), manyetik rezonans (MRI) ve yakın kızılötesi floresans (NIRF) görüntüleme gibi birçok farklı görüntüleme yöntemi vardır ve her birinin farklı avantaj ve dezavantajları vardır. Bu moleküler görüntüleme yöntemlerinin dezavantajlarının üstesinden gelmek için iki veya daha fazla moleküler görüntüleme yönteminin bir kombinasyonu olan multimodal görüntüleme yöntemleri geliştirilmiştir. Bununla birlikte, bu görüntüleme yöntemleri, kullanılan multimodal görüntüleme yöntemlerini geliştirmek için farklı vektörlerle konjuge edilmiştir. Bu alanda görüntüleme yöntemlerinden kaynaklanan bazı dezavantajların üstesinden gelmek için multimodal görüntüleme yöntemlerinin konjugasyonunda ilaç taşıyıcı sistemler, peptitler, proteinler, antikorlar ve aptamerler yaygın olarak kullanılmaktadır. Bu derlemede PET ve NIRF kombinasyonlu görüntüleme modaliteleri anlatılmış ve daha spesifik olarak PET ve NIRF nanoparçacık ikili görüntüleme yöntemleri artıları ve eksileri ile incelenmiştir.*

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Sonuç ve Tartışma: *İkili görüntüleme yöntemleri, tek görüntüleme yöntemlerinin sınırlarını ortadan kaldırır ve biyolojik, anatomik ve fizyolojik süreçlerin daha iyi anlaşılmasını sağlar. Çoklu görüntüleme yöntemleri birkaç dezavantajı olmasına rağmen, düşük maliyet ve toksisite ile birlikte daha yüksek hassasiyet, çözünürlük ve özgüllük sunar.*
Anahtar Kelimeler: *Floresan görüntüleme, ikili görüntüleme, kuantum noktaları, nanopartikül, PET*

INTRODUCTION

Molecular imaging mainly provides the investigation of molecular abnormalities of diseases, not only the differences in the molecular stage. It was defined as an *in vivo* characterization and biological process measurements at a molecular level by Weissleder and Mahmood. Biomarkers, essential for molecular imaging, are used for targeting biological systems and provide high specificity and sensitivity [1,2]. Molecular imaging modalities such as CT, MRI, PET, SPECT, and optical imaging are showing increasing popularity in both clinical and preclinical areas [3]. Especially multimodal imaging, which combines two or three molecular imaging methods, has gained significant importance due to overcoming individual limitations [3,4]. Multimodal imaging techniques have been used to monitor structural, functional, and molecular changes, quantify, and identify biological processes at cellular and molecular levels in living organisms [4]. The limitation of each imaging modality can be overcome by combining different imaging techniques and providing better images in preclinic and clinic applications (Table 1).

Table 1. Molecular imaging modalities [5-7]

	PET	**SPECT**	**CT**	**MRI**	**NIRF**
Form of Energy	Annihilation photons	Gamma rays	X-rays	Radio frequency ways	Infrared light
Spatial Resolution (mm)	1-5	0.5-15	0.5-1	0.01-0.1	<1
Temporal Resolution	min	s-min	s-min	min-h	s-min
Penetration Depth	unlimited	unlimited	unlimited	unlimited	< 2 cm
Sensitivity	10^{-11}-10^{-12} M	10^{-10}-10^{-11} M	10^{-3} M	10^{-3}-10^{-15} M	10^{-9}-10^{-12} M
Cost	High	Medium-high	Medium	High	Low

CT is the technique that produces images depending on the different attenuation of X-rays by tissues. It is a common method used in clinical and shows high resolution, penetration, and fast acquisition time with low cost. However, high radiation doses and low quality of soft tissues are the main limitations of this imaging technique [4].

MRI is the common imaging method in radiology and provides good detection and characterization for soft tissue, unlike CT. This method shows high spatial resolution with high cost and low sensitivity [8,7]. MRI and CT imaging modalities provide better anatomic images and molecular changes [9].

Nuclear imaging techniques, PET and SPECT imaging, are the most common imaging modalities. SPECT is based on the detection of gamma rays decaying from gamma-emitting radionuclides. PET imaging method detects gamma rays from the two gamma photons (180°C direction) after the annihilation reaction between the electrons and positrons of PET radionuclides. These nuclear imaging methods show high sensitivity and quantification but also have a poor resolution [5]. PET and SPECT are also essential imaging modalities for personalized medicine and imaging models. They are the most common modalities for detecting diseases and monitoring treatments [4].

The optical imaging method is based on the detection of fluorophores, emitting the fluorescence after optical excitation by an optical microscope. This method shows high sensitivity and multiplexed imaging with low or medium-high costs, although the energies of fluorescence imaging are limited to

penetrate the tissues [7].

Nanotechnology, which is generally smaller than 100 nm, has been showing great importance for several decades. It is also one of the research subjects used as a medicine for diagnostic, therapeutic, and theranostic purposes. Nanotechnology applications in medicine provide the elimination of some deficiencies in conventional drug applications [10]. Organic and inorganic nanoparticles have been used in several imaging modalities [11].

In this paper, dual imaging modality (PET and fluorescence imaging combination) by using nano-size delivery systems and their advantages and disadvantages are reviewed.

Fluorescence Imaging

NIRF imaging, the most common optical imaging technique, is using for shallow lesion and superficial object imaging [12]. Fluorescent dyes emitting in the NIRF area are mainly divided into two regions: NIRF first window (NIRF-I: 700-900 nm) and NIRF second window (NIRF-II: 1000-1800 nm) [13,14]. The excitation photon travels and reaches the NIRF agents, and in the end, photon absorbance occurs depending on the absorbing components of tissues and organs [15]. The main absorbing components are water, lipids, oxy, and deoxy hemoglobins [16]. Also, the NIRF imaging method depends on many parameters such as dye properties, excitation light properties, biodistribution and pharmacokinetic properties of the imaging system, targeting tissue and cell properties, etc. [16]. The most common fluorophores are fluorescein isothiocyanate (FITC), cyanine and cyanine derivatives, pacific blue and alexa fluor. However, new dyes are investigated by researchers due to their limited photostability [17]. Rhodamines, fluorescein, boron-dipyrromethene, and cyanine dyes are commercial fluorescent dyes, and they are currently on the market [17].

NIRF imaging technique is getting more attention day by day due to their high sensitivity, high spatial and temporal resolution, low optical absorption, and scattering features [18]. Also, fluorescence imaging properties provide some functional features about the activity of molecules [19]. However deep tissue penetration is the main obstacle to clinical use [20]. The main problem of fluorescence imaging is the instability of the fluorophores [21]. Their stability problem affects the fluorescence signal in imaging techniques. Also, this technique faces some difficulty in imaging living organisms due to a lack of deep tissue penetration (Table 2) [11].

Table 2. Advantages and disadvantages of fluorescence imaging technique

Advantages	**Disadvantages**
Deep penetration in tissues and organs	Instability of fluorescent dyes
High sensitivity	Non-specific adsorption to proteins
High resolution	Rapid degradation

Drug delivery systems have been used for NIRF imaging to overcome these disadvantages. These systems could help to overcome the instability of the fluorescent dyes and the rapid degradation of these dyes. Another advantage of drug delivery systems in NIR imaging is higher loading capacity, providing better resolution [22].

Fluorescent dyes are generally placed in the core of nanoparticles which is protected by the shell from photobleaching. Quantum dots (QDs), carbon-based nanoparticles, silica nanoparticles, fluorescent dyes encapsulated nanoparticles, liposomes, and other drug delivery systems are mainly used for fluorescence imaging [23,24].

Pet Imaging

PET imaging is one of the nuclear medicine imaging modalities based on PET radionuclides, which are traditional radionuclides such as ^{18}F, ^{13}N, ^{15}O, and radiometals such as Gallium-68 (^{68}Ga), Zirconium-89 (^{89}Zr), Cupper-64 (^{64}Cu), etc. [25]. The positron, which is emitted from positron-emitting radionuclides, travels and is annihilated with electron [25]. After this annihilation reaction, two 511 keV gamma rays occurred in the opposite direction (180°C apart) and were detected by the PET scanner (Figure 1). [26]

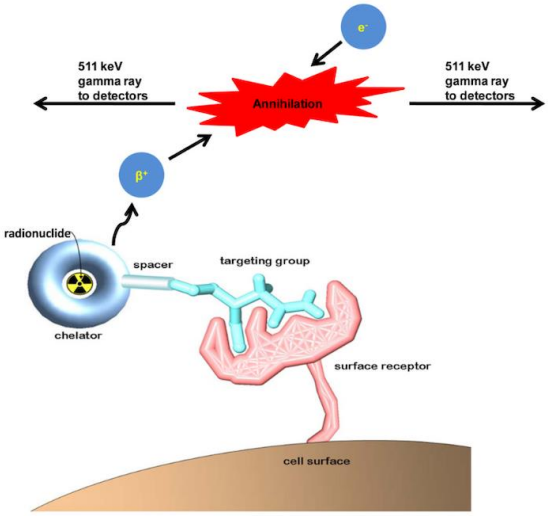

Figure 1. PET imaging principle [26]

PET imaging modality provides some advantages such as better resolution and high sensitivity; however, spatial resolution is the limitation due to the physical characteristics of the scanners (Table 3) [26,27]. Also, the sensitivity is highest among all imaging modalities without any depth limitation [27].

Table 3. Advantages and disadvantages of PET imaging technique

Advantages	**Disadvantages**
High sensitivity	Expensive method
High resolution	Low spatial resolution (about 5mm)
Three dimensional functional method	

The gold standard PET radiopharmaceutical, ^{18}F- fluorodeoxyglucose (FDG), a glucose analog, provides higher tumor uptake depending on the tumor tissue and overexpression on tumor area [9]. FDG PET scans all body with a single injection dose with 96% sensitivity and 77% specificity [28]. This imaging modality is used to detect dynamic changes in the body. It can also be used for basic physiological and molecular mechanisms [29]. Drug delivery systems can be radiolabelled with different radionuclides for many applications besides conventional PET imaging radiopharmaceuticals[30]. Nanoparticles can be radiolabelled with different methods (direct and indirect radiolabelling methods) depending on the various parameters such as half-lives of nanoparticles and radionuclides, energies of radionuclides, and properties of both nanoparticles and radionuclides [30].

Direct radiolabelling methods occurred attachment, incorporation, or encapsulation of radionuclides to nanoparticles. The interactions between nanoparticles and radionuclides can happen with physical interactions such as electrostatic interaction. This method is used mostly for nonmetallic PET radionuclides. Indirect radiolabelling methods, generally preferred ones, require chelators. These chelators act as a bridge between nanoparticles and radionuclides [30,31]. The chelators, also called bifunctional chelators, bind the radionuclides and nanoparticles; because of this, chelator choice is one of the most critical steps [30]. The most common bifunctional chelators for PET radionuclides are 2,2',2'',2'''-(1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), diethylenetriamine-N,N,N',N-pentaacetic acid (DTPA) and desferrioxamine (DFO) [30].

Combination of PET and NIRF Imaging

Dual imaging modalities, especially for diagnosing and monitoring diseases, provide many advantages due to taking advantage of each modality. NIRF imaging modalities have been frequently

used for dual imaging due to their complementary properties (Figure 2) [32]. NIRF's main principle is the selective and repeated activation of dyes with excitation light, while PET imaging is based on the detection of 511 keV gamma energies by the camera [21].

The combination of NIRF and PET imaging is improved the imaging quality and provides better sensitivity, specificity, and real-time visualization for preclinical and clinical applications [33]. These combination imaging agents can be used for surgical planning of whole-body imaging and molecular guiding for surgery, and also provide the correlation between these two imaging, which are for surgical planning and guiding [34].

PET-NIRF dual imaging agents can be synthesis different methods such as coupling and conjugation reactions, and several points should be considered before the synthesis studies:

1. Reaction times should be short if the short-lived radionuclides are used,
2. Reaction should result in as high a yield as possible due to expenses of materials and limited radioactivity,
3. Radiolabelling steps should be controlled because any reaction may occur that cause stability problem for fluorescent dyes.

The stability and optical properties of NIRF dyes can affect the quality and sensitivity of dual imaging, including PET radionuclides and NIRF dyes. Thus, researchers should pay attention to the radiolabelling steps and conditions; and the factors such as radiolabelling conditions, radiolysis, and interactions between the radionuclides and fluorescent dyes should be considered [35].

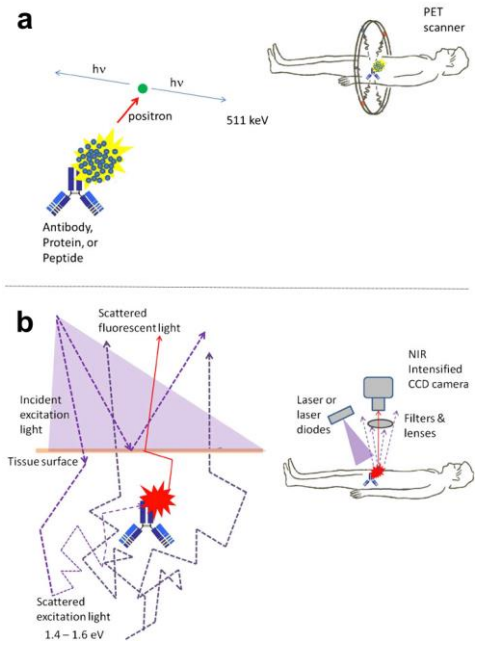

Figure 2. Schematic representation of PET imaging (a) and NIRF imaging (b) modalities [34] [Addepted from Azhdarinia et al. (2011)]

Nanoparticles with PET and NIRF Imaging

Dual imaging modalities with nanoparticles provide deep tissue detection of disease for image-guided surgery owing to PET imaging and also provide tissue resection owing to NIRF imaging [36]. Nanoparticles radiolabelled with PET radionuclides and conjugated or encapsulated fluorescent dyes can be used for tracking macrophages and detecting diseases [36].

Ariztia and co-workers explained the synthesis methods of PET and Fluorescence dual imaging agents with nanoparticles. Synthesis methods were divided into three main categories: 1. Dye approach; 2. Iterative approach; 3. Simultaneous approach (Figure 3) [27].

Dye Approach; This approach is not commonly used because radionuclides-bifunctional agent-fluorescent dyes synthesis is challenging. The radionuclides and fluorescent dye conjugation is in the first step and then followed by the conjugation of this dual imaging and nanoparticles. However, if radiometals will use for PET imaging, radiolabelling process is completed after fluorescent dyes-nanoparticles conjugation [27].

Iterative Approach; This approach is the most commonly used method for the dual imaging agent with radiometals. This strategy allows the radiolabelling in different steps. Thus, this convenience provides the prevention of chemical degradation and instability [27].

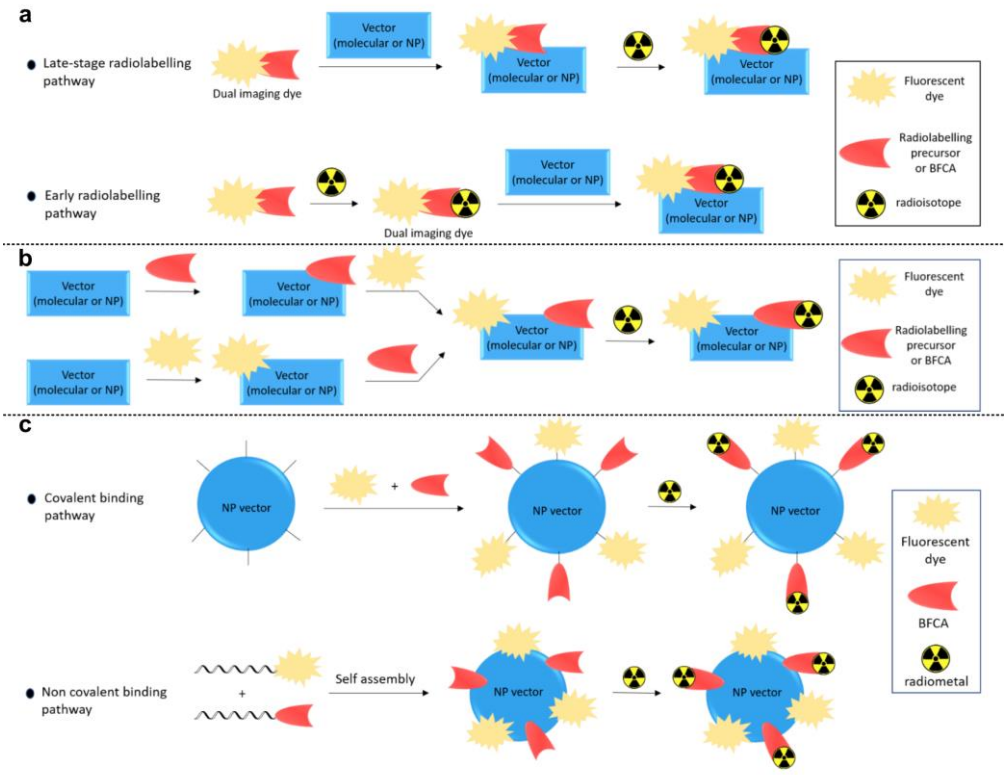

Figure 3. PET and NIRF dual imaging nanoparticle agents synthesis methods (a) dye approach (b) iterative approach (c) simultaneous approach [27] [Adapted with permission from Ariztia et al. (2022) American Chemical Society]

Simultaneous Approach; Covalent and non-covalent binding plays the primary role in this approach. Briefly, fluorescent dyes and bifunctional agents directly bind the surface of nanoparticles with covalent bind. This approach can only be used for radiometals and nanoparticles [27].

Imaging with contrast agents is essential in diagnosis, monitoring, and therapy for distinguishing physiological processes from anatomical processes. Although it has not yet had an Food and Drug Administration (FDA) approved agent, this imaging method is promising, and many preclinical studies have been reported in the literature [17]. PET/NIRF dual imaging combination in nano-size also has promising applications.

Recent studies show nanocrystals have a great potential for optical imaging [37]. QDs are fluorescent nanocrystals between 1-10 nm in size. They have optical and electrical properties and show better stability than other NIRF imaging nanoparticles. QDs consist of an inorganic core and inorganic shell structure [24,16]. They are not water-soluble due to their hydrophobic nature, and surface conjugation should be necessary to make them water-soluble probes[38]. Different types of molecules such as peptides[39], folates[40], dextrans[41,42], aptamer[43], antibodies[44], monoclonal antibodies (mAbs)[45] and commonly polyethylene glycol (PEG) can be conjugated [43,46].

Many studies have been conducted combining QDs with many different imaging methods [47]. NIRF with QDs and PET imaging combination could provide more information about the pharmacokinetics of NIRF QDs [33]. One of the main concerns of QDs imaging agents is the toxicity due to Cadmium (Cd) in QDs. Dual imaging could also solve this problem and decrease the toxicity of QDs [48]. ^{64}Cu radiolabelled PET/NIRF QDs showed significantly lower toxicity potential compared with the NIRF QDs. PET/NIRF imaging agents QDs require smaller amount for tumour imaging due to PET radionuclides, thus, the potential of toxicity decreases [33,49]. The toxicity levels of QDs depend on their size, charge, concentration, other bound groups or coats, and their stability [50,51]. Smaller QDs could be prepared with the combination of PET and NIRF imaging, which provide lower toxicity and reticuloendothelial system (RES) uptake herewith better imaging quality [52]. 21 nm (large) and 12 nm (small) size QDs biodistribution studies did not show any significant biodistribution differences between them. On the other hand, Cd, the reason for the toxicity of QDs, was not detected in *ex-vivo* studies of ^{18}F radiolabelled PEGylated QDs [53].

Studies showed that QDs can be radiolabelled with successful and high yield. Cai and co-workers (2007) radiolabelled the QD s with ^{64}Cu more than 90% yield. ^{64}Cu radiolabelled QDs were compared (in large and small sizes) with and without PEGylation. Both non-PEGylated QDs showed rapid uptake (2 mins) into the liver and spleen, unlike PEGylated QDs (6 mins). QDs size did not affect the biodistribution in their study, which is an unexpected result. However, size can be helpful in RES clearance, and smaller size QDs can affect the RES clearance and can help improve the NIRF image quality [33,38].

Peptide modified ^{18}F labelled QDs (^{18}F-Fluoropropionly (FP)-QD-arginine-glycine-aspartate acid (RGD)-bombesine (BBN)) were evaluated *in vitro* and *in vivo* for tumour detection/accumulation, imaging, biodistribution and compared with same QDs without ^{18}F-labelling (QD-RGD-BBN). ^{18}F-FP-QD-RGD-BBN showed higher uptake in kidney, liver and bladder unlike QDs due to metabolic stability of QD-RGD-BBN. ^{18}F-FP-QD-RGD-BBN dual imaging agent showed reduced toxicity and lower tissue penetration [49].

Radiolabelled QDs can also be used for *in vitro* and *in vivo* imaging. However, only a few *in vivo* imaging studies have been published. QDs show high photostability and brightness with changeable size and fluorescence wavelengths but for *in vivo* imaging, QDs should be more specific and effective to the targeted areas and organs [33]. The shortcomings in the acquired images have increased the search for dual imaging modalities for QDs. Also, tracking and quantification and, consequently, the biodistribution studies of the QDs *in vivo* by NIRF imaging are very limited due to their deep tissue penetration problems. However, due to the heavy metal toxicity of QDs, silica and carbon-based nanoparticles have been developed. Moreover, other nanoparticles may be more advantageous than QDs because their size can be adjusted easily, they can be formed from different materials, and they can be conjugated easily with different groups, which are helpful for desired circulation time [54].

NIRF dye encapsulated nanoparticles are also photostable systems that can overcome the stability problems of NIRF dyes. Moreover, prolonging the circulation half-life of NIRF dyes is the other primary advantage of this system [23]. Lee and co-workers developed the glycol chitosan-based nanoparticles for NIRF and PET dual imaging. Researchers indicated that the dual imaging agent developed by them provides biological features of the tumor as well as quantitative information on tumor targeting [19]. Moreover, dual imaging nanopartics provide better description for biodistribution for tumuors. PET images show better signal to noise ratio compared to NIRF images, however, NIRF provides *in vivo* and *ex vivo* visualization [55].

Silica-based nanoparticles show great potential for clinical applications in the future due to their biocompatibility. FDA also indicated silica-based nanoparticles as “Generally Recognized As Safe”. Silica nanoparticles, dense silica nanoparticles ($dSiO_2$), new generation $dSiO_2$ based Cornell prime dots (C’ dots), mesoporous silica nanoparticles (MSN), and hollow mesoporous silica nanoparticles (HMSN) are considered as a silica-based nanoparticles and are widely investigated for imaging properties. Several studies in radiolabelling silica-based nanoparticles with different radionuclides have been reported [56-59]. They can also be used for NIRF imaging by entrapment of fluorescence dyes [60]. This is an ideal drug delivery system for fluorescence dyes because it is photochemically inert and allows for the excitation and emission of light [23]. Other advantages of this drug delivery system are the water-

dispersible and microbial-resistant features. Their silica matrix allows the light to pass through and also protects the dyes from degradation [61]. Silica nanoparticles are biocompatibility, non-toxic characteristics, and easy modification with other molecules [22]. Different types of dual imaging with silica nanoparticles tagged/loaded with varying types of materials for targeting purposes are been investigated for primer or metastatic tumours. Many studies about aptamer, protein, and antibody conjugated silica nanoparticles for multimodal imaging using PET and NIRF systems have been reported for cancer imaging/therapy. One of them about aptamer-functionalized ^{64}Cu radiolabelled silica nanoparticles, was reported by Tang and co-workers (2012). Mono-disperse aptamer conjugated silica nanoparticles with 20 nm size for PET/NIRF imaging showed advantages for lymphatic imaging. Overcoming the depth insensitivity and low spatial resolution of each imaging modality with dual imaging showed potential for resection of metastatic lymph nodes [62].

MSNs have been widely investigated for drug and gene delivery systems, bioimaging, and cell markers due to their high drug-loading capacity, large surface area/high-surface modification, low toxicity and high stability [63]. MSNs are quite popular system for PET/NIRF imaging [64,65]. ^{64}Cu (PET radionuclide) and 800 CW (fluorescent dye) labelled targeted MSN were synthesized successfully and pharmacokinetics and targeting efficacy were evaluated *in vitro* and *in vivo* by using dual imaging modalities. Results showed that PET/NIRF MSN as a dual imaging agent could be a promising agent for imaging and also could be used for providing more information about accumulation, pharmacokinetics and targeting efficacy of agents [66].

MSN also have appropriate shell-thickness and size with protection for drugs. Hence, ^{64}Cu radiolabelled CuS@MSN nanoparticles tagged with TRC105 were synthesized for theranostic purposes. CuS nanoparticles were coated with MSN and after that, the anticancer drug was loaded into the CuS@MSN. It was followed by ^{64}Cu radiolabelling procedures for the purpose of the evaluation of their biodistribution and pharmacokinetics. Chen and co-workers (2015), mentioned that ^{64}Cu-CuS@MSN with a TRC105 tag was evaluated as a unique theranostic nanoparticle which provides *in vivo* active tumour targeting [67].

HMSN, which show a large drug loading capacity compared with mesoporous silica nanoparticles, were also used for dual imaging modalities [68]. Chen et al. (2014) reported the successfully prepared HMSN for PET and NIRF dual imaging purposes [68]. The chimeric antigen receptor (CAR) T cells tagged PET/NIRF silica nanoparticles were synthesized with success by Harmsen and co-workers (2021). ^{89}Zr radiolabelled NIRF silica (CF680R loaded) nanoparticles, were investigated for long-term whole-body CAR T cell tracking to provide a better understanding of CAR-T cell therapy and its limitations. CAR-T cell-tagged PET/NIRF nanoparticles provided whole-body tracking for 1 week. However, PET/NIRF nanoparticles were released from CAR-T cells after a week post-administration [69].

C'dots, inorganic silica nanoparticles, are Cy5 containing systems which can be used for NIRF images. One of the C'dots type which is ^{124}I-cyclic arginine-glycine-aspartic acid (cRGDY)-PEG-C'dots has been already approved by FDA Investigational New Drug for integrin-expressed cancer imaging This ultra small size nanoparticles evaluated as a bulk renal clearance, appropriate pharmacokinetics, excellent dual imaging modality without acute toxicity [70]. Following that, cRGDY-PEG-C'dots labelling studies with different radionuclides such as ^{89}Zr, ^{131}I etc. have been performed [70,57]. ^{124}I-cRGDY-PEG-C'dots were evaluated *in-vitro* and *in-vivo* by Benezra et al. (2011). In this study, Benezra and co-workers successfully obtained the binding affinity and levels of receptor expression, pharmacokinetic and clearance profiles, dosimetry and blood/tissue ratio of ^{124}I-cRGDY-PEG-C'dots. Results proved that ^{124}I-cRGDY-PEG-C'dots showed advantages in metastatic cancers [71]. ^{124}I-cRGDY-PEG-C'dots were also studied by Phillips and coworkers and this study were describes as a first-in-human trial of C'dots [72]. Phillips and co-workers investigated its safety profiles and also pharmacokinetics by using PET imaging. 124I-cRGDY-PEG-C'dots showed accumulation at tumour site with different pharmacokinetic with good clearance without RES uptake [72]. cRGDY-PEG-C'dots have already been labelled with 89Zr to evaluate as an agent for cancer detection and compare radiolabelling strategies by *in vitro* and *in vivo*. Results proved that ^{89}Zr-cRGDY-PEG-C'dots showed a great potential for clinical studies [57].

Liposomes are unilamellar lipid bilayer drug delivery systems that can trap the hydrophilic and lipophilic molecules in the central core and lipid bilayer [73]. This system is commonly used and preferred because of its bio-compatible, non-toxic, and biodegradable features [18]. Also, their encapsulation ability, modification property, and transportation ability into the tumor are the other reasons for common use [74]. Due to these features, many liposomes have been approved by FDA, and many of them are also available in the market [75]. Liposomes are suitable systems for dual imaging due to their encapsulation and/or attachment capability of different types of molecules [18,76]. Perez-Medina and co-workers developed dual imaging agents, ^{89}Zr radiolabelled Cy5 dyes encapsulated liposomes. Mainly, two different radiolabelling chemistry were investigated, and after that, NIRF dyes were encapsulated into the lipid bilayer. Results proved that dual imaging agents could be prepared with high stability [74].

Table 4. PET/NIRF dual imaging nanoparticles mentioned in this article

	PET radionuclides	**NIRF dyes**	**Dual imaging agent**	**Reference**
QDs	^{64}Cu	-	^{64}Cu-labeled DOTA–QD	[33]
	^{64}Cu	-	^{64}Cu-DOTA-QD525 ^{64}Cu-DOTA-QD800 ^{64}Cu-DOTA-QD525PEG ^{64}Cu-DOTA-QD800PEG	[38]
	^{18}F	-	^{18}F-FP-QD-RGD-BBN	[49]
	^{18}F	-	^{18}F-QDs	[53]
Silica Nanoparticles	^{64}Cu	NC200 NC20	^{64}Cu-NC200 silica nanoparticle ^{64}Cu-NC20 silica nanoparticle	[62]
	^{64}Cu	800CW	^{64}Cu-800CW-MSN	[66]
	^{64}Cu	CuS	^{64}Cu-CuS@MSN-TRC105	[67]
	^{64}Cu	ZW800	^{64}Cu-HMSN-ZW800-TRC105	[68]
	^{89}Zr	CF680R	^{89}Zr-silica nanoparticle	[69]
Chitosan Nanoparticles	^{64}Cu	Cy5.5	^{64}Cu-DOTA-Lys-PEG$_4$-DBCO	[19]
C'dots	^{124}I 131I	Cy5	cRGDY-PEG-Cy5-C'dots	[70]
	^{89}Zr	Cy5	^{89}Zr-DFO- cRGDY-PEG-C'dots	[57]
	^{124}I	Cy5	^{124}I -cRGDY-PEG-Cy5-C'dots	[71,72]
Liposomes	^{89}Zr	DiIC@DFO-L (Cy5 analog 1,1-diododecyl-3,3,3,3-tetramethyl-indodicarbocyanine-5,5-disulfonic acid)	89Zr-liposomes	[74]
	^{64}Cu	IRDye800CW	Liposome-DOX- ^{64}Cu/800CW	[78]

PET and NIRF imaging systems can also be very attractive dual imaging modalities to evaluate in different ways the therapy of drug delivery systems. Lobatto and co-workers used dual imaging modalities to understand the atherosclerosis therapy success of liposomes. Biodistribution and vessel

wall targeting of liposomes were evaluated by PET/CT images, while vascular permeability was evaluated by NIRF imaging. In the clinics, PET/CT imaging systems are used to provide a better understanding of *in vivo* behavior of drug delivery systems [77]. Du and co-workers (2017) studied PD-1 specific doxorubicin loaded, NIRF dye, and ^{64}Cu labeled liposomes to evaluate the tumor detection sensitivity and also therapy approach. Researchers aim to monitor the pharmacokinetics of this agent and also to understand the antitumor activity of the PD-1 targeted doxorubicin-loaded liposomes. The reason for using PET and NIRF combination imaging is the advantages of using both imaging modalities to understand tumor therapy (Table 4) [78].

RESULT AND DISCUSSION

Multimodal imaging modalities have been widely investigated in different combinations such as PET/MRI, SPECT/MRI, PET/optical imaging, etc. The limitations of single imaging modalities can be overcome with these combinations and provide a better understanding of biological, anatomical, and physiological processes. Multimodal imaging modalities offer higher sensitivity, resolution, and specificity with lower cost and toxicity. However, some limitations still exist or still need to develop. Drug delivery systems in nanosize have important advantages in multimodal imaging due to their large surface area, high loading and modification capability, and extended circulation half-life properties. Also, some nanoparticles provide better stability results for NIRF imaging dyes. The combination of PET and NIRF imaging has shown important advantages compared to single imaging modalities and also could be promising properties for clinical application. Moreover, nanoparticles in PET/NIRF dual imaging provide many benefits in preclinical and clinical stages and overcome many of the disadvantages that come from PET/NIRF imaging modalities, such as better stability and blood circulation times, and better images.

Nanoparticles in PET/NIRF, dual imaging modalities, have been found as a promising research area and have an excellent potential for preclinical and clinical applications. However, more research should be done in this field, especially radiolabelled and fluorescent dyes encapsulated nanoparticles should be investigated due to their biocompatible features.

AUTHOR CONTRIBUTIONS

Concept: E.T.S.; Design: E.T.S.; Control: E.T.S.; Sources: E.T.S.; Materials: E.T.S.; Data Collection and/or Processing: E.T.S.; Analysis and/or Interpretation: E.T.S.; Literature Review: E.T.S.; Manuscript Writing: E.T.S.; Critical Review: E.T.S.; Other: -

CONFLICT OF INTEREST

The authors state that there are no actual, potential, or perceived conflicts of interest for this paper.

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DERLEME MAKALE / REVIEW ARTICLE

KİMYASAL SİLAHLARA VE BİYOTERÖRE KARŞI TEDAVİDE KULLANILAN UYGULAMALAR

APPLICATIONS USED IN TREATMENT AGAINST CHEMICAL WEAPONS AND BIOTERRORISM

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ÖZ

Amaç: *Bu çalışmada çeşitli kimyasal savaş ajanlarının kimyasal ve fiziksel özellikleri, tıbbi koruma yöntemleriyle ilgili genel bilgiler, güncel analiz metotları ekipmanı, dekontaminasyon teknikleri ve kimyasal ajanlara maruz kalındığında kullanılan farmasötik formülasyonlar tartışılacaktır.*
Sonuç ve Tartışma: *Kitle imha silahları arasında kimyasal savaş ajanları, biyolojik ve nükleer silahlara göre insanlığın yarattığı en acımasız tehlikelerden biridir. Günlük hayatımızda kolayca elde edilme imkânı olan kimyasallar ile bu savaş ajanları çok ucuz bir şekilde üretilebilir ve küçük terörist gruplar tarafından bile az miktarlarda kitlesel zayiatlar yaratabilir. Kimyasal savaş ajanları vücuda çeşitli yollardan girer; semptomlar buna göre değişebilir. Solunduğunda gazlar, buharlar ve aerosoller, burun ve ağız mukozasından akciğerlerin alveollerine kadar solunum yolunun herhangi bir kısmından emilebilir. Göz ayrıca bu ajanları doğrudan emebilir. Sıvı damlacıkları ve katı partiküller ise, deri ve mukoza zarlarının yüzeyinden emilebilir. Deri üzerinde karakteristik bir etkiye sahip toksik bileşikler, cilt üzerinde katı veya sıvı partiküller halinde biriktiklerinde etkilerini gösterebilirler. Bazı uçucu özellikteki maddelerin buharı sağlam cilde nüfuz edebilir ve bunu takiben zehirlenmeye neden olabilir. Yaralar veya sıyrıklar, sağlam deriye göre daha geçirgendir. Kimyasal savaş ajanları, yiyecek ve içecekleri kontamine edebilir ve dolayısıyla gastrointestinal sistem tarafından absorbe edilebilir. Kimyasal savaş ajanları çeşitli transmukozal yollarla nüfuz ederken, yüzeylerde tahriş veya hasarda oluşturabilirler. Ayrıca çevreye sızan zehirli maddeler yer altı suyunu, toprağı ve havayı kirletebilir ve canlı organizmalar üzerinde uzun süreli zararlı etkilere yol açabilir.*
Anahtar Kelimeler: *Biyoterörizm, dekontaminasyon, ilaç tedavisi, kimyasal savaş ajanları*

ABSTRACT

Objective: *In the present study, the chemical and physical properties of various chemical warfare agents, general information about medical protection methods, current analysis methods equipment, decontamination techniques and pharmaceutical formulations used when exposed to chemical agents will be discussed.*
Result and Discussion: *Among weapons of mass destruction, chemical warfare agents are one of the most brutal dangers posed to humanity compared to biological and nuclear weapons. These war agents can be produced easily, cheaply and can cause mass casualties in small amounts with chemicals that are easily obtained in our daily lives, even by small terrorist groups. Chemical warfare agents can enter the body through various routes; and symptoms may vary accordingly.*

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When inhaled, gases, vapors and aerosols can be absorbed through any part of the respiratory tract, from the mucosa of the nose and mouth to the alveoli of the lungs. The eye may able to absorb these agents directly. Liquid droplets and solid particles can be absorbed from the surface of the skin and mucous membranes. Toxic compounds that have a characteristic effect on the skin can demonstrate their effects when they accumulate on the skin as solid or liquid particles. The vapors of some volatile substances can penetrate intact skin and subsequently cause poisoning. Wounds or abrasions are more permeable than intact skin. Chemical warfare agents can contaminate food and beverages and absorbed into the gastrointestinal tract. While chemical warfare agents penetrate through various transmucosal routes, they can cause irritation or damage to the surfaces. In addition, toxic substances can pollute groundwater, leaking into the environment by soil and air and cause long-term harmful effects on living organisms.
Keywords: *Bioterrorism, chemical warfare agents, decontamination, drug therapy*

GİRİŞ

Kitle imha silahları arasında, kimyasal savaş ajanları muhtemelen insanlık tarafından geliştirilen en tehlikeli silahlardan birisidir. Kimyasal savaş ajanları, gaz, sıvı, aerosol veya partiküllere adsorbe edilen kimyasallar şeklinde kullanılabilen son derece hızlı etki gösteren toksik sentetik maddelerdir. Kimyasal savaş ajanlarının insanlar üzerinde öldürücü veya zayıflatıcı yönde zararlı etkileri bulunmaktadır [1]. Yüzyıllardan beri savaşlarda kullanılan kimyasal patlayıcılardan farklı olarak, basınç-kaynaklı yıkıcı çevresel etkileri yoktur. Günümüzde on binlerce toksik madde rapor edilmiştir, fakat bunlardan sadece bir kısım kimyasal, farklı etki özelliklerine göre "kimyasal savaş ajanı" kabul edilmektedir. Bu özellikler genelde yüksek toksisite, duyuların felç edilmesi, yayılma ve maruziyet süresi sonrası zehirlenme hızı gibi kimyasal silahlar sözleşmesinde ilgili kimyasallar olarak listelenmiştir [2]. Kimyasal silahlar sözleşmesine göre kimyasal silahlar, "toksik kimyasallar ve bunların başlangıç kimyasalları, bu kimyasalların depoları ve bu tür silahlarla doğrudan bağlantılı kullanılmak üzere özel tasarlanmış herhangi bir ekipman" ifadesi ile tanımlanmaktadır.

Tarih boyunca zehirli kimyasalların kullanımı sıklıkla görülmüştür ve halen 2021 yılında kullanımı hakkında haberler, uluslararası medyada bildirilmektedir. Bitkilerden elde edilen zehirli kimyasalların kullanımı orta çağ ve Rönesans boyunca geniş çapta belgelenmiştir, ancak 19. yüzyılda kimyasal harp ajanlarının toplu üretimi ve savaşta kullanılması endüstriyel kimyanın gelişmesinin bir sonucu olarak karşımıza çıkmaktadır. Özellikle Almanya'da gelişen kimya sanayisinin öncülük ettiği araştırmalar sonucunda modern kimyasal savaş ajanlarının çok miktarda üretimi, depolanması ve savaş alanlarında kullanımını görmekteyiz. Kimyasalların savaş alanında ilk kullanımı ise 22 Nisan 1915'te Belçika'nın Ypres kentinde Almanların klorlu gaz saldırısıyla başlamıştır. Fosgen ve hardal gazı gibi kükürt elementi içeren gazlar ve diğer toksik kimyasalların kullanımı 1. Dünya Savaşı'nda 100.000'den fazla can kaybına neden olmuştur [3]. Binlerce masum sivilin, Naziler tarafından 2. Dünya Savaşı sırasında hidrojen siyanür gazı ile öldürüldüğü rapor edilmiştir. Vietnam Savaşı sırasında ABD tarafından kimyasal silahların kullanımı ve 1980'lerde İran-Irak Savaşı sırasında yaklaşık 5.000 kişinin ölümüne yol açan kimyasal saldırısı gerçekleşmiştir. Kuzey Irak'da gerçekleşen bu saldırı, kimyasal savaş ajanlarının kitle imha silahı olarak kabul edilmelerine örnek gösterilmektedir [4,5]. Tarihte ve yakın geçmişte yaşanan bu tür olaylar, kimyasal silahların üretimlerinin basit oluşu, uygun depolama alanlarının nispeten kolay inşası ve kolay taşınma özelliği ile bunları popüler bir terör ajanı haline getirmektedir. Takip ve tespit edilme zorlukları nedeniyle çok tercih edilen kimyasallardan sarin gazı Japonya'nın Matsumoto şehrinde (1994) ve Tokyo metrosunda (1995) bir Japon tarikatı tarafından kullanılmasıyla 5.500 kişinin yaralanması ve 12 kişinin ölümüne neden olmuştur. Sarin gibi tabun, soman, VX (O-etil S-[2-(diizopropilamino)etil]metilfosfonotiyoat) ve botulinum toksini de terör saldırılarında sıkça kullanılmaktadır [6]. Bu kimyasalların kullanımı herhangi bir uyarı olmaksızın tek bir terörist ülke içinde ve dışında kitlesel ölümlere neden olabilir. Terörizm ve suç faaliyetleri, 11 Eylül 2001'de New York'taki Dünya Ticaret Merkezi'ne tekrarlanan saldırılar ve ardından şarbon içeren mektupların dağıtılmasıyla sonuçlanan olaylardan sonra yepyeni bir nitelik kazanmıştır [7]. 6 Nisan 2004'te haber ajansları, İngiliz polisinin El-Kaide üyelerinin Londra'da kimyasal silah içeren bir bomba hazırlamak ve patlatmak için yaptığı bir komployu engellediğini bildirmiştir [8]. Daha önceleri terör faaliyetleri genel olarak bombalama suikast ve rehine alma gibi daha geleneksel şiddet yöntemleri olarak

karşımıza çıkmaktaydı. Bu örneklere baktığımızda özellikle teröristlerin kimyasal silahları masum sivillere karşı kullanma eğiliminde olabileceğinden korkulmaktadır. Bu nedenlerle, bu kimyasalların üretim kontrolü, dağılımı, tespiti ve maruziyet sonrası acil tıbbi müdahale konularının kapsamlı bir şekilde ele alınması ve devletler tarafından yetkili kamu organları tarafından organizasyonların yapılması gerekmektedir. Virüs ve bakteri gibi biyolojik silahlara karşı aşı gibi savunma sistemleri geliştirilebilmesine rağmen, akut zehirleme özelliğine sahip kimyasallara karşı savunma mekanizmalarının geliştirilmesinde büyük zorluklar bulunmaktadır. Bu tür kimyasal silahların üretimi ve kullanımının temel nedenleri çok çeşitli olabilmekte, özellikle yoğun nüfusa sahip metropol şehirlerde kullanıldığında çok etkili olduğu görmektedir [9]. Ayrıca çok düşük dozları dahi siviller arasında hızlı paniğe ve kargaşaya neden olmaktadır. Kimyasal savaş ajanları arasında klor, fosgen ve siyanürler yaygın olarak kullanılmaktadır. Bu tür ajanlar Tokyo metro saldırısında olduğu gibi sarin içeren basit bir plastik torbada bile taşınabilir [10]. Bu tür ajanlar ulusal veya uluslararası düzeyde su şişeleri, yiyecek kutuları, hatta kalem şeklinde kolayca taşınabilmektedir. Kimyasal savaş ajanlarının etkisi, ilgili bileşiğin toksisitesi, uçuculuğu ve konsantrasyonu, maruz kalma yolu, maruziyet süresi ve çevresel koşullar dahil olmak üzere farklı faktörlere bağlı olarak büyük ölçüde değişmektedir. Bu tür ajanların kapalı ortamlarda kullanımı tercih edilmekte olup, bu sayede çok sayıda insanı yaralayacak veya öldürecek kadar yüksek dozlar kullanılabilirken, açık bir alanda kimyasal ajan yayıldıkça etkisi sınırlı olmaktadır.

Günümüzde, tüm dünyada kimyasal terörizm insanlığın güvenliği için ciddi bir tehdittir [11] ve muhtemelen 20. yüzyılda kimyasal terör ajanları yaklaşık 70 farklı kimyasal veya kimyasal karışımı olarak kullanılmış veya depolanmıştır [12]. Teknolojideki son gelişmeler, hammaddelere kolay erişim, internette imalata ilişkin teknik bilgilerin hazır bulunması, artan suç oranları, yolsuzluk ve devlet-destekli terörizm ve küreselleşme gibi faktörler göz önüne alındığında, teröristlerin hedeflerine ulaşmak için kimyasal silah ajanlarını kullanmaları son derece kolaydır [13]. Bu bölümde, mevcut koruyucu ekipmanın durumu, tespit ve dekontaminasyon yöntemlerini içeren bir değerlendirme yapılmıştır.

Kimyasal Ajanların Sınıflandırılması

Kimyasal silah ajanları belirgin farklı kimyasal ve fizikokimyasal özelliklere sahiptir [14,15]. Bu nedenle birçok şekilde sınıflandırılırlar. Uçuculuklarına göre bulundukları ortamda "kalıcı veya kalıcı olmayan ajanlar" olarak sınıflandırılırlar. Bir kimyasal ajan ne kadar uçucu olursa, o kadar çabuk buharlaşır ve dağılma özelliğine sahiptir. Klor, fosgen ve hidrojen siyanür gibi daha uçucu maddeler kalıcı olmayan maddelerken, hardal ve VX gibi daha az uçucu maddeler ise kalıcı maddeler olarak sınıflandırılmaktadır. Kimyasal silah ajanları ayrıca kimyasal yapılarına göre organofosfor, organosülfür ve organoflor bileşikleri ve arsenik olarak da sınıflandırılabilirler. Diğer yandan, kimyasal silah ajanları ile insanlar üzerinde görülen fizyolojik etkiler açısından sınıflandırma da kullanılmaktadır. Bu nedenle savaşlarda kullanılan kimyasal silah ajanları şu şekilde sınıflandırılır: Sinir ajanları, deri irritasyonuna neden olan kimyasal ajanlar, kan zehirlenmesine neden olan kimyasal ajanlar, boğucu ajanlar, göz yaşartıcı gazlar, psikomimetik ajanlar, fentaniller ve diğer potent opioitler, toksinler.

Sinir Ajanları

Sinir sisteminin işleyişini etkiledikleri için bu şekilde isimlendirilen sinir ajanları, doğal olarak bulunmayan organofosfor bileşik grubudur. Bilinen ilk sinir ajanı tabun, ilk olarak 1930'larda Alman kimyager Gerhard Schrader tarafından yeni organofosfor tipi insektisitlerin geliştirilmesine yönelik araştırmaları sırasında geliştirilmiştir. Sonrasında, sarin ve soman gibi ismi "G" ajanı olarak isimlendirilen bir dizi sinir ajanı geliştirilmiştir. Almanya, 2. Dünya Savaşı sırasında sinir gazı stoklarına sahip olmakla beraber bunları kullanmamıştır [15]. 1960 yılına kadar askeri amaçla çeşitli sinir ajanları geliştirilmiş ve etkileri ile çevresel kalıcılıklarını artırmaya çalışılmıştır. Böylece "G" ajanlarının daha kararlı versiyonları olan V-ajanları geliştirilmiştir. Bu nedenle, kükürt içeren bir organofosfor olan VX, sarinden daha etkili, stabil, az uçucu ve suda daha az çözünür özelliktedir. VX doğrudan cilt teması yoluyla vücuda girmekte ve serbest bırakıldıktan sonra birkaç haftaya kadar çevrede kalabilmektedir. Sinir ajanları, diğer rapor edilen kimyasal ajanlarından daha toksiktir ve konsantrasyona bağlı olarak maruz kaldıktan sonra birkaç dakika ila birkaç saat içinde ölüme neden olabilir. Genel kanının aksine, aslında 2. Dünya Savaşı sırasında sinir ajanları kullanılmamıştır. Sinir ajanlarının savaş alanı olarak

bilinen tek kullanımı 1980-1988 Irak-İran savaşı esnasında olmuştur. Irak'ın İran ordu birliklerine ve daha sonra Kuzey Irak'taki Kürt nüfusa karşı sinir ajanları kullandığı rapor edilmiştir [16]. Tüm sinir kimyasal ajanları saf haldeyken renksiz sıvı formundadır. G ajanları meyvemsi bir kokuya sahipken, V ajanları amin kokusu vermektedir. Sarin suda çok iyi çözünür, soman ise suda az çözünür. Tabun ve VX'in çözünürlüğü ise bu ikisi arasında bulunmaktadır. G ajanlarının reaksiyonları, P-X bağı parçalanması yoluyla gerçekleşir. Bu nedenle, G-ajanlarının alkali ile reaksiyonu sonucu toksik olmayan fosfonik asit türevleri açığa çıkmaktadır. Öte yandan, VX'in hidroliz ürünü ise aşırı derecede toksik kimyasal üretmektedir. Sinir ajanlarının etki şekli bilimsel literatürde kapsamlı bir şekilde rapor edilmiştir [17]. Sinir ajanları, asetilkolinesteraz (AChE) adlı enzimi geri dönüşümsüz şekilde inhibe ederek biyolojik etkilerini göstermektedir. Bu enzim, serbest bırakılan bir nörotransmitter olan asetilkolinin (ACh) hidroliz edilmesinden sorumludur. Normal bir bireyde, az miktarda ACh, AChE tarafından sürekli olarak serbest bırakılır ve hidroliz edilir. AChE'ın inhibisyonu, aşırı uyarılma veya felce yol açan ACh birikmesine neden olmaktadır. Buna bağlı olarak, sinir ajanları sisteme girer girmez akut zehirlenme belirtileri ortaya çıkmaktadır. Sinir ajanlarının toksik etkileri, merkezi sinir sisteminde (MSS) bulunan muskarinik ve nikotinik reseptörler üzerindeki etkilerinin bir sonucudur. Bunlar arasında göz bebeğinin küçülmesi, tükürük üretiminin artması, burun akıntısı, terleme, idrara çıkma, bronkosekresyon, bronkokonstriksiyon, kalp hızı ve kan basıncının azalması, kas seğirmeleri ve kramplar, kardiyak aritmiler ile titreme bulunur. En kritik etkiler solunum kaslarının felci ve solunum merkezinin engellenmesidir. Sonuçta, solunum felci nedeniyle ölümle sonuçlanır. Sinir ajanının konsantrasyonu yüksekse, ölüm çok kısa sürede hatta saniyeler içerisinde olmaktadır.

Tedavi Yöntemi

Sinir gazı zehirlenmesinin tedavisi esnasında sağlık personelinin sürekli dikkatli olması gerekmektedir [18]. Sinir ajanı maruziyetini tedavi etmek için atropin, pralidoksim klorür ve diazepam olmak üzere üç ilaç etkin maddesi kullanılır. Atropin, muskarinik ACh reseptörleri için ACh ile rekabet eder ve böylece sinir ajanı zehirlenmesi sırasında fazla ACh birikiminin önüne geçilmesine yardımcı olur. Atropin tüm sinir ajanlarına karşı aktif bir bileşiktir. Bu nedenle, atropine 2 mg'lık bir başlangıç dozu ile hemen intramüsküler veya intravenöz olarak uygulanmalı ve atropin belirli aralıklarla tekrarlanmalıdır. Atropin uygulamasına burun ve ağız mukozasının kuruluğu ve kalp atış hızındaki artış ile gösterildiği gibi yeterli olana kadar devam edilmelidir. Atropin, çoğu muskarinik etkiyi kendi başına etkili bir şekilde iyileştirir, ancak kas seğirmesi, gevşeklik gibi nikotinik etkiler üzerinde çok az etkiye sahiptir. Oksimler, nikotinik etkilere karşı koymada yardımcı olarak kullanılır. Atropinin etkilerini güçlendirirler. Kas fasikülasyonu ve ardından depolarizasyon felci dahil sinir ajanlarının nikotinik etkileri için 2-piridin aldoksim klorür (2-PAM veya pralidoksim) kullanılabilir [19]. Bazen, 2-PAM AChE'ın yenilenmesine yardımcı olarak kas repolarizasyonunu geri yükler. Pralidoksim dışında, toksogonin olarak da bilinen obidoksim de kullanılabilir. Ancak bu oksimler soman zehirlenmesinde etkili değildir. Bunun için H-serisi oksimler (HI-6) tercih edilir. Oksimler, atropin ile kombinasyon halinde uygulanmalıdır. Yavaş intravenöz enjeksiyonla pralidoksim klorür dozu 15-25 mg/kg'dır. Normal toksogonin dozu 300 mg'dır. Bu oksimler hızla atıldığından, daha yüksek doz gerekebilir. Benzodiazepinler, merkezi sinir sistemi tahrişini iyileştirmede kullanılır. Sinir gazı zehirlenmesinin neden olduğu kasılmalar beyin hasarına neden olabilir. Diazepam konvülsiyonları azaltmak için yardımcı olarak kullanılır. Diazepamın normal dozu intramüsküler yoldan 5-10 mg'dır. Panzehirlerin sahada çok hızlı bir şekilde ilk yardım şeklinde uygulanması önemlidir. İlacı enjekte etmek için sağlık personelinin yardımına ihtiyaç bırakmayan otomatik enjektörler kullanılır. Atropin ve PAM klorür oto enjektörleri akut kullanım için mevcuttur. Sinir ajanları zehirlenmesine karşı kabul edilmiş profilaktik antidotlar, yani ajana maruz kalmadan önce uygulanan ilaçlar mevcut değildir. Ancak profilaktik bir ilaç olarak piyasaya sürülen parasempatomimetik etkili piridostigmin bromürün dozu günde üç kez 30 mg'dır. Sinir gazı zehirlenmesine karşı bir miktar koruma sağlasa da bulantı, diyare, bağırsak spazmı, bronşiyal sekresyonda artış, siyalore, göz yaşarması, bradikardi ve miyozis gibi yan etkileri vardır. Aslında, atropinin kümülatif dozu 24 saat içinde hasta başına 100 mg'dan fazla olabilir.

Lokal atropin göz damlalarının (%0.25 ila %1) oküler semptomları ve göz ağrısını hafiflettiği düşünülebilir. Nitekim yüksek uçuculuğa sahip G ajanları, göz ağrısına ve görme bozukluklarına neden olabilir. Tokyo sarin saldırısında, sadece görme bozukluklarından şikayetçi olan hastalar, atropin içeren

göz damlaları ile tedavi edilmiştir. Sinir ajanı intoksikasyonu durumunda oksim seçimi çok önemlidir. En olası aday ilaç oksimin dimetansülfonat tuzu olan HI-6'dır. Fakat HI-6 sulu çözelti içinde kararlı değildir ve iki bölmeli ıslak-kuru enjektör cihazı ile taşınması gerekmektedir. Ne yazık ki HI-6 bazı organofosfatlı pestisitlere karşı etkili bir oksim değildir. Bu nedenle, genel sağlık bakımında obidoksim gibi oksimlere ihtiyaç duyulmaktadır. Avrupa'da, çeşitli pralidoksim tuzlarının yanı sıra obidoksim dahil olmak üzere birkaç oksim şu anda mevcuttur. Oksimlerin etkinliği, organofosfatın kimyasal yapısına, fosforile enzime bağlı olarak tedavideki gecikmeye, etkinliklerini değerlendirmek için kullanılan yöntemlere bağlıdır.

Orta ve şiddetli yaralılarda önerilen dozda oksim uygulaması düşünülmelidir. Tedavi süresi, organofosfat tipine bağlıdır. Sinir ajanlarında, yaklaşık 24 saat tedavi gereklidir ve tedavi duruma göre uzatılabilir. Organofosfatlar için genellikle birkaç günlük tedaviye ihtiyaç vardır.

Deri İrritasyonuna Neden Olan Kimyasal Ajanlar

Deri irritasyonuna neden olan ajanlar "yakıcı ajanlar" (vezikanlar) olarak isimlendirilir. Vezikanlar yanıkların neden olduğu yaralanmalara benzer cilt yaralarına neden olan toksik bileşiklerdir. Bu ajanlar inhale edildiklerinde, akciğerlerin yanı sıra üst solunum yollarını da etkiler ve pulmoner ödem oluşturabilir, ayrıca ciddi göz yaralanmalarına da neden olmaktadırlar. Hardal ve arsenik olmak üzere iki tip vezikan bulunmaktadır. Bu gruptaki en önemli kimyasal ajan hardal gazıdır ve kimyasal ajanların en yaygın bilinenidir. Hardal veya sarımsağa benzer karakteristik bir kokuya sahip olması nedeniyle "hardal" adı verilen kimyasal ajan, renksiz ve kokusuz bir sıvıdır ve uçuculuğu düşüktür. Organik çözücülerde kolaylıkla, ancak suda çok az çözünür (0.8 g/l). Azot içeren hardallar ise saf halde renksiz sıvılardır. Daha az uçucu, daha az çözünürlüğe sahip, ancak depolama esnasındaki stabiliteleri daha düşüktür. Organoarsenik yapısındaki vezikanlar ise metalik kokulu ve renksiz sıvılar olup, sudaki çözünürlükleri kükürt içeren hardal kimyasalına benzerdir. Fakat kararsız özellikleri nedeniyle bu kimyasal ajanların depolanması zordur. Kükürt içeren hardal kimyasalları, 1. Dünya Savaşında kullanılmasından bu yana askerî açıdan önemli kimyasallardır. Azot içeren hardallar 1930'larda sentezlenmesine rağmen, büyük miktarlarda üretilmemiştir. Azot içeren hardal, bir kanser kemoterapötik ajanı olarak uygulama alanı bulmuş ve uzun yıllar bu amaca yönelik referans bileşik olarak kalmıştır. Organoarsenik kimyasalları, yanıcı olmamaları ve hardala benzer toksisitesi nedeniyle 1918'de askerî amaçla sentezlenmiştir, ancak herhangi bir savaşta henüz kullanılmamıştır. Hardal kimyasalları hücre bölünmesi için son derece toksiktir. Lipofilik özelliğe sahip olan hardallar cilde, hatta çoğu tekstil ürünlerine kolayca nüfuz eder. Hücre membranından geçtikten sonra, kükürt içeren hardal yüksek reaktiviteye sahip sülfonyum iyonuna dönüşür. Geri dönüşsüz bir şekilde DNA, RNA ve proteini alkile ederek hücre ölümüne neden olur; en önemli hedef ise DNA'dır. Hardal, DNA'nın pürin bazlarını alkilleştirir [20]. Organoarsenikler, cilt tarafından 10 kat daha hızlı emilir ve etkilenen organda ani ağrı ve tahrişe neden olur. Ayrıca daha sistematik semptomlara neden olmaktadır. Hardal maruziyetinin klinik olarak ayırt edici özelliği, maruziyetin ardından belirgin semptomların görülmemesidir [21]. Bu gizli dönemin uzunluğu ve semptom gelişiminin hızı ve yoğunluğu, maruziyet şekline, kimyasal ajanın konsantrasyonuna ve çevresel koşullara bağlıdır. Gaz veya aerosol formundaki hardal cilde, gözlere ve solunum sistemine temas eder. Kimyasal hasar temastan 1-2 dakika sonra başlar, ancak ağrı 4-6 saat sonra başlamaktadır. Güneş yanığına benzer şekilde cilt hasarı meydana gelir. Sıvı formuna maruz kalma ile hardal dermiste pıhtılaşmaya neden olabilir [22]. Deride ilk belirti genellikle kaşıntıdır. İlk olarak ince, ılık ve nemli cilde sahip bölgelerde kızarıklık, maruziyetten 4-12 saat sonra ortaya çıkar. Lezyonlar ilerleyen saatlerde artar ve 48-72 saat sonra maksimuma ulaşacak şekilde sarı içerikli ince duvarlı ağrılı veziküllerde birlikte akan kabarcıkların oluşmasına neden olur. Veziküller, yoğun maruziyetten sonra nekrotik yaraların etrafında oluşabilir. Tipik tam gelişmiş klinik tablo, birinci ve ikinci derece yanıklara benzeyecektir. Sıvı hardalla temastan sonra yanıklar meydana gelebilir. Şiddetli ve büyük lezyonlar, ikincil enfeksiyonlara karşı özellikle hassasiyeti olan yanık hastalarına benzer komplikasyonlara yol açabilir. Şiddetli vakalar, özel yanık birimlerinde tedavi edilmeli ve potansiyel olarak bağışıklığı baskılanmış hastalar olarak düşünülmelidir. İyileşme normalde 4-6 hafta sürer ve bunu etkilenen bölgelerde pigmentasyon değişiklikleri ve nöropatik semptomlar izleyebilir. Göz hasarı, konjunktivitten korneal opasifikasyona, akabinde ülserasyona ve yırtılmaya kadar değişir. Semptomlar, fotofobi, lakrimasyon ve ağrılı konjunktivit, kornea lezyonları ve nadiren irit gibi klinik belirtilerin

ortaya çıkmasına kadar 1 ile 6 saat sürebilir. Solunum semptomları 4 ile 24 saatlik bir gecikmeden sonra ortaya çıkar ve öksürük, kanlı balgam çıkarma, ciddi vakalarda pulmoner ödem gibi klinik belirtilerle birlikte bronşite yol açar. Hardal gazının solunum yolu üzerindeki etkisi aynı zamanda maruziyet derecesine de bağlıdır. Maruziyet hafifse burunda, gırtlakta ve soluk borusunda şişlik ve kızarıklık gözlenmektedir. Akciğerlerde oluşabilecek bakteriyel enfeksiyon riski nedeniyle bronkopnömoniye neden olabilir. Sıvı hardalla kontamine olmuş besinler veya suyun yutulması mide bulantısı, kusma, ağrı ve ishale neden olur. Tek başına cilde teması bile halsizlik, kusma ve kalpte anormalliklere yol açabilir. Hardala aşırı derecede maruz kalmak kemik iliği hasarına neden olarak lökopeniye yol açabilir. Sonuçta, hardala maruz kaldıktan sonra ölüm riski çok yüksektir. Arsenikler, hardallara kıyasla daha hızlı klinik etkiler üretir. Maruziyetten hemen sonra (sıvı teması veya buharın solunması), ciltte ve gözlerde ağrı ile birlikte göz tahrişi, öksürme, hapşırma, gözyaşı ve tükürük salgısı meydana gelir. Etkiler 4-8 saat sonra maksimuma ulaşır. Gözlerin maruz kalması, gözün ön kısmında nekroza ve körlüğe yol açabilir. Şiddetli maruz kalma, akciğer ödemine ve solunum yetmezliğine neden olabilir. Sistemik emilim durumunda karaciğer ve böbrek hasarı, ensefalopati ve nöropati, hemolitik anemi, rabdomiyoliz veya miyokardiyal hasar gibi toksik etkiler görülebilir. Kılcal sızıntıya bağlı hemodinamik şok ve akut böbrek yetmezliği meydana gelebilir.

Tedavi Yöntemi

İlk yardım önlemleri acil olarak alınmalıdır ve hiçbir belirti oluşmasa bile uygulanmalıdır. Daha sonra dekontaminasyon uygulanması ile lezyonların şiddetini azaltabilir ve hardalın yayılmasını önlenebilir. Genel olarak, kontaminasyon kaynağından uzaklaştırma, kontamine giysilerin, saatlerin vb. çıkarılması, su ve sabunla yıkayarak dekontaminasyon yapılır. Bu önlemler, gözlerde sıvı hardal kontaminasyonunun ilk 5 dakikasında uygulandığında sonuç vermektedir. I. Dünya Savaşı sırasında hardal gazı yaralanmaları sonrası tedavi yönetiminde önemli deneyim elde edilmiştir. Spesifik bir antidot hali hazırda bulunmamaktadır ve hardalın proteine hızlı bağlanması, hardalın vücuttan antidot kullanılarak çıkarılmasını imkânsız hale getirir. Hardala bağlanması amaçlanan kükürt (veya -SH) grupları sağlayan ilaçlarla yapılan çalışmalar, bu tür bileşiklerin maruziyetten hemen sonra verilmesi gerektiğini göstermiştir. Spesifik bir panzehirin bulunmadığı durumlarda, birçok maddenin kullanılması tavsiye edilmiştir. Hardal toksisitesi için spesifik bir antidot mevcut olmamakla birlikte, tedavisi yanık yaralanmalarına benzerdir [23]. Gözler temiz su ile yıkanmalıdır. Göz kapakları yapışkan ise steril vazelin sürülebilir. Blefarospazm, bir damla atropin solüsyonunun (%1) 3-4 kez uygulanmasıyla giderilebilir ve siprofloksasin damlatılarak tedavi edilebilir. Deri bölgesi, Fuller's Earth kullanılarak hemen dekontamine edilmelidir. Hardal kabarcıklarının üzerine povidon-iyot merhem veya framisetin merhem sürülmelidir. Kaşıntı ve ağrıyı gidermek için sistemik analjezikler ve antihistaminikler kullanılabilir. Soluma yoluyla maruziyete bağlı görülen farenjit, alkali gargara alınarak hafifletilebilir. Kalıcı öksürük için kodein alınabilir. Hardalın aksine, arsenik içeren kimyasalların zehirlenmesine karşı özel bir tedavi mevcuttur. Yaygın olarak İngiliz Anti Lewisit (BAL) olarak bilinen dimerkaprol, arsenikler için özel bir birleştirme kimyasalıdır [17]. Lewisit, BAL ile geri dönüşümlü etkili bir anaerobik glikoliz inhibitörüdür. Göz veya cilt kontamine olmuşsa hemen BAL merhem sürülmelidir. Çok yoğun kontaminasyon durumunda BAL sistematik olarak uygulanmalıdır. BAL (%10'luk çözelti) 5 mg/kg dozda derin intramüsküler enjeksiyonla verilmeli; 4, 8 ve 12 saat sonra tekrarlanmalıdır. BAL'ın toksisitesi ve yan etkileri nedeniyle, mezo-dimerkaptosüksinik asit (DMSA), 2,3-dimerkapto-1-propansülfonik asit (DMPS) ve 2,3-ditiyoeritritol gibi daha az toksik ve daha spesifik yeni arsenik antidotlar üzerinde çalışılmaktadır [24].

Kan Zehirlenmesine Neden Olan Kimyasal Ajanlar

Kan zehirlenmesine neden olan kimyasallar vücut dokuları tarafından oksijenin normal kullanımını engelleyerek vücut fonksiyonlarını tahrip eden siyanür kimyasallar grubudur [25]. Bu tip kimyasal ajanlar için zaman zaman kullanılan "kan ajanı" yanlış bir terimdir. Zira bu ajanlar aslında kanı doğrudan etkilemezler, ancak kan bileşenlerinin üretilmesini kesintiye uğratabilirler. Aksine toksik etkilerini hücresel düzeyde mitokondrinin iç zarlarındaki elektron taşıma zincirini kesintiye uğratarak gösterirler. Bu ajanlar, belirli spesifik enzimleri inhibe ettikleri için sistemik ajanlar olarak da bilinir. Hidrojen siyanür (HCN) ve siyanojen klorür (CNCl), bu sınıftaki ana kimyasal ajanlardır. İlk defa İsveçli

bir kimyager tarafından 1872'de keşfedilen hidrojen siyanür, 1. Dünya Savaşı sırasında bir kimyasal savaş ajanı potansiyeli göstermesinden çok önce, endüstriyel bir kimyasal olarak kullanılmıştır. Fransızlar tarafından bu amaçla 1916'da Somme savaşında bu malzemeden yapılmış mermiler kullanılmıştır. Siyanojen klorür, 1. Dünya Savaşı sırasında endüstriyel ara ürün olarak uygulamaları olan ve bol miktarda bulunan ticari bir ürün olarak bilinmektedir. Siyanojen klorür ayrıca güçlü göz yaşartıcı ve boğucu etkilere sahip olduğundan, bir gaz maskesinin filtre elemanlarına diğer herhangi bir ajandan daha kolay nüfuz etmektedir. Dünya çapındaki endüstriyel gelişmeler, siyanür söz konusu olduğunda büyük bir tehdit haline gelmiştir. Siyanür, Irak tarafından 1983-88 savaşı sırasında İran ile olan çatışmalarda kullanılmıştır. Kan kimyasallarının uçuculuğunun çok yüksek olması, kimyasal savaş ajanı olarak kullanımını kısıtlamakla beraber, kapalı alanlara uygulandıklarında etkinliklerinin arttığı bilinmektedir. Siyanür, ferrik (Fe^{+3}) formdaki demire çok yüksek bir afiniteye sahiptir. Biyolojik sisteme girdiğinde, hücresel solunumun son zincir enzimi olan sitokrom oksidazın üç değerlikli demiriyle kolayca reaksiyona girerek dokulardaki oksijen kullanımını bozar. Sonunda, solunum yetmezliğinin bir sonucu olarak ölüm meydana gelir [26]. Semptomların başlangıcı ve şiddeti, solunan toksik buhar konsantrasyonuna ve maruziyet süresine bağlıdır. Düşük dozlarda hidrojen siyanüre maruz kalmanın semptomları zayıflık, baş dönmesi, baş ağrısı, kafa karışıklığı ve bazen mide bulantısı ve kusmadır. Klinik belirtiler yalnızca hızlı ve ağrılı solunum, hareket koordinasyon eksikliği, kardiyak düzensizlikler, hipoksik konvülsiyonlar, koma ve ölümle sonuçlanan solunum yetmezliğini içeren yüksek düzeyde maruziyette ortaya çıkar. Teşhis, karakteristik siyanür kokusu (acı badem) veya soluk kırmızı bir cilt tonu ile yapılabilir. Semptomlar inhalasyondan sonra saniyeler ila dakikalar içinde ortaya çıkar ve ani ölüm meydana gelebilir. Bu ciddi vakalarda, solunum merkezinin doğrudan uyarılması ve metabolik asidoz yoluyla hiperventilasyonun klinik belirtileri başlar. Bunu konvülsiyonlarla bilinç kaybı, kalp veya solunum durması izler. Diğer klinik belirtiler arasında baş dönmesi, halsizlik, çarpıntı ve anksiyete, ardından pulmoner ödem ve felç yer alır. Laktat plazma konsantrasyonu değeri = 10 mmol/l olması, siyanür zehirlenmesinin hassas ancak özgül olmayan bir belirtecidir.

Tedavi Yöntemi

Hasta kontamine ortamdan uzaklaştırılmalıdır. Genel olarak, gazın dağılması çok hızlı olduğundan dekontaminasyon gerekli değildir. Deriye maruz kalma durumunda, giysilerin dikkatlice çıkarılması ve cildin su ve sabunla iyice yıkanması gerekir. Ağızdan zehirlenmede, hasta stabilize edilmişse ve acil önlemlerin alınabileceği nadir durumlarda, gastrointestinal dekontaminasyonun birincil yöntemi olarak aktif kömür verilmesi önerilir. Sağlık personeli için gaz maskeleri ve koruyucu giysiler gereklidir. Tedavinin amacı siyanür iyonunu sitokrom oksidaz-siyanür kompleksinden ayırmaktır. Bu, amil nitrit, sodyum nitrit ve 4-dimetilaminofenol (DMAP) gibi, hemoglobini methemoglobine oksitleyen ve daha sonra siyanür iyonunu siyanmethemoglobin oluşturmak için ayıran bağlayıcılarla gerçekleştirilebilir. Semptomatik tedavi olarak %100 oksijenasyon dahil destekleyici bakım ve gerekirse mekanik ventilasyon yapılabilir. Tıbbi tedavi olarak birkaç panzehir mevcuttur. Farklı panzehirlerin eşzamanlı veya art arda uygulanması tavsiye edilmiştir. Amil nitrit genellikle inhalasyon yoluyla verilirken, sodyum nitrit (%3, 10 ml) intravenöz (i.v.) olarak verilir. Karaciğerde bulunan rodanaz enzimi için bir kükürt donörü olan sodyum tiyosülfat (%25'lik çözelti, 50 ml, i.v.), tiyosiyanat olarak siyanürün eliminasyonunu artırır. Hidroksikobalamin (vitamin B12a, 20 mg, i.v.) ve kelosiyanor (kobalt-EDTA) gibi diğer şelatlayıcı bileşikler de etkili panzehirlerdir. Dikobalt edetat, siyanüre karşı etkili bir kompleksleşme panzehiridir ancak siyanür zehirlenmesi tanısının kesin olduğu durumlarda kullanılmalıdır. Çünkü siyanür olmadığında ciddi kardiyovasküler yan etkilere sahip olduğu için yalnızca ciddi zehirlenmelerde kullanılması gerekmektedir. Hidroksokobalamin en iyi tedavi seçeneğidir. Ardışık tedavide tiyosülfat diğer siyanür antidotları ile birlikte düşünülmeli ancak hidroksokobalamin ile aynı zamanda bir karışım olarak uygulanmamalıdır. Hidroksokobalamin veya dikobalt edetatın bulunmaması durumunda, siyanürün methemoglobine dolaylı olarak kompleksleşmesi yoluyla etki gösteren alternatif antidotlar, değerlendirilebilir. Methemoglobin oluşturan antidotlar arasında sodyum nitrit, amil nitrit ve 4 dimetilaminofenol bulunur. Son çalışmalar, disodyum 2-ketoglutaratın, tek başına veya sodyum tiyosülfat ile kombinasyon halinde, deney hayvanlarında ölümcül siyanür zehirlenmesini antagonize etmede çok etkili olduğunu göstermiştir. Hiperbarik oksijen ayrıca bilinen panzehirlerin etkinliğini de güçlendirmektedir. Diğer destekleyici tedaviler arasında

diazepam (3 mg × 10 mg, i.v.), sodyum bikarbonat ve metilen mavisi (%1, 30 ml, i.v.) bulunur. Siyanür zehirlenmesinin tedavisinin etkili olabilmesi için hızlı olunması gerekir [25].

Boğucu Ajanlar

Boğucu ajanlar, maruz olan kişiyi esas olarak solunum yolunda, yani burun, boğaz ve özellikle akciğerlerde etkilemektedir. Yüksek maruziyet durumunda, akciğerler sıvıyla dolmakta ve oksijen eksikliğine bağlı ölüm meydana gelmesi nedeniyle, bu ajanlara "boğucu ajanlar" denmektedir. Bu tür ölümler "kuru boğulmalar" olarak da adlandırılır [14]. Solunum irritanları, tümü solunum yollarında enflamatuar bir tepkiye ve doku tahribatına neden olma potansiyeline sahip asitleri, bazları ve oksitleyici ve alkilleyici özelliklere sahip maddeleri içerir. Etkileri oksidasyon, serbest radikallerin üretimi, bunların kombinasyonu ve yaralanmaya karşı fizyolojik savunma mekanizmaları dahil diğer mekanizmalar yoluyla olmaktadır. Etkinin yeri büyük ölçüde maddenin suda çözünürlüğüne bağlıdır. Asitler, SO_2 ve amonyak gibi suda yüksek oranda çözünür maddeler, etkisini esas olarak üst solunum yollarında gösterir. Fosgen, ozon ve nitrojen oksitleri gibi yağda çözünen maddeler, alt solunum yolları ve alveoller üzerinde birincil etkiye sahiptir. Bununla birlikte, yüksek konsantrasyonlarda, tahriş edici maddeler solunum sisteminde yaygın hasara neden olur. Aerosoller için, akciğerde birikme partiküllerin "aerodinamik çapına" bağlıdır. Suda çözünür maddeler, bir aerosol içinde solunabilir parçacıklara veya damlacıklara adsorbe edilir. Suda çözünürlüğü yüksek olan gazlar, üst solunum yollarında, gözlerde ve hatta deriden anında semptomlara neden olur. Daha yüksek maruziyetle, laringeal ödem, mukoza zarında hasar, bronkospazm ve hatta Akut Solunum Sıkıntısı Sendromu gibi daha şiddetli belirtiler ve etkiler ortaya çıkabilir. Daha az suda çözünür gazlar, özellikle fosgen ve nitrojen oksitleri, başlangıç semptomları orta derecede olsa bile akciğerlerde hasara neden olabilir. En şiddetli vakalarda akut akciğer hasarı veya çoğu şiddetli vakada akut solunum sıkıntısı sendromu görülür. Etkiler 24 veya 48 saate kadar gecikebilir ancak yoğun maruziyetten birkaç saat sonra da ortaya çıkabilir. Uzun vadeli etkiler, reaktif hava yolu disfonksiyon sendromu, fibroz ve bronşektaziyi içerebilir. Klor ve fosgen bu sınıf arasında en iyi bilinen kimyasallar olmasına rağmen, difosgenler, nitrik oksit ve perfloroizobutilen (PFIB) de bu gruba aittir. Boğucu ajanlar, büyük miktarlarda üretilen ilk kimyasal harp maddeleri arasında olmuştur ve 1. Dünya Savaşı sırasında yaygın olarak kullanılmıştır. Genellikle havadan ağır olan hem klor hem de fosgenin gibi birçok kimyasalın endüstriyel işlemlerde de kullanılması bu bileşiklerin kontrolünü zorlaştırmakta ve teröristlerin elinde düşük teknolojili yıkıcı bir silah olarak kullanılmasına vesile olmaktadır. Klor oda sıcaklığında keskin kokulu, yeşil-sarı bir gazdır ve orta basınç altında sıvılaştırılabilir. Fosgen gazının toksik seviyelerde bile ayırt edici kokusu çok azdır ve kurbanlarını genellikle 24 saat sonra öldürür. Fosgen, 1. Dünya Savaşı'nda kimyasalların neden olduğu ölümlerin yaklaşık %80'inden tek başına sorumlu olmuştur [14]. Oda sıcaklığında sıvı olan difosgen, 1. Dünya Savaşı'nda kullanılan bir diğer ajandır. Genellikle fosgenden daha kolay kullanılır ve klor veya fosgene göre maruziyeti daha fazladır. PFIB, yüksek ısıda ve politetrafloroetilen (teflon) üretimi sırasında bir yan ürün olarak üretilen endüstriyel bir gazdır. Fosgen gibi, PFIB'e de maruziyet ve semptomları arasında bir gecikme süresi vardır. Fosgenden yaklaşık 10 kat daha yüksek toksisitesi ve koruyucu filtreleri kolayca geçmesi nedeniyle PFIB, kimyasal savaş ajanı olarak listelenmiştir. Fosgen zehirlenmesinin etki mekanizmasına ilişkin muhtelif görüşler vardır. Fosgen oldukça reaktif olduğundan, enzimler dahil biyolojik makromoleküllerin -SH, $-NH_2$ ve -OH grupları ile birleşmesi toksik etkilerini açıklayabilir. Fosgen zehirlenmesi, esas olarak akciğerlerin belirli doku elemanlarına etkisi ve alveolar mukoza zarının geçirgenliğinin artmasına bağlı olarak anoksi ve ölümle sonuçlanan pulmoner ödemle sonuçlanır. Düşük fosgen konsantrasyonlarının solunması hızlı ve sığ solunum, solunum hacminde azalma, bradikardi ve hipotansiyonu tetikler. Artan tükürük salgısı, bulantı, vs gibi semptomlar da gözlenir. Daha yüksek konsantrasyona maruz kalma, akciğerlerin hayati fonksiyonları üzerinde daha spesifik etkiler yaratabilir ve sonunda ölümle sonuçlanan pulmoner ödem gelişebilir. Ani ölüm nedeni genellikle anoksiye bağlı solunum merkezinin felç olmasıdır [27].

Tedavi Yöntemi

Dekontaminasyon için tek seçenek maruziyeti sona erdirmektir. Tedavi temelde klinik belirtilere göre yapılır. Oksijen tedavisi yaşamsal işlevlerin desteklenmesini sağlar. Mağdurlar dinlenmeli ve sıvı alımı kısıtlanmalıdır. Oksidatif stresin patogenezdeki rolü nedeniyle, %100 oksijen ile tedaviye yeterli

oksijenasyonu sağlamak için gerektiği sürece devam edilmelidir. Tıbbi tedavi olarak pulmoner irritanların hiçbiri için spesifik bir antidot yoktur. Fosgen zehirlenmesinin tedavisi esasen palyatiftir. Tedavinin temel amacı, anoksiden kaynaklanan pulmoner ödem ve diğer ikincil etkilerin gelişmesini önlemektir. Tedavi üç aşamada uzatılır. İlk yardım sırasında, mağdura temiz hava verilmeli ve sıcak tutulmalıdır. Tedavi, maruziyetten sonraki 30 dakika içinde temel tedaviyi sağlayacak şekilde aşamalandırılır ve ardından seçilen ek tedavi uygulanır. Acil tıbbi yardım, pozitif basınçlı solunumla desteklenen kortizon (hekzametazon veya beklemetazon) ve sodyum bikarbonat uygulamasının yanı sıra suni solunum içermektedir. Öksürük prognozu kötüleştirir ve kodein ile baskılanabilir. Sakinleştiriciler tavsiye edilmemektedir. Bronşit veya pnömoni geliştiğinde antibiyotik tedavisi önerilir. Ek tedavi olarak, oksijen ve i.v. sodyum bikarbonat enjeksiyonu yapılabilir. Hava yolu tıkanıklığından kurtulma teofilin ve prostaglandin E_1 (PGE_1) ve ardından dipalmitoil fosfatidilkolin veya kolesterol palmitat aerosolleri ile yüzey aktif madde takviyesi ile sağlanabilir. Zehirlenme durumunda 24 saatten fazla yoğun bakım ve gözetim gereklidir [28].

Göz Yaşartıcı Gazlar

"Kargaşa kontrol ajanları" olarak da bilinen, gözleri tahriş ederek, kapanmalarına ve üst solunum yollarının tahriş olmasına neden olarak geçici rahatsızlığa neden olan bileşiklerdir. Genellikle "tahriş edici, göz yaşartıcı ve taciz edici ajanlar", halk arasında ise "göz yaşartıcı gazlar/biber gazı" olarak adlandırılır. Birçok gözyaşı uyarıcı madde, kimyasal silah ajanları olarak test edilmiştir [29]. Maruz kalındığında gözlerde ağrıya, öksürme, hapşırma, gözyaşı akmasına ve ciltte tahrişe neden olurlar. Genellikle bu kalıcı doku hasarına neden olmaz. Daha sonra göğüste sıkışma ve öksürük, nefes darlığı, dil ve ağızda yanma, tükürük ve kusma gelişebilir. Yüksek konsantrasyonlara maruz kalınması durumunda gözde kimyasal yanıklara neden olabilir. Deride yanma hissi, ardından dermatit, gelişebilir. Önceden var olan akciğer hastalığı olan kişilerde nadiren bronkospazm gelişebilir ve bu maruziyetten 48 saate kadar gecikebilir. Maruziyetten 24 saat sonrasına kadar son derece nadir pulmoner ödem vakaları bildirilmiştir. Gözyaşı gazlarının neden olduğu cilt tahrişi ve gözyaşı gibi güçlü duyumlar, maruz kalan kişilerin rasyonel davranamamalarına, koordineli faaliyetlerinin engellenmesine ve güçsüzleşmelerine neden olmaktadır. Üç tip kargaşa kontrol ajanı olarak; "göz yaşarmasına ve göz tahrişine neden olan göz yaşartıcılar", "hapşırmaya ve üst solunum yolunda tahrişe neden olan sternutatörler" (hapşırmaya neden olan gazlar) ve "ek olarak kusmaya neden olan kusma ajanları" bilinmektedir. Bu grupta yer alan kimyasal ajanlar arasında özellikle CN, CS ve CR çok önemlidir [14]. Oda sıcaklığında katı olan CN, CS ve CR aerosoller formunda kullanılır. Suda nispeten çözünmezler, ancak pek çok organik çözücüde çözünürler ve kısa etki süresi özelliği vardır. Konjunktivit, blefarospazm ve gözyaşı ile birlikte hızla ortaya çıkan ağrı nedeniyle göz en hassas organdır [30]. Belirtiler 10-30 saniye içinde hissedilir. Akut ağrı semptomlarının her biri için olası etki modu, nikotinamit adenin dinükleotit hidrojenaz (NADH)-bağımlı enzimatik süreci içeren deri ve mukozadaki duyu reseptörleri üzerinde doğrudan kimyasal bir etkidir. Etki alanlarının çevresel doğası, bu ajanları, psikokimyasallar gibi merkezi sinir sistemini etkileyen diğer kimyasal ajanlardan farmakolojik olarak ayırmaktadır.

Tedavi Yöntemi

Göz yaşartıcı ajanlara maruziyet sonrası tedavi, giysilerin ve gözlerin derhal dekontaminasyonunu gerektirir. Mağdur hemen temiz havaya çıkarılmalı, gözleri ve cildi su ile dekontamine edilmelidir. Deri iltihabı kalamin losyonu ile tedavi edilebilir [31].

Psikomimetik Ajanlar

Otonom sinir sisteminde büyük bir rahatsızlığa veya diğer ciddi sakatlığa neden olmadan sürekli olarak düşünce, algı ve ruh halinde değişiklikleri tetikleyen kimyasal ajanlar "psikomimetik ajanlar" olarak adlandırılır [14]. Bu nedenle, bu ajan grubu genellikle düşük dozlarda (<10 mg) uygulandığında, psikotik bozukluklara benzer durumlara veya merkezi sinir sisteminden kaynaklanan duygu kaybı, felç, halüsinasyonlar vb diğer semptomlara neden olan maddeleri içerir. Öne çıkan periferal antimuskarinik semptomlar, kuru, kızarık cilt ve mukoz membranların kuruması, hipertermi, taşikardi, azalmış bağırsak hareketliliği ve idrar gecikmesidir. Önemli merkezi sinir sistemi semptomları kafa karışıklığı, ajitasyon,

titreme, zayıf koordinasyon, algılama bozuklukları, biliş ve hafıza işlevleri, halüsinasyonlar (genellikle görsel) ve deliryumdur. Solunum depresyonu ile birlikte nöbetler ve koma, şiddetli zehirlenmelerde ortaya çıkabilir. Bu tür ajanların kullanımı antik çağlara kadar uzanır. Örneğin antikolinerjik etkili alkaloitler içeren *Datura stramonium* gibi bitkilerin kullanımını içerir. Psikomimetik ajanlar ilk kez M.Ö. 600'de savaş sırasında kullanılmış olup, Solon'un askerleri düşman birliklerine su sağlayan derelere *Helleborus* bitkisinin köklerini atmış ve ardından ishal başlamasına neden olmuştur. Hannibal'in ordusu M.Ö. 184'te *Atropa belladonna* bitkisini düşmanda yönelim bozukluğuna neden olmak için kullanmıştır. 2. Dünya Savaşı sırasında, ABD ordusu, çavdar mahmuzunda bulunan ergotaminden hareketle elde edilen indol türevi yarı-sentetik bir psikoaktif madde olan lizerjik asit dietilamit (LSD) ve çeşitli glikolat antikolinerjikler de dahil ölümcül olmayan, ancak psiko-davranışlara etkili çeşitli olası kimyasal ajanları araştırmıştır. Antikolinerjik bileşiklerden biri olan 3-kinüklidinil benzilat, 1960'larda bir psikokimyasal olarak savaş alanında kullanılmak üzere yeni bir kimyasal ajan olarak keşfedilmiş ve BZ (3-kinüklidinil benzilat) kodu ile silah haline getirilmiştir [14]. BZ ve analogları, uzun süreli antikolinerjik sendroma neden olan glikolik asit esterleridir. BZ'nin klinik profili, etki süresi ve potens açısından önemli ölçüde farklılık gösterse de atropininkine çok benzer. Bu bileşiğe maruz kalma, büyük olasılıkla bir aerosol olarak veya duman üreten cephanelerde gerçekleşir. Bu nedenle solunum sistemi emilimin birincil yoludur. Teneffüs ettikten sonra, semptomların başlangıcı yaklaşık bir saat içinde görülür ve semptomlar yaklaşık 8-10 saat içinde pik yapar. Cilt maruziyetinden sonra (genellikle propilen glikol içinde çözülür), emilim %5-10'dur ve semptomlar 24-36 saate kadar gecikebilir. Yuttuktan sonra, bu nadir de olsa, emilimin yaklaşık %80 olduğu tahmin edilmektedir. Etkisiz hale getiren bir dozun ardından, iyileşme kademeli olarak yaklaşık 48 saat sonra başlar ve 96 saate kadar sürer. LSD (D-lizerjik asit dietilamit), bir alkaloidinin sentetik bir türevidir ve klasik halüsinojenlere aittir. Aerosol yoluyla uygulandığında semptomların başlangıcı dakikalar içinde gerçekleşir. Yutulduktan sonra semptomlar 30-60 dakika sonra başlar. Tepe etkilerine 2-5 saat içinde ulaşılır. Eylem süresi 8-12 saattir. LSD'nin olası kimyasal bir ajan olarak geliştirilmesi, 1959'dan 1965'e kadar gerçekleşmiştir. LSD'nin yaklaşık 2.5 μg/kg'lık bir oral doz verilmesiyle öngörülemeyen davranışlara yol açan, oldukça güçlü bir kimyasal ajan olduğu kanıtlanmıştır. Etkilenen bireyler genellikle kendilerine verilen talimatı uygulayamaz veya herhangi bir göreve konsantre olamaz. Simüle edilmiş askeri tatbikatlarda yapılan araştırmalar, iyi eğitilmiş birimlerin bile <200 μg oral doz verilmesiyle tamamen düzensiz hale geldiğini göstermiştir. Maruz kalan bireylerin %50'sinde psikomimetik belirtileri görmemizi sağlayan (ID_{50}) aerosol formunda LSD dozunun 6 μg/kg olduğu tahmin edilmektedir [32]. LSD'nin insan algısı üzerinde böylesine derin etkilere neden olduğu mekanizma halen çözülememiştir [33]. LSD orta beyindeki sempatik sinir sistemi merkezini uyararak pupiller genişlemeye, vücut ısısında artışa ve kan şekeri seviyesinde yükselmeye neden olur. LSD ayrıca serotonin bloke edici bir etkiye sahiptir. LSD ayrıca doğal olarak oluşan hormon benzeri başka bir madde olan dopamin ile bağlantılı nörofizyolojik fonksiyonları da etkiler. LSD'nin kimyasal yapısı, indol halkası içeren meskalin ve psilosibin gibi diğer halüsinojenik ilaçlara çok benzediğinden, benzer etkiler de göstermektedir. Psikomimetik ajanların neden olduğu ortak belirti ve semptomlar; huzursuzluk, baş dönmesi, emirlere uyulmaması, kafa karışıklığı, düzensiz davranış, tökezleme, kusma, ağızda kuruluk, taşikardi, yüksek ateş, yüzde kızarıklık, bulanık görme, gözbebeği genişlemesi, geveleyerek veya anlamsız konuşma, halüsinasyon davranışı, soyunma, mırıldanma, uyuşukluk ve anlamsız gülümseme veya kahkaha, irrasyonel korku, dikkat dağınıklığı, kendini ifade etmede zorluk, algısal çarpıklıklar ve fobiler olarak bilinmektedir. Halüsinojenik etkiler genellikle mide bulantısı ve midriyazis, taşikardi, hipertansiyon, hipertermi ve terleme gibi sempatik etkilerden önce gelir. Psikolojik etkiler, uyarılma, duygu, algı (algısal bozulmalar), düşünce süreci ve öz imajdaki değişiklikleri içerir. "Beden dışı deneyimlerle" duyarsızlaşma ve duyusal yanlış algılamalar sıktır. Halüsinasyonlar görsel (en yaygın), işitsel, dokunsal veya koku alma olabilir. Uzun süreli psikotik reaksiyonları içeren panik reaksiyonlar ve psikoz ortaya çıkabilir.

Tedavi Yöntemi

Kontamine hastalar başkalarında ikincil maruziyete neden olabilir. Kontamine giysiler çıkarılmalı ve izole edilmelidir. Su ile duş almak veya su ve sabunla yıkamak yeterlidir. Tedavi semptomatik olabilir. Güvenli çevre ve yakın denetim gereklidir. Bunlar mümkün değilse, etkilenen bireylerin küçük

gruplara ayrılması büyük gruplara tercih edilir. Ajitasyon veya nöbetleri kontrol etmek için i.v. benzodiazepinler kullanılabilir. Hipertermi harici soğutma ile tedavi edilmelidir. Tıbbi tedavi olarak panzehir fizostigminin kullanımı, kısa etki süresi nedeniyle (sadece 20-60 dakika) genellikle tavsiye edilmez. Ayrıca fizostigmin, nöbetleri ve disritmiyi hızlandırabilir. Aktive edilmiş odun kömürü ile gastrointestinal dekontaminasyon, yakın zamanda maruz olmuş hastalar için düşünülebilir, ancak klinik semptomlar ortaya çıktığında yararlı değildir. Semptomatik tedavide minimum uyaranla sessiz bir çevre faydalı olabilir. Benzodiazepinler, ajitasyonu ve hipertermiyi kontrol etmek için kullanılabilir. Şiddetli psikotik reaksiyonlar, antipsikotik ilaç kullanımını gerektirebilir. Bu kimyasalların yutulması veya solunmasından kaynaklanan klinik etkiler, 30 dakikadan 20 saate kadar uzayabilen, asemptomatik veya gizli bir dönemden sonra ortaya çıkmasına rağmen, normal aralık 0.5-4 saat arasındadır. Bununla birlikte, cildin BZ kimyasalına maruz kalmasından 36 saat sonra bile etkisi görülmemektedir. Hastanın genel korunma yöntemi, hastanın giysilerinin çıkarılması ile kimyasalların ve diğer ilgili öğelerden dekontamine edilmesini gerektirmektedir. Hasta için en büyük risk, özellikle sıcak veya nemli ortamlarda bulunan veya aşırı efor veya yetersiz su alımı nedeniyle susuz kalmış hastalarda kendi düzensiz davranışları ve hipertermiden kaynaklanan yaralanmalardır.

Fentaniller ve Diğer Potent Opioitler

Tüm fentaniller, cerrahi ve/veya post-operatif analjezi üretmek için klinik olarak kullanılan sentetik opiatlardır. Klinik olarak kullanılan fentaniller genel olarak morfinden 100 kat daha etkilidir. Fentanillerin tıbbi kullanımı genellikle güvenlidir çünkü bu ürünler genellikle düşük doz seviyelerinde kısa bir süre için ve tıbbi gözlem altında uygulanır. Halen tıpta kullanılmayan çeşitli fentanil analogları da sentezlenmiştir ve fentanillerden yaklaşık 10-50 kat daha etkilidir. Veterinerlik tıbbında kullanılan tebain grubundan diğer opioitler (örneğin etorfin, asetorfin) da çok güçlü opioit ajanlardır ve potansiyel olarak bir kimyasal saldırıda kullanılabilir. Normal tıbbi uygulamada, fentaniller genellikle bir transdermal yama olarak enjekte edilir veya uygulanır. Fentaniller biyolojik zarlardan kolaylıkla geçerek beyne hızla ulaşır. Fentanillerin bir aerosol olarak veya inhalasyon yoluyla maruz kalan bir dumana verilmesi, bir terör saldırısında en olası yoldur. Toplu su kaynaklarına fentanil eklemek, dikkate alınması gereken başka bir olası zehirlenme yoludur. Morfin gibi, tüm fentaniller güçlü opioit reseptör agonistleridir. Toksisiteleri temel olarak doza bağlı solunum depresyonundan kaynaklanır. Hayatı tehdit eden ana etki, siyanoz, bilinç kaybı ve solunum durması ile birlikte solunum depresyonudur. Diğer klinik belirtiler arasında analjezi, miyoz, nöbet benzeri aktivite ve kas sertliği yer alır. Çok yüksek dozların enjeksiyonu veya solunması dakikalar içinde bilinç kaybı ve solunum durmasına neden olabilir. Hızlı tıbbi müdahale olmaksızın sonuç genellikle ölümcül olacaktır.

Tedavi Yöntemi

İlk yardım önlemi olarak hava yolunu güvence altına alınması, oksijen sağlanması ve destekli veya kontrollü ventilasyonu başlatılması gereklidir. Partikül ve karbon filtreli bir solunum maskesi, fentanil ve benzerlerinin solunmasına karşı koruma sağlayacaktır. Daha güçlü fentanillerden biri kullanılıyorsa vücut koruması gerekebilir. Tıbbi tedavi olarak nalokson (i.m. veya i.v.) veya nalmefenin (i.v.) parenteral uygulaması, semptomlar ve belirtiler için etkili bir antagonizm elde edilene kadar gerekirse tekrarlanan dozlar halinde önerilir. Standart ilaca kıyasla bu panzehirlerden önemli ölçüde daha yüksek dozlar (10 kattan fazla) gerekebilir. Şiddetli zehirlenme durumunda, her iki panzehirin i.v. alımı gerekebilir. Naloksonun kısa yarılanma ömrü nedeniyle, tekrarlanan uygulama gereklidir. Destekli veya kontrollü ventilasyonu kolaylaştırmak için belirgin kas sertliği ve eşzamanlı solunum depresyonu durumlarında miyorelaksan ajanlar gerekebilir.

Toksinler

Doğada bakteri, mantar, kara veya deniz hayvanları gibi canlı organizmalar tarafından sentezlenen zehirli kimyasal bileşiklere "toksinler" denir [34]. Toksinler kimyasal yapı, molekül ağırlığı, vücutta tercih edilen hedefler ve etki mekanizmalarına göre sınıflandırılırlar. Toksinler düşük molekül ağırlığına sahip kimyasallar olduğu gibi, amino asit zincirlerinden ve genellikle karmaşık bir kimyasal yapıya sahip peptit veya proteinlerden de oluşmaktadır. Toksinlerin doğal yapıları nedeniyle, bazen kimyasal, bazen de biyolojik savaş ajanları olarak kabul edilmişlerdir. Benzer şekilde etki

mekanizmalarına bağlı olarak, kardiyotoksinler, dermatotoksinler, hepatotoksinler, nörotoksinler, vb. olarak gruplandırılırlar. En güçlü doğal/biyotoksinler, botulinum toksini ve tetanoz toksini gibi nörotoksinlerdir, ancak stafilokok enterotoksini gibi diğerleri de vardır. Bu biyotoksinlerin her birini farklı bakteriler üretir. *Clostridium botulinum* tarafından üretilen botulinum toksini, şu ana kadar bilinen en toksik maddedir [35]. Botulinum toksini aynı zamanda "ajan X" olarak da bilinir. Bu toksinin 1 gramının aerosol haline getirilmesi durumunda bir milyondan fazla insanı öldüreceği tahmin edilmektedir [36]. 70 kg'lık bir insan tarafından solunduğunda ölümcül doz yaklaşık 0.7 µg veya yutulduğunda 70 µg olduğu tahmin edilmektedir [37]. Botulinum toksini ve aflatoksin gibi mikroorganizma kaynaklı doğal toksinin yanısıra, risin *Ricinus communis* (hint yağı) tohumlarından izole edilmiş, çok güçlü bir bitkisel toksindir. Toksalbümin türevi olan risin ribozom proteinlerini inhibe eder, yavaş etkilidir ve insanlar için toksik doz, uygulama şekline bağlı olarak yaklaşık 0.05-1.0 µg/kg'dır [38]. 1978'de Bulgar muhalif Georgi Markov Londra'da şemsiye ucuna sürülen risin ile zehirlenerek suikasta uğramış ve öldürülmüştür. "Şemsiye suikastı" olarak bilinen bu olayda, Scotland Yard'ın olayı incelemesi sonucunda, Markov'un akciğerlerinin sıvı dolduğu (kalp yetmezliğine bağlı), kan zehirlenmesine bağlı karaciğer hasarı, çok yüksek akyuvar hücre sayısı ile bağırsakta ve lenflerde kanama gözlenmiştir. Otopsi sonucunda ölüm sebebi tam olarak saptanamamış, ancak İngiliz anti-terör uzmanları Markov'un sağ kalçasında 2 mm çapında bir kesik yarası ve yarada 1.52 mm çapında platin pellet bulmuştur. Pellette 0.34 mm çapta iki minik delik mevcut olup, balmumu ile kaplanmış olan bu pellette balmumunun içindeki letal bileşiğin risin olduğu anlaşılmıştır. Şemsiye benzeri bir aparat ile yürürken tesadüf gibi dokundurularak, pellet Markov'un vücuduna sokulmuştur. Daha sonra adli bilimciler, bir başka Bulgar muhalif olan Vladimir Kostov'un sırtındaki yaradan da balmumu ile kaplanmış aynı pelletten çıkarmıştır. Kostov da aynı şekilde risin ile suikaste uğrayarak ölmüştür. Savaş ajanları olarak toksinlerin kullanımı, suikastlarla veya sınırlı terörist saldırılarla sınırlıdır. En önemli iki toksin, toksisitesi nedeniyle botulinum toksini ve stafilokok enterotoksini B'dir. Özellikle yaz aylarında görülen gıda zehirlenmesi vakaları genellikle bu bakteriyel toksine bağlıdır. Risin ve T-2 mikotoksin dahil trikotesen mikotoksinleri daha az zararlı olmakla birlikte, yine de potansiyel tehdit olarak kabul edilmektedir. Toksinlerle oluşan zehirlenmelerin tedavisinde, çeşitli antitoksinlerle pasif aşılama uygulanmaktadır [39].

Botulinum Toksini

Botulinum toksini, yaygın bir çubuk şeklindeki toprak bakterisi *Clostridium botulinum* tarafından üretilir. *C. botulinum*, kendisine düşman olan bir ortamda sporlar oluşturacak ve böylece, örneğin yüksek bir sıcaklığa, hayatta kalma ve direnç gösterme yeteneğini önemli ölçüde artıracaktır. Farklı türlerde toksinler (A-G) üretilir. Bakteriyel enfeksiyonlarda en yaygın olanı A, B ve E'dir. A tipi toksin tıbbi olarak kullanılır ve çeşitli nörolojik ve oftalmolojik bozuklukların tedavisinde lokal olarak enjekte edilir. Terörizmde, kültüre alınmış *C. botulinum* ve sporlarının bir karışımı beklenebilir, ancak muhtemelen saflaştırılmış bir toksin değildir. Muhtemelen toksin ve/veya bakteri ve sporlarla kontamine olmuş su ve yiyeceklerin yutulmasıyla maruziyet gerçekleşir. Toksin, yutulduktan 3 gün sonra emilir, bozulur veya elimine edilir, ancak toksinin etkileri birkaç hafta veya ay sürebilir. Toksinin solunması olası bir maruz kalma yoludur, ancak etkileri tam olarak belgelenmemiştir. Botulinum toksini, nörotransmitter olan asetilkolinin presinaptik salınımını bozar. Ek etki mekanizmaları için bazı kanıtlar olsa bile, gözlemlenen hemen hemen tüm belirti ve semptomlar, botulinum toksininin kolinerjik nöronlar üzerindeki etkileri ile açıklanmaktadır. Saflaştırılmış toksin muhtemelen bilinen en güçlü toksindir. Farelere enjeksiyondan sonra ortalama öldürücü doz 0.03 ng/kg'dır. Etkili ise toksinin solunması da oldukça toksik olabilir. Bununla birlikte toksisitenin, toksin saflığına ve farmasötik ürünün kalitesine bağlı olarak önemli ölçüde değişmesi beklenebilir. *C. botulinum*'un neden olduğu bir enfeksiyondan sonra, inkübasyon süresi herhangi bir belirti ve bulgu ortaya çıkmadan 0-7 gün öncedir. Bunlar, saflaştırılmış botulinum toksini ile zehirlendikten sonra ortaya çıkmaktadır. Nefes darlığı, uyumsuzluk ve kuru veya boğaz ağrısı gibi semptomların ortaya çıkması muhtemeldir. Ek olarak, felç ve solunum yetmezliği ortaya çıkabilir.

Tedavi Yöntemi

Maskeli solunum koruması, toksin veya sporların solunmasına karşı etkili bir şekilde koruma

sağlayacaktır. Tedavi olarak mide dekontaminasyonu, oral maruziyet şüphesi durumunda ve semptomların başlangıcından önce öncelikle aktif kömür veya alternatif olarak mide lavajı ile yapılmalıdır. *C. botulinum*'un sporlarını yok etmek için yiyecek ve suyun 100 ºC'de 10 dakika kaynatılmasıyla zehirlenme önlenebilir. *C. botulinum* sporlarını yok etmek için 100 ºC'den daha yüksek bir sıcaklıkta ısıtmak gerekir.

Risin ve *Abrin*

Risin, "hintyağı bitkisi" olarak bilinen *Ricinus communis* tohumlarının lifli kısmında bulunan toksik bir glikoprotein olan bir lektindir. Ayrıca *Abrus precatorius* bitkisi de risine benzer bir lektin olan, yüksek toksisiteye sahip abrin içerir. Her iki toksin de bahsigeçen bitkilerin tohumlarında veya suda çözünür ekstrelerinde bulunur. Risin ve abrin sağlam cilde nüfuz etmez. Midede yaygın bir risin bozulması meydana gelir, böylece oral alımdan sonra %10'dan daha azı emilir. Bazı kasıtsız risin zehirlenmesi vakaları da bildirilmiştir. Risin veya abrinin partikül olarak veya çözelti içindeki toksin damlacıklarının aerosolü olarak yayılması, diğer olası maruz kalma yollarıdır. Hintyağı tohumunun lifli kısmında risin I ve risin II olarak iki tip lektin mevcuttur. Risin II, bilinen en toksik lektindir. Disülfür bağlarıyla birbirine bağlanmış iki amino asit zinciri A ve B içerir. B zinciri (MA 33.000 Dalton) hücre zarına bağlanır ve A zincirinin (MA 30.000 Dalton) hücreye endositozunu kolaylaştırır. Sitozolde, A zinciri ribozomların güçlü bir inaktivatörüdür ve protein sentezini geri döndürülemez şekilde bloke eder. *A. precatorius* tohumları ise izoabrin adı verilen dört tip lektin içerir. Ayrıca disülfür bağları ile birbirine bağlanmış iki amino asit A ve B zincirinden oluşurlar. Dört izoabrinden biri olan izoabrin-A, protein sentezi üzerinde en yüksek önleyici etkiye sahiptir. İnsanlarda tahmini oral öldürücü doz 1 mg/kg'dır. Tohumlar bir bütün olarak yutulursa, semptomların gelişmesi, tohumların çiğnenmesine veya iyice kırılmasına göre çok daha az olasıdır. Ağızdan risin alımından sonra zehirlenen insanların çoğu, uygun tıbbi müdahale ve destekleyici tedaviden sonra hayatta kalmaktadır. Hintyağı tohumları ayrıca alerjenik glikoproteinler içerdiğinden, duyarlı kişilerde anafilaktik veya diğer alerjik reaksiyon riski vardır. Oral alımdan sonra abrinin toksisitesi hakkında yeterli bilgi yoktur, ancak muhtemelen en az risin kadar toksiktir. İnsanlarda, tahmini minimum öldürücü risin dozu oral olarak 1 mg/kg ve i.m. enjeksiyonla 10-30 µg/kg'dır. Risin veya hintyağı tohumlarının yutulmasından sonra, semptomlar 4-6 saat ile birkaç gün arasında bir süre geçmektedir. Semptomlar iştahsızlık, mide bulantısı, kusma ve ishal ile başlar, ardından kalıcı kusma ve dehidratasyon ile birlikte şiddetli gastroenterit belirtileri izler. 6-8 gün içinde ölüm meydana gelebilir. Risin veya abrin inhalasyonundan sonra belirti ve bulgulara ilişkin çok az insan verisi mevcuttur. Solunum yolunun lokal tahrişi sonrasında zehirlenme belirtileri ortaya çıkabilir.

Tedavi Yöntemi

Solunum maskeleri risin ve abrin inhalasyonuna karşı koruma sağlamaktadır. Risin veya abrin alımından sonra, mide dekontaminasyonu öncelikle aktif kömür veya alternatif olarak mide yıkama ile yapılmalıdır. Semptomatik olmayan bir hasta için herhangi bir önemli maruziyetten sonra en az 8 saatlik bir gözlem süresi gereklidir. Daha şiddetli vakalarda, i.v. sıvılar, destekleyici bakım, elektrolit replasmanı, hipoglisemi izleme, hemoliz ve hipovoleminin komplikasyonları gibi yoğun tedavi (genellikle hastanede) gerekebilir. Abrin ve risin diyalize uygun olmadığından hemodiyaliz işleminin bir faydası yoktur. Deneysel olarak risine karşı bir aşı geliştirme olasılığı mevcut olmakla birlikte, şu anda bir aşı veya başka etkili bir panzehir bulunmamaktadır.

Saksitoksinler (STX) ve *Tetrodotoksinler (TTX)*

Saksitoksinler (STX) ve tetrodotoksinler (TTX), bazı deniz yosunu ve balık türleri tarafından üretilen bir grup kimyasal bileşiktir. Bir insan için öldürücü doz, toksin vücuda gıda yoluyla girdiğinde yaklaşık 0.5-2.0 mg ve enjeksiyon anında 0.05 mg ile olan zehirlenmedir. Aerosol durumunda insan için öldürücü doz 5 mg/dk/m'dir. STX ve TTX zehirlenmesi çoğunlukla sinir sisteminde dudaklarda, dilde, diş etlerinde, uzuvların distal segmentlerinde parestezi, baş ağrısı, disfoni, astigmatizm, yüzer hissetme, kas güçsüzlüğü, kraniyal ve periferik sinirlerde felç şeklinde semptomlara neden olur. STX ve TTX için spesifik bir panzehir yoktur. Destekleyici tedavi önerilmektedir [40].

Kimyasal Acil Durumlarda Korunma

Bilinmeyen kimyasalların veya kimyasal savaş ajanlarının salımından şüphelenildiğinde veya kanıtlandığında, acil durum müdahalesinde, ajanın tespiti ve belirlenmesi, ajana karşı fiziksel ve tıbbi korunma ile dekontaminasyon çok önemli unsurlardır.

Kimyasal Savaş Ajanının Tespiti

Bilinmeyen ajanın hızlı, nicel ve nitel analizi için yeterli koruyucu önlemlerin (koruyucu maskeler ve giysilerin yanı sıra tıbbi tedavi), kontaminasyon alanının haritalanması ve dekontaminasyon işlemleri gereklidir [41]. Özellikle kimyasal savaş ajanlarını yerinde doğrulama için, üç renkli detektör kâğıdı, atık buhar algılama kiti, su zehir tespit kiti ve kimyasal madde monitörleri dahil olmak üzere birkaç tespit cihazı mevcuttur. Ancak bu cihazların, düşük özgüllük ve tüm kimyasal savaş ajanlarını tespit edememe gibi çeşitli dezavantajları vardır. Bir ajanın kesin tanımlanması, yerinde bir mobil analitik laboratuvarda veya saha dışında bir laboratuvarda gerçekleştirilebilir ve bu analiz genellikle çok uzun sürmektedir. Maruz kalan kişilerdeki klinik semptomlar ve belirtiler, olası ajanın en belirgin göstergeleri olabilir. Bunun için kâğıt tabanlı spot testler ile yerinde analiz yapılır. Reaktiflerle muamele edilen kâğıt, sıvı kimyasal maddeler ile reaksiyona girerek farklı renkler vermektedir. Kâğıt herhangi bir yüzeye yapışır ve 30 saniye içinde renk değişimi olur, hardal ajanı kırmızıya, G-sinir ajanı sarı ve V-sinir ajanı ise yeşil renk vermektedir. Kâğıt tabanlı spot testin dezavantajı ise diğer birçok maddeyle yanlış pozitif sonuçlar verebilmesidir. Atık buhar algılama kiti, havadaki kimyasal savaş buharlarını yüksek özgüllükle tespit edebilen, taşınabilir, tek kullanımlık bir kimyasal savaş ajan algılama kitidir. Bu kit, silika üzerine immobilize edilmiş kimyasallarla doldurulmuş cam sondadan oluşur. Atmosferdeki sinir ajanları, fosgen, siyanojen klorür ve hidrojen siyanür ile su kaynaklarında bulunan zehirli maddeler de bu kit yardımıyla tespit edilebilir. Kimyasal savaş ajanlarının nokta tespiti için birçok enstrümantal cihaz bulunmaktadır. Bunlar temel olarak iyon mobilite spektrometreleri (IMS), gaz kromatografisi ve yüzey akustik dalga sensörleri olmak üzere başlıca üç tekniğe dayanmaktadır. İyon mobilite spektrometresi (IMS) kimyasal savaş ajanlarının, özellikle de sinir ajanlarının saptanması için kullanılan en yaygın cihazlardır [42]. Son yıllarda gaz analizleri için IMS kullanımı giderek artmaktadır. Birçok laboratuvarda sabit veya elde taşınan geliştirilmiş kimyasal ajan monitörü IMS cihazı bulunmaktadır. Körfez savaşı sırasında kullanılan IMS-tabanlı otomatik kimyasal savaş ajanı alarm detektörü geliştirilmiştir. IMS cihazı kimyasal ajanları birkaç saniye içinde tespit edebilir ve bir dakika içinde yeni bir tespit yapabilir. Alev fotometri prensibine dayanan elde taşınan kimyasal ajan detektörleri de geliştirilmiştir. Bir hidrojen/hava alevinde kükürt veya fosfor içeren organik bileşiklerin kemolüminesans ölçümleri yapılmaktadır. Yüzey akustik dalga sensörleri ile kuvars kristal mikrobalans daha yüksek hassasiyetlerde çalışmaktadır. Bu detektörlerden başka elektrokimyasal hücre-temelli sensör sistemleri de kullanılmaktadır. Taşınabilir gaz kromatografisi (GC), otomatik olarak gerçek zamanlı sürekli kimyasal izleme için kullanılır. Burada taşıyıcı gaz aracılığı ile hava örneği, adsorban malzeme ile doldurulmuş bir ön yoğunlaştırıcı kolon içinden geçirilir ve muhtemel ajanları tespit etmek için fotoiyonizasyon detektörü kullanılır. Örnek toplamadan saptamaya kadar tüm döngü yaklaşık 5-10 dakika sürer. Bir kütle detektörü ile birleştirilmiş gaz kromatografisi (GC-MS), çok düşük konsantrasyonlarda kimyasal savaş ajanları dahil organik bileşiklerin çoğunun kesin tespiti için kullanılabilir [43]. Bununla birlikte GC-MS'in komplike cihaz yapısı nedeniyle bir uzman tarafından opere edilmesi gerekmektedir. Ayrıca cihazın bakımı zordur ve kimyasal saldırı meydana geldiğinde sahada çok fazla avantaj sağlamaz. Diğer yandan, ticari olarak temin edilebilen, GC-MS tabanlı taşınabilir cihazlar bulunmaktadır. Sinir ajanları için elektrokimyasal bazlı detektörlere ek olarak, uzaktan algılamalı kimyasal madde alarmı, bir kızılötesi detektör ölçümü ile 5.000 m'ye kadar bir mesafeden buhar fazındaki sinir ve deriyi tahriş yapan ajanları tespit edebilir [14]. Moleküler baskılı polimer (MIP) sensörleri, biyosensörler, yüzey plazmon rezonansı (SPR), iletken polimer sensörleri vb. gibi diğer tekniklere dayalı aletler geliştirme aşamasındadır. Tüm saptama yöntemlerinin yanlış pozitif sonuçlara duyarlılığı vardır. En iyi yöntem, doğru verileri elde etmek için farklı prensipler üzerinde çalışan iki farklı tür detektör kullanmaktır [44].

Kimyasal Savaş Ajanına Karşı Fiziksel ve Tıbbi Korunma

Fiziksel korumanın temeli, toksik ajan ve bireyler arasında yapay bir bariyer oluşturulması ve "solunabilir hava" sağlanmasıdır [45]. Bariyer sıvılara, aerosollere veya gazlara karşı koruma sağlamalıdır. Solunabilir hava; doğrudan bir kaynağa (oksijen veya saf hava silindiri) bağlanma veya kirli havanın bir filtreden geçerek detoksifiye edilme şeklinde iki yolla sağlanabilir. Bununla birlikte, solunum cihazının kullanılması, solunum direnci, ısı stresi ve görme gibi fizyolojik rahatsızlıklara ve iletişim sorunlarına neden olabilir. Maske, aerosol tutma için yüksek verimli bir partikül aerosol (HEPA) filtre içerir ve havadaki gaz halindeki kimyasallar, karbon siyahi tarafından adsorpsiyon, katalitik ayrışma ve kemisorpsiyon işlemleri yoluyla uzaklaştırılır. Böylece tam yüz koruyucu maskeler hem solunum yolu hem de gözler için iyi koruma sağlar. Yüz maskesi, iyi bir görüş alanına ve bir mikrofonla konuşma aktarımına sahip olmalıdır. Farklı boyutlardaki bu koruyucu solunum maskelerinin farklı yüz boyutlarına uygun olması gerekir. Tüm vücut koruması durumunda, kimyasallar ile kontamine olmuş alanlar için sıvı geçirmez tam koruyucu malzemeler kullanılmalıdır. Örnek olarak dışı neopren ve diğer tarafı bütil kauçuk ile kaplanmış naylon kumaştan yapılmış ve toksik buhar geçirimsiz/hava geçirgen koruyucu giysiler kullanılabilir. Geçirgen koruyucu giysi üç katmandan oluşur. Üst katman yağ, su ve alev geciktirici naylon kumaştır, orta katman karbon kaplı dokumasız bir kumaş olup, üçüncü katman ise basit bir pamuklu kumaş katmandır. Koruyucu eldivenler, kimyasal koruma için bir bütil kauçuk dış eldivenden ve ter suyunun emilmesi için bir iç pamuk eldivenden oluşur. Eldivenlere benzer şekilde, bütil kauçuktan yapılmış botlar, ayakkabıyı kontaminasyona karşı korumak için ayakkabıların üzerine giyilir. "Kişisel koruyucu ekipman" (KKE) terimi, tam koruyucu donanımları, yani yüz maskesi, elbiseler, eldivenler ve botları belirtmek için kullanılır. Araçlarda veya barınaklardaki toplu koruma işlemi, kimyasallar ile kontamine olmuş havanın büyük filtrelerden süzülmesiyle arıtılmış hava sağlanmasıyla sağlanır. Bunun avantajı, korunan alan içindeki kişilerin ayrı ayrı maske takmalarına gerek olmamasıdır. Tehlikeli maddelerle uğraşmak için dört seviyede kişisel koruma vardır. Bunlar farklı solunum koruma türleri ile birlikte kimyasal koruyucu giysiler için A, B, C ve D seviyeleri olarak tanımlanmaktadır [46]. A Düzeyinde korunma, en yüksek düzeyde solunum, cilt, göz ve mukoza zarının korunması gerektiğinde uygulanmalıdır. Bu koruma, tamamen kapalı, buhar geçirmez, kimyasala dayanıklı bir elbise, botlar ve eldivenler ile birlikte bağımsız bir solunum aparatından oluşur. Seviye B koruması, en yüksek düzeyde solunum koruması gerektiğinde, ancak daha az derecede cilt ve göz koruması gerekli olduğunda seçilmelidir. Bu ekipman, kimyasala dayanıklı elbise, kimyasallara dayanıklı bot ve eldivenlerden oluşur. C Düzeyi koruma, havada taşınan madde türleri bilindiğinde, konsantrasyon ölçüldüğünde, hava temizleyici solunum cihazlarını kullanma kriterleri karşılandığında ve cilt veya göze maruz kalma olasılığı düşük olduğunda seçilmelidir. Bu KKE, kimyasala dirençli bir giysi, hava temizleyici filtre ile donatılmış tam yüz maskesi ve kimyasala dayanıklı bot ve eldivenlerden oluşur. D Seviyesinde korunma ise, solunum koruması ve cilt koruması sağlamamasının yanı sıra solunum veya cilt için riski olduğunda hiçbir yerde giyilmemelidir.

Dekontaminasyon

Dekontaminasyon, toksik kimyasalların imhası veya detoksifikasyonu yoluyla zararsız ürünlere dönüştürülmesidir [47]. Dekontaminasyon, bir bileşenden veya yüzeyden kişinin alabileceği dozu azaltmak, havadaki kimyasal, biyolojik, radyolojik ve nükleer (KBRN) ajanların potansiyelini azaltmak veya bileşen ya da malzemeyle ilişkili bertaraf maliyetini azaltmak için kullanılır [48]. Dekontaminasyon, çeşitli özelliklere sahip yüksek derecede toksik kimyasalları, olumsuz koşulları ve tedavi edilecek çok çeşitli konuları içeren örneğin insan derisi ve gözleri, metal/ahşap/plastik yüzeyli ekipman, alan vb karmaşık bir süreçtir. Kimyasal savaş ajanları açısından dekontaminasyon, kimyasal ajanların azaltılması veya uzaklaştırılması olarak tanımlanabilir. Bu, ajanların fiziksel olarak çıkarılmasıyla veya kimyasal olarak nötralize edilmesiyle gerçekleştirilebilir. Dekontaminasyon maruziyetten sonraki bir dakika içinde yapıldığında çok etkilidir, ancak bu pratik olarak nadiren mümkün olmaktadır [14,49].

Fiziksel Dekontaminasyon

Fiziksel dekontaminasyon kolay olmasına rağmen, kimyasal dekontaminasyon kadar etkili

değildir. Cildin sıvı maddelere maruz kaldığından şüpheleniliyorsa, bir dakika içinde cilt dekontamine edilmelidir. Tüm deneyimler, en önemli faktörün zaman olduğuna işaret etmektedir; talk pudrası, un, sabun ve su, özel dekontaminantlar veya bazı polimerler gibi çok farklı yöntemleri kullanmak suretiyle iyi sonuçlar elde edilebilir [47]. Fuller'ın toprağı, kaolin, talk, aktif karbon vb. gibi adsorbanlara toksik kimyasalın adsorpsiyonunu veya su/sabunlu su püskürterek ajanların basınç altında uzaklaştırılmasını içerir. Acil durumlarda, adsorban un, talaş veya toprak dahi olabilir. Fiziksel yöntemin temel dezavantajı, dekontaminasyon için kullanılan adsorban veya suyun da daha sonra detoksifiye edilmesi ve dikkatlice atılması zorunluluğudur. Diğer vücut yüzeylerine sıçrayacağı için baş veya derinin hardal ile kontamine olması durumunda su püskürtülerek (sprey şeklinde) kullanılmamalıdır.

Kimyasal Dekontaminasyon

Kimyasal dekontaminasyon, toksik kimyasal savaş ajanlarını, güvenle kullanılabilecek zararsız ürünlere dönüştürür. Kimyasal dekontaminasyon yöntemlerinde genellikle uygulanan kimyasal reaksiyonlar, nükleofilik reaksiyonlar veya oksidasyonlardır. Yaygın olarak kullanılan bir cilt dekontaminantı, %0,5 hipoklorit solüsyonu veya ev tipi ağartıcıdır. Metanol veya etanolde çözünmüş alkali (sodyum veya potasyum hidroksit), kimyasal silah ajanlarının çoğunu detoksifiye eder, ancak çok acil durumlar dışında cildin dekontamine edilmesinde kullanılmamalıdır. Kloraminler, hardallara ve V-ajanlarına karşı etkilidir, ancak G-ajanlarına karşı etkisizdir. Sodyum karbonat çözeltisi, G tipi sinir ajanlarını zararsız hale getirir, ancak V ajanları ile toksik bir ürün üretir. Kullanıma hazır, ticari olarak temin edilebilen birçok etkili kimyasal formülasyon geliştirilmiştir. Örneğin sodyum hidroksit (%2), etilen glikol monometileter (%28) ve dietilentriamin (%70) bileşimi DS2 olarak isimlendirilir. DS2, ekipman ve saha kullanımı için bir dekontaminasyon çözümüdür. Bu formülasyondaki aktif bileşen, bir nükleofil görevi gören ve kimyasal savaş ajanlarını toksik olmayan ürünlere hidrolize eden etilen glikol monometileterdir. DS2, tehlikelerini 5 dakika içinde etkili bir şekilde azaltmak için kimyasallar ile reaksiyona girer. 30 dakikalık temas süresi içinde DS2, bilinen tüm toksik kimyasal maddeleri nötralize eder. Boyaların yanı sıra plastik, kauçuk ve deri malzemelere de zarar verebilir. Amerikan dekontaminasyon kiti etanol (%72), fenol (%10), NaOH (%5), amonyak (%0.2) ve su (%12) etken maddelerini içerir. Benzer şekilde, Alman emülsiyonu, tetrakloroetilen (%15), su (%76), anyonik yüzey aktif madde (%1) ve kalsiyum hipokloritten (%8) oluşur. Mikroemülsiyon bazlı (çok amaçlı kimyasal, biyolojik dekontaminant) dekontaminasyon sistemi su (%60), tetrakloroetilen (%7), *N*-setil trimetil amonyum klorür (%28) ve eser miktarda yardımcı yüzey aktif madde içerir. Bu mikroemülsiyona, maddelerle reaksiyonlar için Fichlor (%4), sodyum 2-nitro-4-iyodoksibenzoat (%0.1) ve sodyum borat eklenir. Kimyasal savaş ajanları için en iyi sıvı dekontaminasyon ajanı %0.5'lik hipoklorit çözeltisidir. Ev tipi ağartıcıyı 1/10 oranında seyreltilerek hazırlanır. Hipoklorit çözeltisi, maddenin fiziksel olarak uzaklaştırılması, oksidasyonu ve/veya hidrolizi yoluyla çalışır; su bunu çok daha yavaş bir hızda yapar. Hipoklorit çözeltileri, cilt ve yumuşak doku yaralanmalarında kullanılabilirler. Ancak bu kimyasal gözde, açık göğüs yaralarında, açık beyin veya omurilik yaralanmalarında kullanılmaz. Bu bölgeleri bol miktarda steril tuzlu su ile yıkamak gerekir. Deri veya yumuşak doku yaralarında hipoklorit çözeltisi kullandıktan sonra, daha sonra bu alanları steril tuzlu su solüsyonu ile yıkamak gerekir.

Dekontaminasyonun Etkinliği

Dekontaminasyonun etkinliğini belirleyen değişkenler kontaminasyon süresi, sıcaklık, kontaminasyon yoğunluğu ve dekontaminasyon ortamı, ajanın doğası ve dekontaminantların doğasıdır. İdeal bir dekontaminasyon veya dekontaminasyon formülasyonunun gereksinimleri, hızlı eylem, verimlilik, insanlara zararsız, aşındırıcı olmayan, uzun süreli depolamada stabilite ve suyla yıkanabilirdir. Dekontaminasyon ajanları ve stratejileri hakkında son zamanlarda çok sayıda tartışma ve yeniden değerlendirme yapılmaktadır. Herhangi bir kimyasal saldırı durumu için planlama, tespit, koruma ve dekontaminasyonda bireysel eğitim ve düzenli alıştırma tatbikatları gereklidir. Herhangi bir olay başlamadan önce herhangi bir kimyasal ajanının varlığını kontrol etmek için alan izleme yapılmalıdır. En yakın hastaneler belirlenmeli ve herhangi bir acil durumla başa çıkmak için gerekli düzenlemeler (dekontaminasyon tesisi, panzehir stoğu, izole servisler, koruyucu donanımlar, sağlık bakanlığı tarafından eğitilmiş personel) yapılmalıdır. Giysiler kontaminasyona maruz kalmışsa, kimyasal savaş ajanının cilde geçişini önlemek için soyunurken çok dikkatli olunmalıdır. Yaralı kişilerin

giysilerini keserek çıkarmak gerekebilir. Tedavi sırasında, tıbbi personelin kimyasal savaş ajanına maruz kalmasını engellemek için hastanın tamamen dekontamine edilmesini sağlamak çok önemlidir. Acil servis personeli, tehlikeli bir maddeye maruz kalmış, kontamine olmuş ve hastaneye gelmeden önce yeterli dekontaminasyondan geçmemiş bir hastayı tedavi ederken, kimyasal kontaminasyonu giderilmelidir. Hastane personelini, diğer hastaları ve ziyaretçileri korurken, hastalar uygun şekilde dekontamine edilmeli ve tedavi edilmelidir. Normal sağlık hizmeti olabildiğince çabuk yeniden kurulmalıdır. Hastaya bakan sağlık personeli, kontamine hastalarla temas etmeden önce uygun kişisel koruyucu donanımları giymelidir. İdeal olarak, dekontaminasyon hastane dışında özel ekipler tarafından yapılmalıdır. Bu mümkün değilse, hasta için bir dekontaminasyon alanı hazırlanmalıdır. En ideal konum dış mekândır. İç mekânda dekontaminasyon gerekliyse, bir sonraki ideal yer bir dekontaminasyon odası olmalıdır. İç mekân dekontaminasyonu yalnızca kontrollü bir iç ortamın güvenli bir şekilde sürdürülebildiği durumlarda yapılmalıdır. Kimyasal tehlikeyi etkili bir şekilde izlemek için, hastane kimyasalı, ortam hava konsantrasyonunu ve ortam oksijen konsantrasyonlarını tanımlayabilen sensörlere ihtiyaç duyulmaktadır. Böyle bir oda yoksa, hasta büyük bir odada izole edilmeli ve oda diğer hasta bakım alanlarından uzakta olmalıdır. Hastanın bulunduğu bölgenin havalandırması sürekli kontrol edilmeli ve hastanenin diğer kısımları ile bu havanın teması önlenmelidir. Hastanede yapılacak diğer bir uygulama sarı bantla güvenli bir bölge oluşturmak ve gerektiğinde sadece uygun bir şekilde korunan kişilerin bu alana girmesine izin verilmesidir. Hasta hastaneye geldiğinde, herhangi bir acil hayat kurtarıcı müdahaleye ihtiyaç duyup duymadığı belirlenmeli ve hastanın dekontaminasyon öncesinde veya sırasında stabilize edilmesi gerekmektedir [14].

Dekontaminasyon İçin Tedavi Alanı

Tedavi alanlarının oluşturulması için çeşitli yönergeler vardır ve kimyasal acil durumlar için dekontaminasyon tesislerinin özellikleri sırasıyla; 1. Tehlikeli olayları içeren bir felakete müdahale ederken malzeme ve kitle imha silahları, tedavi alanının rüzgâra karşı en az 300 metre yukarısında olmalıdır. 2. Hastanın kıyafetleri ve takıları çıkarılmalıdır. 3. Hasta su ve sabun ile baştan aşağı yıkanmalıdır. Cildin tahrişini önlemek için şiddetli ovalamadan kaçınılmalıdır. 4. Tuzlu su ile irigasyon yapılarak açık yaralar dekontamine edilmelidir. 5. Hastanın kimyasala maruz kalmamış cilt bölgeleri kontamine edilmemelidir. Gerekirse cerrahi örtüler kullanılmalıdır. 6. Maruz kalan bölgeleri 10 ile 15 dakika sabun ve suyla yıkanmalı ve açıkta kalan gözleri 10 ile 15 dakika tuzlu su ile yıkanmalıdır. 7. İdeal olarak, mümkünse atık suyu çelik bidonlarda toplanmalıdır. Kimyasallara karşı etkili olabilmek için hızlı ve kapsamlı dekontaminasyon çok önemlidir ve bu dekontaminasyon işleminin bireylerden absorpsiyonu azaltmak ve toksik maddenin yayılmasını önlenmesi olarak iki temel unsuru bulunmaktadır. Dekontaminasyon işlemleri genel olarak ilgili kimyasal ajanın fiziksel durumuna, yani sıvı veya gaz halde olma durumuna bağlı olarak uygulanır.[14] Gazlar bilinen tek maruz kalma kaynağıysa, cilt dekontaminasyonu genelde gerekli görülmemektedir. Herhangi bir gaz maruziyet durumunda, mağdurun maruziyet alanından derhal uzaklaştırılması ilk acil durum önlemidir. Daha sonra giysiler çıkarılmalı ve ağzı kapalı çift plastik torbalarda izole edilmelidir. Sıvı haldeki kimyasallara maruz kaldıktan sonra, hızlı dekontaminasyon çok önemli olduğundan cilt dekontaminasyonu ilgili solüsyon ile yapılmalıdır. Su ve sabun veya tek başına bol su kullanımı ilk tercihtir, çünkü çoğu durumda yeterli miktarlarda suya ulaşım kolaydır. Saç, tırnak, cilt kırışıklıkları, koltuk altı, kasık ve genital bölge ve anüs çevresinin temizliğine özel dikkat gösterilmelidir. Saatler, mücevherler ve kontakt lensler de çıkarılmalıdır. Suyun bulunmadığı acil durumlarda ise toprak, un ve sabun gibi kuru tozlar dekontaminasyon için kullanılabilir. Özel dekontaminasyon çözümleri yalnızca istisnai durumlarda geçerli olacaktır. Aerosollere (buhar veya duman) maruz kaldıktan sonra, giysilerin çıkarılıp izole edilmesi ve sıvılar için yukarıda açıklandığı gibi cilt dekontaminasyonu yapılır. Genellikle sabun ve su ile duş almak yeterli kabul edilir. Aerosol içindeki kargaşa kontrol ajanlarının kullanılması durumunda, dekontaminasyon için hipoklorit solüsyonlarından kaçınılmasına özel önem verilmelidir, çünkü bu solüsyonlar cilt lezyonlarını şiddetlendirebilir. Göze maruz kalma acil olarak tedavi edilmelidir. Gözler nazikçe su, salin (%0.9 sodyum klorür solüsyonu) veya diğer göz yıkama solüsyonları ile yaklaşık 15 dakika yıkanmalıdır. Bir danışman göz doktorundan bakım tavsiye edilir. Tıbbi personelin ikincil kontaminasyonu, dekontaminasyon sırasında bir tehlikedir ve uygun koruyucu ekipman kullanılmalıdır. Bir terörist saldırının ardından kimyasal ajanlara maruz kalma durumunda gerekli olacak genel halk

sağlığı önlemleri aşağıda özetlenmiştir. Spesifik kimyasal maddelerle ilgili olarak, dekontaminasyon prosedürlerinden yalnızca su ve sabun kullanımı dışındaki yöntemlerin farklı avantajları bulunmaktadır. Dekontaminasyon ile beraber spesifik antidotların bulunması gerekmektedir. Etkinliği iyi belgelenmiş ve yaygın olarak kullanılan farmasötiklerin uygulanması gerekebilir. Anında tedavi (ilk doz) ve sonraki idame tedavisi (24 saat) ile ilgili bilgilerin sağlık personeli tarafından bilinmesi gerekmektedir. Bu bilgi, büyük çaplı terör eylemleri durumunda belirli bir ürüne ne zaman ve ne miktarda ihtiyaç duyulabileceğini tahmin etmede faydalı olacaktır. Farmasötiklerin veya dekontaminasyon ürünlerinin seçimini etkileyebilecek yasal, pratik ve lojistik özellikler halk sağlığı yetkilileri tarafından planlanmalıdır. Dekontaminasyonun ardından, kimyasal olarak yaralanmış hastaların tedavisi, destekleyici tedaviye ve mümkün olduğunda spesifik antidot tedavisine dayanmaktadır. Organ yetmezliğinin destekleyici tedavisi, temel yaşam desteği ve ileri yaşam desteği olarak sınıflandırılabilir. Bilindiği üzere acil tıpta temel yaşam desteği çok önemli bir konudur ve kimyasal saldırılar, çok sayıda yaralıya destek tedavisinin hemen sağlanması gibi büyük bir sorunu ortaya çıkarmaktadır. Kimyasallara maruz kalma, çeşitli organ yaralanmalarına ve arızalarına neden olabilmekte ve çeşitli kimyasal maddeler tahrişe neden olmaktadır. Biyolojik toksinler şiddetli ishale neden olabilir. Çok sayıda hastada görülen anksiyete ve huzursuzluk, olay yerindeki tıbbi personeli zor duruma sokabilir [14].

Kimyasal Ajanlara Maruz Kalindiğinda Kullanilan Farmasötik Formülasyonlar

Kimyasal ajanlara maruz kalındığında uygulanan tedavi ayrıntılarına bakıldığında; öncelikle uygulama kolaylığı olması ve hızlı etki göstermesi nedeniyle haricen veya dahilen kullanılan çözelti türü dozaj şeklinin sıklıkla kullanıldığı görülmektedir. Oral kullanımda çözelti türü preparatların tercih edilmesinin sebepleri; katı preparatlara göre alımları kolay olması (bebek, çocuk, yaşlı, Parkinson hastalığı gibi durumlarda yutma kolaylığı sağladığı için), etkin madde katı dozaj şekline göre daha kolay emilmesi (katı dozaj şeklinin dağılması, çözünmesi için belli bir zaman geçmesi gereklidir), biyoyararlanımı katı ilaç şekline göre daha iyi olması, etkin madde çözeltinin her tarafında eşit miktarda bulunarak doz homojenliği sağlaması, üretimlerinin kolay ve ucuz olması ve bazı etkin maddelerin mukoza üzerindeki irritasyon etkisinin çözelti şeklinde verilmeleri nedeniyle oldukça daha az olması (bunlar mide sıvısıyla bir anda seyrelerek irritasyon etkisini kaybederler) şeklinde sıralanabilir. Farklı etkin maddeler ile birlikte hazırlanabilen şurup, posyon, eliksir, spirit, tentür, aromatik sular ve damlalar da çözelti türü preparatlardandır. Oral rehidrasyon çözeltileri, kolera gibi salgın hastalıklar da dahil olmak üzere şiddetli ishal, hipovalemik şok, asidoz durumlarında kullanılan hayati tehlikeyi ortadan kaldıran önemli çözelti türü preparatlardandır. Maruz kalınan kimyasalın hızlıca uzaklaşmasını sağlamak ve yıkamak için irrigasyon çözeltileri kullanılmaktadır. Ağız yıkama çözeltileri, gargara ve kollutuvar şeklinde farklı etkin maddeler ile değişik farmakolojik etki için hazırlanmış çözelti türü preparatlar bulunmaktadır. Temizleme ve antiseptik amaçla duşlar kullanılmaktadır. Göz duşları, yabancı partikülleri uzaklaştırmak için, nazal duşlar nazal boşluğu yıkamak için, vajinal duşlar ise vajinayı temizlemek için kullanılmaktadır. Müshil amaçlı, anti enflamatuvar, sedatif etki ve X-ışını görüntüleme amacıyla rektal çözeltiler kullanılmaktadır. Bağırsakların kısa sürede temizlenmesi amacıyla oral kolonik lavaj çözeltileri kullanılmaktadır. Ayrıca hastaya rahatlık sağlamak, nefes alıp vermesini kolaylaştırmak için uygun ventilasyon cihazları ile birlikte inhalasyon çözeltileri kullanılmaktadır. Lokal ve sistemik etki amacıyla tedavide kullanılan göz, kulak ve burun damlaları da çözelti şeklinde hazırlanan dozaj formlarıdır. Enjeksiyon yoluyla kullanılan parenteral preparatlar öncelikli tedaviler arasında olduğunu görülmektedir. Parenteral preparatların tercih edilme sebepleri, hızlı veriliş, çabuk ve tahmin edilebilir etki sağlaması, tam biyoyararlanım sağlamaları, gastrointestinal sistemde karşılaşılabilecek problemlerin olmaması, hasta veya koma halindeki hastalarda güvenilir bir ilaç uygulaması olması gibi sebeplerdir. Parenteral preparatlar, enjeksiyonlar (hemen kullanılacak şekilde çözelti, emülsiyon veya süspansiyon şeklindeki preparatlar), infüzyonlar (büyük hacimli steril ve pirojensiz, sulu çözelti veya sürekli fazı su olan emülsiyon şeklindeki preparatlar), enjeksiyonlar veya infüzyonlar için konsantreler (bunlar konsantre olarak hazırlanıp kullanım sırasında istenilen doz veya hacme getirilen preparatlar), enjeksiyonlar veya infüzyonlar için tozlar (çözelti veya süspansiyon halinde dayanıklı olmayan ilaçlar steril toz halinde hazırlanıp kullanılmadan hemen önce su ile karıştırılarak çözelti veya süspansiyon haline getirilenler) ve implantlar (parenteral implantasyon için uygun büyüklük ve şekilde olan, etkin madde/maddeleri uzun sürede salan steril, katı ve birer dozluk

steril kaplarda sunulan preparatlar) şeklinde sınıflandırılmaktadır. Parenteral preparatların veriliş yolları intradermal veya intrakütan, (i.c., deri arasına), subkütan, (s.c., deri altına), intramüsküler, (i.m., kas içine), intravenöz, (i.v., damar içine), intratekal, (i.t., subaraknoit bölgeye), intraartiküler, (i.a., eklem içine), intrasisternal, (i.s., beynin arka ve alt kısmına), intrakardiyak, (kalp içine) ve intraoküler uygulama gibi pek çok veriliş yolları olduğu görülmektedir. Ayrıca kullanıma hazır enjektörler ve PEN'ler de hem hızlı etki hem de kullanımda kolaylık sağladığı için tercih edilmektedir. Ayrıca losyon, krem, merhem, pomat gibi deriye uygulanan lokal ve sistemik etkili yarı katı preparatların da kullanıldığı görülmektedir. Antienflamatuvar, antiseptik, antibiyotik etkili, lokal anestezik, antihistaminik ürünler, sistemik etkili merhemler, göze uygulanan göz merhemleri vb ürünler kullanılmaktadır. Kimyasal ajanlara maruz kalındığında ilk tedavi ve sonrasında yapılacak destekleyici tedavi yöntemleri aşağıda özetlenmiştir [14].

Kimyasal Kaynaklı Solunum Yetmezliği Durumunda Tedavi

Çok sayıda kimyasal silah, maruziyetten sonra birkaç saniye veya birkaç dakika ile birkaç saat içinde solunum yetmezliğine neden olabilir. Bunlara boğucu maddeler, siyanür, organofosfatlar, fentanil gibi opioitler ve kapalı bir atmosferde kullanılan göz yaşartıcı gazlar sayılabilir. Solunum semptomlarından muzdarip hastalar için ilk bakım, onları dinlenme pozisyonuna getirmektir. Boğucu ajanlara maruz kalındığında, bir tedavi olarak acil endotrakeal entübasyon gerekebilir. Üst solunum yolunun açıklığı bu hastalarda büyük bir sorundur ve birkaç saat içinde yakından izlenmelidir. Solunum yetmezliğinin tedavisi esas olarak yetişkinlerde genellikle 12 l/dk gerektiren yüksek oksijen akışının (%100) uygulanmasına dayanır. Olay yerinde çok sayıda hastanın tedavisi, hafif yaralanmalar için yüz maskesi, orta dereceli yaralanmalarda sürekli pozitif hava yolu basıncı dahil olmak üzere birkaç hastaya aynı anda yüksek akış uygulaması için yeterli oksijen tedarikinin ve uygun ekipmanın bulunmasını gerektirirken, ciddi yaralanmalarda ventilasyon cihazlarının kullanılması gerekebilir. Aşırı salgı ve bronşiyal birikintiler, sık hava yolu aspirasyonu gerektirebilir ve bronkospazm semptomatik olarak adrenerjik beta 2-agonistlerle tedavi edilmeli, gerekirse standart protokollere göre bir metilksantin ile desteklenmelidir. Kimyasal kaynaklı solunum hasarının erken evresinde steroitler önerilmemekle birlikte, şiddetli bronkospazm, steroitlerin inhalasyon veya i.v. yolla uygulanmasını gerektirebilir. Solunum yolunun şiddetli tahrişi, antibiyotik tedavisi gerektiren ikincil solunum yolu enfeksiyonu ile de ilişkilendirilebilir. Akciğer tahriş edicileri (örneğin klor, fosgen) maruziyet durumunda antidot olarak *N*-asetilsistein (NAC) kullanımı faydalı olabilir. İkincil tedavi olarak hastayı yarı dik pozisyonda dinlendirip, sıcak tutulmalıdır. Oksijen ve bronkodilatörler semptomatik tedavi olarak uygulanmalıdır. Yardımlı ventilasyon gerekebilir ve öksürük günde 30-60 mg kodein ile tedavi edilmelidir. Organofosfat zehirlenmesi durumunda, oksijen ventilasyon ile birlikte atropinin hemen kullanımına ihtiyaç duyulabilir. Opioit zehirlenmesi, hayatı tehdit eden merkezi solunum hasarına neden olabilir ve bir opioid antagonistinin uygulanması, ventilasyon ihtiyacını ortadan kaldırabilir. Önemli siyanür zehirlenmesinde acil oksijen uygulaması gerekmektedir. Siyanür maruziyetinde ilk yardım amil nitrit kullanımıdır. İlk tedavide 5 saniye solunan ezilmiş 0.3 ml ampul, 3-5 dakika sonra tekrar kullanılabilir. Hasta nefes almıyorsa, amil nitrit ampuller bir Ambu torbasına konulabilir. Destekleyici tedavi olarak %100 oksijen tedavisi, gerekirse havalandırma ve hastanın entübe edilmesi gerekebilir. Siyanürü teneffüs ettikten sonra canlı olarak hastaneye ulaşan hastalar, siyanür hızlı etki ettiği için muhtemelen antidot tedavisine ihtiyaç duymazlar. Hasta metabolik asidoz açısından izlenmelidir ve %8.4 sodyum bikarbonat çözeltisi kullanılabilir. Elektrolit dengesizliği düzeltilmelidir. Siyanür zehirlenmesi durumunda antidot olarak sodyum tiyosülfat kullanılabilir. Yetişkinler için sodyum tiyosülfat 10 dakika boyunca 400 mg/kg ile maksimum 12.5 g (örn. 1.6 ml/kg ila maksimum 50 ml %25 çözelti). Gerekirse ek dozlar verilmelidir. Çocuklar için sodyum tiyosülfat 10 dakika boyunca 400 mg/kg ile maksimum 12.5 g (örn. 1.6 ml/kg ile maksimum 50 ml %25 çözelti) kullanılmalıdır. Gerekirse ek dozlar verilmelidir. Destekleyici tedavi olarak sodyum tiyosülfat, sodyum nitrit veya 4-DMAP ile birlikte kullanılır. Sodyum tiyosülfat tek başına hafif siyanür zehirlenmesinde kullanılabilir. Sodyum tiyosülfat bazen hidroksokobalamin ile birlikte kullanılabilir. Siyanür için diğer kulanım potansiyeli olan antidot sodyum nitrittir. Yetişkinlerde sodyum nitrit 300 mg (örn. 10 ml, %3 çözelti), 5-10 dakikada yavaş i.v. ile verilebilir. İyileşme yoksa 30 dakika sonra sodyum nitrit orijinal dozun %50'sinde tekrarlanabilir. Çocuklarda sodyum nitrit kullanımı yavaş i.v. ile 4-10 mg/kg ile maksimum 300 mg (%3 çözelti, 0.13-

0.33 ml/kg) şeklindedir. Sodyum nitrit, iyileşme yoksa 30 dakika sonra orijinal dozun %50'sinde tekrarlanabilir. Destekleyici tedavi olarak sodyum tiyosülfat ile kullanmalıdır. Alternatif olarak 4-DMAP 3-4 mg/kg i.v. olarak kullanılabilir. Siyanür maruziyetinde ayrıca hiddroksokobalamin içeren bir kit bulunmaktadır.

Kimyasal Kaynaklı Kardiyovasküler Yetmezlik ve Tedavisi

Hipotansiyon, hastayı sırtüstü yatırarak, alt ekstremiteleri kalp seviyesinin üzerine çıkarıp venöz dönüşü kolaylaştırarak tedavi edilir. Yüksek oksijen akışı verilmeli ve sıvı uygulaması için i.v. bir katater yerleştirilmelidir. İlaca bağlı kardiyovasküler şok durumunda, hasta başına 1 ile 2 l i.v. izotonik sıvı yaygın olarak kullanılır. Daha sonra, katekolaminlerin bir infüzyon pompası ile uygulanması düşünülmelidir. Kardiyovasküler yetmezlik, ciddi solunum yaralanmasından kaynaklanabilir. Bu hastalarda adrenalin infüzyonu ile birlikte endotrakeal entübasyon sonrası mekanik ventilasyon düşünülmelidir. Kardiyovasküler yetmezlik gösteren ve siyanür ile ciddi şekilde zehirlenen hastalar, hemodinamik dengeyi hızla düzeltebilen sıvı takviyesi ile bir kobalt türevi ile tedavi edilmelidir. Organofosfat zehirlenmesinde standart tedaviye ek olarak, özellikle kalp hızı yavaş olan hastalarda acil atropin uygulaması daha çok düşünülmelidir. İshala veya kusmaya bağlı kardiyovasküler şok, büyük miktarda sıvı verilmesini gerektirmektedir. Kimyasal maruziyette, solunum veya kalp yetmezliği neden olduğu beyindeki düşük oksijenlenme de komaya yol olabilir. Buradaki tedavi, serbest hava yollarının ve solunumun güvenliğini sağlaması, oksijen verilmesini içerir. Hasta, sağ yan yatar pozisyonda yatırılmalıdır. Sinir gazlarına (sarin, GB, VX, tabun) maruz kalındığı zaman ilk tedavide antidot olarak atropin yetişkinler için her 5-10 dakikada bir 2 mg i.m. veya i.v. şiddetli semptomlar için, ilk dozda 6 mg'a kadar verilebilir. Çocuklarda ise her 5-10 dakikada bir 0.05-0.1 mg/kg i.m. veya 0.02 mg/kg i.v. (doz başına 2 mg'ı geçmemelidir) uygulanabilir. Daha yüksek atropin dozları gerekebilir, ancak klinik yanıta göre değerlendirilmelidir. Yeterli dozun verilmesini sağlamak ve aşırı dozu önlemek için atropin kullanımında dikkatli olunmalıdır, hasta yakından takip edilmelidir. Destekleyici tedavi ise tüm orta ve ağır vakalara uygulanmalıdır. Hayati tehlike arz eden aritmiyi önlemek için atropinden önce mümkünse hipoksi düzeltilmelidir. Şiddetli maruziyet durumunda ventilasyon gerekebilir. Sinir gazlarına maruz kalındığında kullanılan diğer bir antidot oksimlerdir. Yetişkinlerde ilk tedavide kullanılan oksim, pralidoksim (klorür veya mesilat tuzu olarak) 30 mg/kg (2 g'a kadar) yavaş i.v. olarak 4-6 saatte bir tekrarlanır veya 8-10 mg/kg/saat infüzyon verilebilir. Bunun haricinde alternatif tedavi olarak obidoksim 250 mg i.m. veya yavaş i.v., ardından 24 saat içinde 750 mg infüzyon uygulaması yapılabilir. Maksimum günlük doz 1000 mg olarak kullanılabilir. Çocuklarda ilk tedavi pralidoksim (klorür veya mesilat tuzu olarak) 15-30 mg/kg yavaş i.v. gerektiğinde 30-60 dakikada bir, ardından 1-2 doz için bir saatlik aralıklarla tekrarlanmalıdır. Alternatif tedavide ise obidoksim, 4-8 mg/kg yavaş i.v., ihtiyaç halinde ise 10 mg/kg/24 saat infüzyon ile kullanılmalıdır. Oksim kullanıldığında destekleyici tedavi olarak asetilkolinesterazı yenilemek için HI-6 gibi diğer oksimler kullanılabilir ancak yaygın olarak bulunmazlar. Bu panzehirler için otomatik enjektör formülasyonları mevcuttur. Sinir gazları maruziyetinde diğer bir antidot benzodiazepinlerdir. Yetişkinlerde ilk tedavi protokolü olarak diazepam 5-10 mg i.v./i.m. (daha yüksek dozlar 40 mg gerekli olabilir) kullanılmalıdır. Çocuklarda ise diazepam 0.05 ila 0.3 mg/kg i.v./i.m. (maksimum 10 mg) kullanılmalıdır. Destekleyici tedavi olarak ağır vakalarda nöbetlerin tedavisi için veya ampirik olarak diğer benzodiazepinler (örn. lorazepam, midazolam) kullanılabilir.

Kimyasal Nedenli Nöbetler ve Tedavisi

Nöbetin ilk tedavisi koma ile aynı iken, sürekli nöbetler beyin hasarına neden olabilir ve benzodiazepinler gibi antikonvülsanlarla tedavi gerektirebilir. Hem sinir ajanları hem de siyanür, hızla solunum durmasının ardından gelen nöbetlere neden olabilir. Bu durumlarda temel yaşam desteğinin ardından ilgili panzehir verilmelidir. BZ kimyasal ajanına maruz kalındığında ilk tedavi olarak fizostigmin (2-3 mg i.v., sonra idame 2-4 mg/saat) kullanımıdır. Mümkün olan en kısa sürede (2-5 mg/1-2 saat) oral uygulamaya geçilmelidir. Tedavi çok erken kesilmemelidir, zehirlenmenin şiddetine bağlı olarak 4-5 gün devam edebilir. Destekleyici tedavi olarak hastanın eylemleriyle kendine zarar vermesini önlemek amacıyla hastanın durumu, fiziksel veya kimyasal kısıtlama olmaksızın izlenmelidir. Bununla birlikte, gerekirse bir benzodiazepin ile yatıştırılır. Yetişkinlerde midazolam 1-2 mg i.v. hasta

güvenli bir şekilde yönetilene kadar 2-3 dakikada bir veya i.v. erişim sağlanamıyorsa 5-10 mg i.m. olarak tedavi uygulanmalıdır. Opioitlere maruz kalındığında ise başlıca antidot nalokson kullanımıdır. Başlangıç dozu 0.4 ile 2 mg i.v. kullanımıdır. Solunum fonksiyonunda istenen düzelme elde edilmez ise her 2-3 dakikada bir tekrarlanmalıdır. 10 mg nalokson uygulandıktan sonra hiçbir yanıt gözlenmezse, opioit kaynaklı veya kısmi opioit kaynaklı toksisite tanısı sorgulanmalıdır.

Kimyasal Kaynaklı İshal ve Kusma ve Tedavisi

Sürekli ve aşırı ishal ve kusma, dehidrasyona, metabolik bozukluklara, böbrek yetmezliğine, hipovolemik kardiyovasküler şoka ve sonunda ölüme neden olabilir. Sıvıların oral veya intravenöz yoldan verilmesi etkili bir tedavidir. Bir yetişkin hastanın rehidrasyonu, günde 8 l kadar sıvı hacmi gerektirebilir.

Kimyasal Kaynaklı Cilt ve Göz Yanıkları ve Tedavisi

Yanıklara neden olan kimyasallar için standart termal yanık tedavisi yeterlidir. Sıvı formdaki kimyasallarla dermal temastan sonra tedavi için gerekli panzehirlerin otomatik enjektör formülasyonları mevcuttur. Standart otomatik enjektörler yetişkinlere yöneliktir. İğneler çok küçük çocukların kas hacmi için çok uzundur ve panzehir dozu çok yüksektir. Acil tedaviye ihtiyaç duyan ciddi şekilde zehirlenmiş bir çocuk olduğunda standart bir otomatik enjektör kullanımında dozun hepsi verilmemelidir. Yakıcı ajanlara (blister ajanlar örneğin sülfür mustard, lewisite) maruz kalındığı zaman destekleyici tedavide sodyum tiyosülfat kullanılır. Hardala şiddetli maruziyetten şüpheleniliyorsa, i.v. sodyum tiyosülfat kullanımını düşünülmelidir. Sistemik etkileri azaltmak için ilk saat ve tercihen maruziyetten 20 dakika sonra kullanılmalıdır. Gözler için destekleyici tedavi yapılmalıdır. Distile su veya %0.9 steril NaCl çözeltisi ile yıkanır. Göz kapaklarının birbirine yapışmasını önlemek için %5 borik asit içeren oftalmik merhemler kullanılarak enfeksiyon önlenmelidir. Topikal bir antibiyotik, örn. sulfasetamit içeren göz damlası kullanılmalıdır. Blefarospazmı hafifletmek ve göz muayenesini etkinleştirmek için gerekirse lokal anestezik damlalar uygulanmalıdır. Ancak anestezik damlalar korneaya zarar verme riskini artırabilir, o nedenle dikkatli kullanılmalıdır. Yakıcı ajanlardan etkilenen deri bol miktarda sabun ve suyla yıkanmalıdır. Kaşıntı, soğutma preparatlarının lokal uygulamaları ile azaltılabilir, örn. kalamin losyonu veya su ile yıkanmalıdır. Gümüş sülfadiazin krem uygulanıp ve steril bir örtü ile kapatılmalıdır. Analjezikler uygun dozda kullanılmalıdır. Yakıcı ajanlara maruziyette solunum sistemini rahatlatmak, öksürüğü hafifletmek için kodein kullanılmalıdır. Şiddetli vakalarda mekanik ventilasyon/pozitif son ekspiratuar basınç (positive end expiratory pressure, PEEP) ile bronşiyal lavaj ve oksijen uygulaması gerekli olabilir. Önemli üst hava yolu tutulumu olduğunda endotrakeal entübasyondan ziyade krikotirotomi uygun olabilir. Bronkospazm varsa bronkodilatörlerin yanısıra, *N*-asetilsistein gibi mukolitikler faydalı olabilir. Yutulması halinde kusturmaya çalışılmamalıdır. Yakıcı ajanlara tedavide kullanılan diğer antidot lewisite DMPS (Dimaval®) olup, i.v. ve oral olarak kullanımı mevcuttur.[14] Yetişkinler için lewisit ile şiddetli zehirlenmede doz ayarı şu şekildedir: 1. gün; her 3-4 saatte bir 250 mg DMPS i.v. (1.5-2.0 g DMPS/gün), 2. gün; 4-6 saatte bir 250 mg DMPS i.v. (1.0-1.5 g DMPS/gün), 3. gün; 250 mg DMPS i.v. her 6-8 saatte bir (0.75-1.0 g DMPS/gün). Daha sonra 8-12 saatte bir 250 mg DMPS i.v. (0.5-0.75 g DMPS/gün), ardından 250 mg DMPS günde 1-3 kez veya oral doza geçiş (günde 100 mg 1-4 kez) uygulanmalıdır. DMPS mevcut değilse, destekleyici tedavi olarak demirkaprol kullanımıyla ilgili olarak uzman tavsiyesi alınmalıdır. Yukarıdaki öneriler, biyolojik ajanlarla terörist saldırılarda kullanılacak tedavilere ilişkin mevcut CPMP kılavuz belgesine tamamlayıcı olarak yapılır (EMEA / CPMP / 4048/01).

SONUÇ VE TARTIŞMA

Bu derleme makalesinde çeşitli kimyasal savaş ajanlarının kimyasal ve fiziksel özellikleri, tıbbi koruma yöntemleriyle ilgili genel bilgiler, güncel analiz metotları ekipmanı, dekontaminasyon teknikleri ve kimyasal ajanlara maruz kalındığında kullanılan uygulamalar genel hatları ile incelenmiştir. Kimyasal savaş ajanlarına maruziyet sonrası yapılacak ilk müdahale ve devamındaki tıbbi tedaviler hayati öneme sahiptir.

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REVIEW ARTICLE / DERLEME MAKALE

MUCOADHESIVE POLYMERS IN COLON TARGETED DRUG DELIVERY SYSTEMS: A COMPREHENSIVE REVIEW

MUKOADEZİF POLİMERLERİN KOLON HEDEFLİ İLAÇ TAŞIYICI SİSTEMLERDE KULLANIMI: DETAYLI BİR İNCELEME

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ABSTRACT

Objective: *Mucoadhesive polymers have emerged as crucial components in the realm of drug delivery systems, particularly in the context of targeted treatments within the colon. These polymers possess adhesive properties that enable them to form temporary bonds with mucosal surfaces, extending the contact time of drugs with the colonic mucosa. This review provides a comprehensive overview of mucoadhesive polymers for colon drug delivery systems. Natural polymers such as chitosan and alginate, along with synthetic counterparts like polyacrylic acid derivatives, find application in these systems. The advantages of mucoadhesive polymers lie in their ability to facilitate site-specific drug delivery, thereby minimizing systemic side effects, and in enabling controlled and sustained release of drugs for improved bioavailability. Despite these benefits, challenges including variability in mucosal conditions and the imperative need for biocompatibility must be addressed. The applications of mucoadhesive polymers span diverse medical conditions, including targeted delivery of anti-inflammatory drugs for inflammatory bowel diseases, localized administration of chemotherapeutic agents for colon cancer treatment, and precise delivery of antibiotics for colonic infections.*
Result and Discussion: *As a promising avenue for optimizing colon drug delivery, mucoadhesive polymers offer great potential for the development of effective and well-tolerated treatments for various colonic disorders.*
Keywords: *Colon, colon drug delivery systems, mucosa, mucoadhesion, mucoadhesive polymers*

ÖZ

Amaç: *Mukoadezif polimerler, özellikle kolon bölgesinde hedefe yönelik tedaviler bağlamında, ilaç taşıyıcı sistemler alanında çok önemli bileşenler olarak ortaya çıkmıştır. Bu polimerler, mukozal yüzeylerle geçici bağlar oluşturmalarını sağlayan ve ilaçların kolon mukozası ile temas süresini uzatan yapışkan özelliklere sahiptir. Bu derleme, kolon ilaç taşıyıcı sistemleri için mukoadezif polimerlere kapsamlı bir genel bakış sunmaktadır. Kitosan ve aljinat gibi doğal polimerlerin yanı sıra poliakrilik asit türevleri gibi sentetik muadilleri de bu sistemlerde uygulama alanı bulmaktadır. Mukoadhezif polimerlerin avantajları, bölgeye özgü ilaç dağıtımını kolaylaştırma, böylece sistemik yan etkileri en aza indirme ve gelişmiş biyoyararlanım için ilaçların kontrollü ve sürekli salınımını sağlama yeteneklerinde yatmaktadır. Bu avantajlara rağmen, mukozal koşullardaki değişkenlik ve biyouyumluluk için zorunlu ihtiyaç gibi zorluklar ele alınmalıdır. Mukoadezif polimerlerin uygulamaları, enflamatuar bağırsak hastalıkları için anti-enflamatuar ilaçların hedefe yönelik*

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olarak verilmesi, kolon kanseri tedavisi için kemoterapötik ajanların lokalize olarak verilmesi ve kolon enfeksiyonları için antibiyotiklerin hassas bir şekilde verilmesi dahil olmak üzere çeşitli tıbbi koşulları kapsamaktadır.

Sonuç ve Tartışma: *Kolon ilaç dağıtımını optimize etmek için umut verici bir yol olan mukoadezif polimerler, çeşitli kolonik hastalıklar için etkili ve iyi tolere edilen tedavilerin geliştirilmesi için büyük bir potansiyel sunmaktadır.*

Anahtar Kelimeler: *Kolon, kolon ilaç taşıyıcı sistemler, mukoza, mukoadhezyon, mukoadhezif polimerler*

INTRODUCTION

The field of colon-specific pharmaceutical delivery systems is constantly evolving. Colonic drug administration has grown in significance for the systemic distribution of anti-asthmatic, anti-hypertensive, and anti-diabetic agents as well as for the delivery of pharmaceuticals for the local treatment of colon disorders including Crohn's illness and other similar conditions. To handle the limitations of the prior method and target the colon, new technologies and systems have been created.

Solid dosage forms for oral administration have historically been created to release their drug content in the upper gastrointestinal tract (GIT), where the environment is typically more favorable for drug absorption and dissolution. Managing the rate and position of the drug's release from drugs taken orally has recently received more attention in order to improve the patient's compliance and therapeutic effectiveness [1,2].

The term "targeted drug delivery system" refers to a method of introducing a therapeutic dose of pharmaceutical to a specific location in the body to achieve the required concentration of drug. Several factors, including unstable situation, a weak solubility, limited half-life, wide volume of the dissemination, weak absorption, weak specificity, and therapeutic index, can cause a pharmaceutical to be targeted toward an area of interest. The benefits of aimed drug delivery include raising the drug's therapeutic potency, limiting drug degradation, minimizing unwanted effects, and lowering the drug's unsafe dose [3].

For localized therapy of a number of colonic diseases, the colon was used as an aim for delivery of pharmaceuticals into the lower gastrointestinal system. The colon-specific system for drug delivery must be able to preserve the drug while it is being transported to the colon, which means that neither drug release nor absorption should take place in the stomach as well as small bowel, nor should the bioactive ingredient degrade during either of the the dissolution tests, but only once the system has arrived at the colon [4].

A higher concentration of the pharmaceuticals can reach the colon with less systemic absorption when it is delivered particularly to the colon rather than initially being absorbed in the upper digestive tract. The colon is the primary location for drug delivery, as the colonic mucosa is known to aid in the absorption of various medications and colonic contents have a longer retention time (up to five days). An oral or rectal route might be used to deliver a medication to the colon. Due to their simplicity, oral dosage forms are an extremely popular method of delivery for colon-specific administration [5,6].

Additionally, oral dose forms don't require sterile preparation, provide more design and manufacturing flexibility, improve patient compliance, and are generally safe to administer [7]. Targeting a medicine to particular areas in the colon is difficult with direct rectal delivery of pharmaceuticals [8]. The rectum provides the quickest route for administering medications to the colon, although it is challenging to reach the proximal region of the colon by the rectal route. The rectal route is challenging and uncomfortable for the patient, which reduces adherence among patients [9].

Colon drug delivery systems (CDDS) protect peptide drugs from hydrolysis and enzymatic degradation in the duodenum and jejunum and eventually release the drug into ileum or colon which results in greater systemic bioavailability. The colon is believed to be a suitable absorption site for peptides and protein drugs such as insulin and vasopressin for the following reasons: a lower variety and quantity of digestive enzymes; comparative proteolytic activity of colon mucosa is much lower than that detected in the small intestin [4].

Colonic Anatomy

The length of the large intestine is approximately 1.5 meters. It starts at the cecum in the right iliac fossa and ends at the rectum and anal canal, which are located deep within the pelvis. The average human large intestine is 1.5 meters long. The colon is made up of a series of tubes that are connected by mucosa, a moist, delicate pink lining [10,11]. The stomach, small intestine, and large intestine make up the GIT. There are three primary sections that make up the large intestine, which runs from the ileocecal connection to the anus. These are the rectum, the anal canal, and the colon [1,12]. The cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, and sigmoid colon make up the actual anatomy of the colon. Before the anus, the rectum is the final anatomical section [3,4]. Colonic anatomy is also depicted in Figure 1 [11].

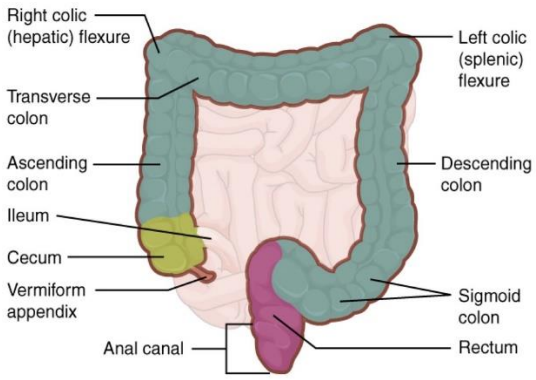

Figure 1. Colonic anatomy [11]

Colonic pH

Both within- and between subject changes can affect the GIT's pH. The pH of the GIT is influenced by disease severity, eating habits, and what is eaten. The creation of colon tailored medication delivery systems is based on the variations in pH in various GIT sections. To direct the medicine to the region, encapsulation is used with various polymers [13]. A diet high in carbohydrates may affect the pH level inside of the colon. This results from the colonic bacteria's fermentation of polysaccharides and subsequent synthesis of short fatty acid chains. Because it influences the medications' solubility in colonic fluid, the pH of the colon has an influence on the pharmacokinetics and pharmacodynamic action of a CDDS. For instance, the influence of colonic pH on drug release is significantly more pronounced if the CDDS formulation has a pH-sensitive coated membrane [14]. Length and pH of different parts of GIT were shown in Table 1 [8].

Table 1. Length and pH of different parts of gastrointestinal tract (GIT) [8]

Part of GIT	Length (cm)	pH
Stomach		1.5-2
Small intestine	550-700	6.6-7.5
Colon Ascending colon	20-25	6.4
Transverse colon	40-45	6.6
Descending colon	10-15	7.6

Colonic Transit Time

The colorectal bioavailability of medicines is significantly influenced by the passage through the colon time. The passage of dose forms is typically influenced by the administration timing, the presence

or lack of nutrition, and the kind of dosage form. The physical state, the size of the dosage form, or the presence of nutrition in the stomach have no effect on small intestinal transit. The dose form takes a consistent 3 to 4 hours to get from its source to the ileocecal tunction. Colon passage time greatly affects the bioavailability of pharmaceuticals released from dosage forms. Gender, dose form size, physiological variables like stress, the presence of nutrition, and sick status are the factors that affect colonic transit time. Small particles and solution move slowly in the proximal colon. In comparison to men, women exhibit a shorter colonic passage time [4,14]. Transit time of different parts of GIT was shown in Table 2 [8].

Table 2. Transit time of different parts of gastrointestinal tract (GIT) [8]

Organ	Transit time
Stomach	1-2 hr
Small intestine	3-4 hr
Colon	20-30 hr

Microflora and Colonic Enzymes

More than 400 different kinds of aerobic and anaerobic microorganisms, including *Escherichia coli* and *Clostridium species*, are found in the human colon. Numerous hydrolytic and reductive metabolizing enzymes are produced by these bacteria. These enzymes catalyze a variety of processes, such as the metabolism of pharmaceuticals and biomolecules such as bile acid, the inactivation of potentially toxic metabolites, as well as the fermentation of carbohydrates and proteins. Polysaccharides like chitosan, guargum, pectin and others are frequently used as release rate-controlling ingredients in dosage forms that target the colon. These polysaccharides can withstand intestinal and stomach enzymes. Nevertheless, are broken down by anaerobic bacteria found in the colon [14].

In different areas of the GIT, medication release is triggered by gut enzymes. These enzymes are typically produced by gut bacteria that is abundant in the colon. These enzymes are employed to break bindings between an active agent and an inert carrier (it means release of a drug from a prodrug) as well as to breakdown coatings or matrices [15]. The many metabolic reactions that occur in the GIT are caused by the enzymes secreted by various microorganisms, including *Clostridia, E. Coli, Lactobacilli, Streptococci* and *Eubacteria.* At least 10^{11}-10^{12} colony forming units of bacteria are present in the colon [13,16].

Advantages of CDDS

CDDS offer numerous advantages in the field of medication administration. The colon, being an ideal site for addressing local colonic illnesses, allows for targeted treatment with lower doses of medication, minimizing the potential for side effects and drug interactions. This, in turn, reduces dosing frequency, leading to cost savings, especially with expensive drugs. The extended retention period in the colon, lasting up to five days, enhances the bioavailability of therapeutic molecules that may not be well absorbed elsewhere. CDDS also facilitates the delivery of peptides, insulin, oral vaccinations, and growth hormones due to the colon's lower peptidase activity compared to other organs.

Moreover, administering medications through the colon mitigates stomach irritation, particularly for drugs like nonsteroidal anti-inflammatory drugs (NSAIDs), by bypassing upper gastrointestinal absorption. The avoidance of first-pass metabolism further contributes to the efficacy of treatment. The prolonged activity in the colon, whether during the day or night, enhances patient adherence to medication regimens. Importantly, CDDS ensures that the treatment directly targets the affected area, preventing medication breakdown and extending the effects and duration of therapy.

The colon’s capacity for both local and systemic treatment enables versatile applications, with local delivery allowing for effective topical therapy. Additionally, the colon's high water absorption and the viscous nature of its contents restrict access to the absorbent membrane for many drugs. Furthermore, the colon serves as a crucial site for various drug metabolism processes, including azo reduction and

enzymatic cleavage. In summary, CDDS offer a comprehensive approach to medication delivery, optimizing therapeutic outcomes across a spectrum of medical conditions [3,17-19].

Disadvantages of CDDS

While CDDS offer notable advantages, they also present certain challenges and limitations. The colon's small luminal surface area and tight connections contribute to delayed systemic absorption, leading to a sluggish onset of action for medications. Developing medications specifically targeted for the colon proves challenging due to various biological barriers. In the colonic mucosa, drug-metabolizing enzymes of the cytochrome (p450) class exhibit lowered affinity, affecting the metabolism of certain drugs.

The prolonged residence period of 3-5 days in the colon may lead to increased plasma levels of pharmaceuticals and enhanced bioavailability, particularly for medications that are substrates for these enzymes. However, the colon's lower and more viscous fluid levels compared to the upper gastrointestinal tract pose challenges in drug formulation. Additionally, multiple production steps are often required, further complicating the manufacturing process.

Issues such as non-specific drug binding to food residue, feces, mucus, and digestive secretions can limit a medicine's bioavailability. The metabolic degradation of medications by local microflora in the colon may also influence colonic function, adding another layer of complexity. Moreover, the requirement for drugs to be in a solution form prior to absorption can be a rate-limiting step for poorly soluble medicines. Finally, the distal location of the colon in the alimentary canal makes it challenging to access. In conclusion, while CDDS offer targeted therapeutic benefits, overcoming these inherent disadvantages is essential for optimizing their effectiveness in clinical applications [4,19-21].

Colon Absorption

In comparison to the small intestine, the colon has a substantially smaller surface area. The slower pace of transit in the colon allows the medicine to remain in contact with the mucosa for a longer amount of time than in the small intestine, compensating up for the much smaller surface area. Drugs are passively absorbed either through the paracellular or transcellular pathway. Paracellular absorption includes the transfer of pharmaceuticals through the tight connection between cells and is the route most hydrophilic drugs take, whereas transcellular absorption involves the transit of drugs through cells, which is the route most lipophilic drugs use.

The movement of water, electrolytes, and ammonia across the mucosa affects the absorption. The usage of absorption enhancers increases the drug's absorption in the colon, and it exhibits efficient absorption via various membranes. The absorption enhancers alter epithelial permeability by denaturing membrane proteins, open the paracellular route, change lipid-protein interactions, and disrupt the integrity of the lipid barrier by colonic electrolytes. They also cause disruption of the intracellular occluding junction complex. The release and adsorption characteristics of colon-specific drug delivery systems may be impacted by gastrointestinal disorders such as crohn's disease, constipation, diarrhea and so on [1,4,22].

Drug Criteria for CDDS

Drug Candidates: The most suitable medications for CDDS are those that exhibit low absorption from the stomach or intestine, including peptides, as well as those that exhibit stability at an alkaline pH of the gastrointestinal system. The medications used to treat intestinal disorders like inflammatory bowel syndrome (IBS), diarrhea, and colon cancer are perfect candidates for local colon administration. Because of the colon's long retention time, more poorly absorbed substances are absorbed, which increases overall absorption.

Drug Carrier: The decision of which drug carrier to use for a specific drug candidate is based on both the physiochemical character of the drug and the illness for which the system is intended to be used. The choice of drug carrier is also influenced by the chemical structure of the drug, its stability, partition coefficient, and the kind of absorption enhancers used. Finally, the choice of drug carrier is also influenced by the functional groups of the drug molecule. The carriers, which contain additives such polymers, may affect the systems' efficacy and release properties [3,17,23,24].

Mucoadhesion

Bioadhesion, which is defined as the formation of an attachment between a biological substance and an artificial substrate, is important for the development of drug delivery systems. Biopolymers often exhibit bioadhesive characteristics and are utilized for a variety of therapeutic goals [25,26]. Pharmaceutical formulations that are bioadhesive are often created to increase medication bioavailability through localizing the impact at the desired place and lengthening the residence duration of therapeutic agents. They also aid in the formulation design of local drug delivery systems, increasing bioavailability by avoiding metabolic pathways [27,28].

The natural defenses of the body against the deposition of impurities onto the mucous membrane might compromise the mucoadhesion of a system, although mucoadhesive compounds can increase contact with a particular site or tissue. Therefore, it is necessary to have the right properties in order to maintain an effective drug concentration at the action site, manage drug release, enable a reduction in the frequency of drug administration, and improve patient compliance with the therapy [29].

In drug delivery applications, mucoadhesion has rekindled interest in extending the residence period of mucoadhesive dosage forms via different mocusal routes. The bioavailability of topical and local systems based on mucoadhesives has increased. Due to its significant surface area and high blood flow, mucoadhesive drug administration provides quick absorption and good bioavailability. Drug delivery through the mucosa avoids the first-pass hepatic metabolism and gastrointestinal enzyme degradation [30].

Mechanism of Mucoadhesion

One way to characterize mucoadhesion is as an interfacial phenomenon where two materials are kept together by interfacial forces of attraction. One of the materials may be an artificial substance like a mucoadhesive polymer, while the other could be the mucin layer of the mucosal tissue. An artificial material that interacts with mucous membranes to be retained on them or hold them together for a long time is called a "mucoadhesive." Typically, there are two steps to the adhesion process, which are listed below. These mucoadhesion stages are also depicted in Figure 2. [31].

Contact stage: In this phase, an intimate wetting takes place between the mucoadhesive substance and the mucosal membrane as a result of contact. The mucosal membrane's mucus is responsible for wetting the mucoadhesive.

Consolidation stage: The mucoadhesive substance attaches itself to the mucus membrane through a variety of physicochemical factors of attraction, creating a mucoadhesion that is long-lasting. We refer to this phase as the consolidation stage. The process of mucoadhesion ends after these two phases [31].

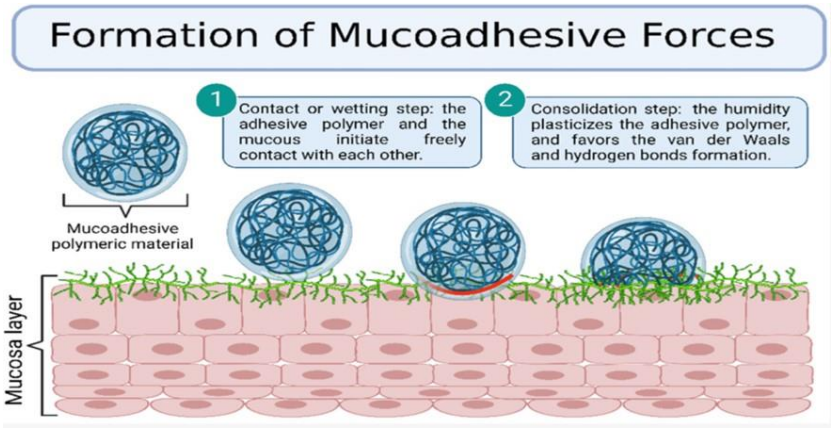

Figure 2. Mucoadhesion mechanism: There are two steps to mucoadhesion. (1) Contact stage: A bioadhesive and a membrane come into close contact (wetting or swelling phenomena). (2) Interactive stage: Interpenetration, or the bioadhesive's entry into the tissue or mucous membrane surface [31]

Challenges in Colon Drug Delivery

A colon-targeted drug delivery system's design must take into account the variations in the GI tract's segments' anatomy, physiology, and absorption properties, in addition to the dosage form's transit kinetics and the GI tract's site of drug release. Additionally, one should take into account the notable distinctions between each individual's healthy and sick GI tract. Understanding the GI tract environment is essential for developing effective dosage forms with enhanced in vitro and pre-clinical in vivo testing when it comes to colon-targeted drug delivery [32]. Consequently, in order to guarantee optimal efficacy following administration, we address here a few of the physiological and pathological aspects of the GI tract that are important in the design of colon-targeted drug delivery.

pH

The pH of the GIT varies depending on its segments. The small intestine has a pH range of 5.9-7.8, which is slightly acidic to neutral, while the stomach has an acidic pH range of 1-3. The pH of the colon varies from 5 to 8 [33]. Since the pH gradient makes it possible to create colon-targeted drug delivery systems that are specifically activated by colonic pH, pH-dependent drug delivery is a crucial tactic from the perspective of drug delivery. One way to prevent medication and carrier degradation in the stomach's acidic environment is to coat the complexes with Eudragit® S100 (polymethacrylate) polymers, which exhibit stability in an acidic pH [34].

However, the intrinsic inter-individual differences and intra-individual variability in GI tract pH values are the key issues with pH-dependent drug delivery devices. Furthermore, it has been observed that individuals with both Ulcerative Colitis and Crohn disease had decreased colonic pH values, which raises questions about the effectiveness of pH-dependent colon-targeted drug delivery regimens [35].

Mucus Barrier

Mucus is a hydrogel layer primarily made up of mucin and other big glycoproteins. When oral dosage forms are used, it is the initial physical barrier to drug absorption in the GI tract [34]. Human intestinal mucus is composed of two layers: a thicker, more adherent basal layer and a looser, luminal layer. The mucosal layer's total thickness varies between 10 and 200 μm (jejunum to colon). The GI mucus has several purposes, such as lubricating chyme, shielding the epithelium from mechanical damage, and adhering to and preventing pathogens from penetrating the epithelial cells. Poor therapeutic outcomes may arise from the majority of foreign particles, including conventional particulate-based drug delivery methods, being effectively retained in human GI mucosal layers by adhesion and eliminated in feces. This effectively limits the period of sustained local drug administration [36].

Transit Time

GI transit times are used by time-dependent release systems to deliver drugs to the colon. In the small intestine, a transit time of 4 hours is commonly acknowledged, with slight inter-individual variations ranging from 2 to 6 hours. On the other hand, colon transit times can differ greatly, ranging from 6 to 70 hours. Furthermore, it has been noted that individuals with colon illnesses such active Ulcerative Colitis have noticeably quicker intestinal transit. In patients with inflammatory bowel disease (IBD), diarrhea and bowel resection may lead to a shorter transit time for traditional oral formulations. Since diseased colon segments may be less exposed to topically active oral medicines as a result of this shortened transit time, therapeutic efficacy against active disease may be significantly diminished [37].

Colonic Microbiota

The human colon is home to around 400 distinct types of both aerobic and anaerobic bacteria. The majority of the anaerobic bacteria in the colon produce a variety of reductive and hydrolytic enzymes to break down polysaccharides in order to meet their energy needs. The large concentration of bacteria in the colon creates a unique environment that can be used to influence the behavior of drugs and dosage forms. Therefore, pro-drugs and polysaccharides that are broken down by colonic microbiota enzymes to release a medication are frequently used in colon-targeted drug delivery systems, such as guar gum, pectin, and chitosan. Nonetheless, a drug's bacterial metabolism may result in toxicity,

activity, or inactivity. Additionally, dietary changes, medication therapy, and illness can all cause variations in the colonic microbiota. These results highlight the ways in which these circumstances might alter the release of drugs from bacterial-enzyme dependent formulations, and they should be taken into account when developing a drug delivery system tailored to the colon [38,39].

Role of Mucoadhesive Polymers in Overcoming Challenges

Formulations with mucoadhesion characteristics can lengthen transit times by adhering to the GI tract's mucosal layers [40]. The mucus binds drug delivery systems by hydrophobic interactions. Alternatively, charged regions of mucin proteins may engage with charged carrier particles and hold them within the mucosal barrier. Drug delivery systems surface chemistry can be changed to boost or decrease adhesion to target-specific cells or biological membranes. Numerous studies showed that cationic drug delivery systems can stick to mucus in the colon and may therefore improve systemic medication absorption to a greater extent [41]. On the basis of this idea, current research has suggested that cationic drug delivery systems may improve mucoadhesion in the colon's surrounding tissues that contain ulcers, and that inflammatory cells' subsequent absorption of the substance may boost treatment outcomes. However, the benefits of cationic particulars in an effective colon delivery may be compromised by non-specific mucoadhesion in the proximal GI tract. It uses a clever strategy that involves shielding the cationic surface of the nano-drug delivery system before the formulation enters the colon and then pH-triggered de-shielding in the colon to get around this issue in oral colon-targeted formulations [42].

If loaded medicinal compounds could be adequately supplied in a sustained manner to the underlying mucosal tissues, colon-specific disorders may be treated more effectively and with fewer adverse effects. It has been highlighted that the GI tract mucosal layers that shield epithelial surfaces are important obstacles to drug delivery systems penetration. In this context, it has been reported on the creation of a mucus-penetrating drug delivery system through the coating of particulate surfaces with a thick layer of polyethylene glycol (PEG) [43].

Mucoadhesive Drug Delivery Systems

Different types of particles with surface modification (cationic or thiolated particulate system) and chemical or physical entrapment of drugs (system made of hydrogels and nanoparticles) are examples of mucoadhesive drug delivery systems. Non-floating in situ gelations (non-floating composite systems of polymeric nanoparticles and hydrogels) can still be achieved in a system made up of hydrogels and nanoparticles [44]. A system's mucoadhesion depends on how the hydrogel and mucosa structure interact. When the hydrogel comes into touch with the mucosa, it swells, which aids in cellular absorption and bioadhesion through chemical or physical interactions. Features like the polymer's molecular weight, hydration, hydrogen bonding ability, chain flexibility, charge, and biological environmental parameters all influence how the hydrogel and mucosa interact [45].

The gastrointestinal system has long been seen as a viable location for the creation of formulations based on mucoadhesives. The use of mucoadhesive polymers to modify the transit duration of delivery systems in a specific area of the gastrointestinal system has piqued the interest of researchers worldwide [46,47].

Mucoadhesive drug delivery system has some advantages and disadvantages and these are;

- Extend the duration of the drug's residency at the tumor site and raise its bioavailability,
- Lower the frequency of dosing,
- Enhance the permeability of drugs,
- Lower the medication dosage that is given,
- Swift onset of action,

But;

- It's possible for the formulation to come loose,
- Overhydration could cause the structure of the formulation to break down [48,49].

Mucoadhesive Polymers

Some mucoadhesive polymers used in colon drug delivery systems are listed below along with some information on how they are used.

Chitosan

A carbohydrate derived from natural sources; chitin is present in many organisms that live. It is present in the cell walls of fungus and yeast as well as being a component of the structure of the exoskeleton of crustaceans. On the chemical basis, it is a poly-β-(1➔4)-N-acetyl-D-glucosamine linear chain. Chitin's unique physicochemical characteristics make it challenging to employ, although it may be treated in an alkaline environment to produce chitosan, a deacetylate derivative [50]. Chitosan is a cationic polymer made up of a linear co-polymer of β-(1➔4)-N-acetyl-D-glucosamine with branches of β-(1➔4)-D-glucosamine that may be randomly distributed. The beneficial biological characteristics of chitosan, including its nontoxicity, biocompatibility, non-immunogenicity, and biodegradability, are widely acknowledged [51]. Chemical structure of chitin and chitosan are represented in Figure 3. [52].

Because free amino groups in its backbone chain are protonated, chitosan can dissolve into a non-Newtonian, shear-thinning fluid in aqueous solutions with pH values lower than its pKa (around 6.5). pH values above 7.5–8 are conducive to regeneration. Chitosan becomes insoluble under these circumstances, causing films, sponges, powders, and fibers to develop [53,54]. Since more amine groups become protonable as deacetylation rises, chitosan solubility is directly correlated with the degree of deacetylation, because chitosan turns unstable at low pH values in the upper gastrointestinal system, it must be coated with a protective layer to stop from solubilizing [55].

Applying various chemical or physical modifications to chitosan is another tactic used to stop it from quickly solubilizing. Crosslinking procedures like grafting, thiolation, carboxymethylation, succinylation, radiation, enzymatic modification, or copolymerization are the main examples of these procedures [56]. To try to increase its structural integrity and stability under various situations, chitosan can be successfully coupled with other natural polymers such alginate, pectin, gums, gelatin, carrageenan, or hyaluronic acid [57].

Because of its hydroxyl groups capacity to create hydrogen bonds, chitosan also possesses mucoadhesive qualities. Through electrostatic interactions, positively charged amino groups from chitosan bind to negatively charged sialic acid that is present on the mucus surface. Mucoadhesion is enhanced by high molecular weight chitosan as well as other elements with an elevated viscosity and degree of deacetylation. Since the sialic acid in mucus is negatively charged at colonic pH, positively charged chitosan/carboxymethyl chitosan nanogels demonstrated superior mucin adsorption and mucoadhesive characteristics. Numerous colonic drug delivery formulations have been tested to examine the mucoadhesive characteristics of chitosan [58].

The mucoadhesive polymer-drug conjugate system is investigated by Shen et al. for the treatment of inflammatory bowel disease (IBD). The quercetin conjugated glycol chitosan product micelles have been produced. They conjugated the medication to the polymer using a ROS responsive linker. Reactive oxygen species, or ROS, can be employed as a stimulus for targeted delivery since they are overexpressed near the site of inflammation in the colon. Less than 20% at physiological pH and with H_2O_2 present means that the entire medication is discharged. Micelle accumulation was seen in the colitis mouse model by biodistribution analysis. In the model of DSS mice, micellar suspension effectively reduced YNF-α, IL-6, and iNOS. This work showed quercetin's inflammatory focused administration for a better IBD therapeutic impact. This work supports the development of sophisticated drug delivery systems for IBD [59].

In order to treat inflammatory bowel disease (IBD), Nalinbenjapun et al. investigated the conjugation of 5-aminosalicylic acid with *N*-(4-aminobenzoyl)-chitosan for colon focused administration. 4-amino benzoyl serves as a divider. In simulated gastric fluid, simulated intestinal fluid, and simulated colon fluid, the medication sulfasalazine has not been released. However, 70% of the medication was released in a 24-hour period in all of the mediums above that contained rat stomach contents. But in just 24 hours, the chitosan-5-ASA conjugate releases only 25% of the medication. This study demonstrated the efficacy of the mucoadhesive polymer-drug conjugate approach in colon

targeted delivery of IBD patients [60].

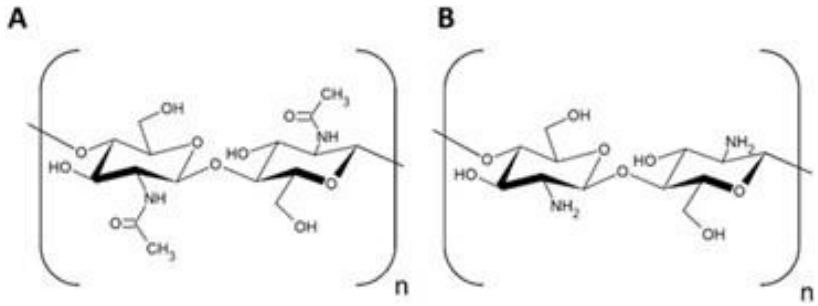

Figure 3. Chemical structure of A: Chitin and B: Chitosan [52]

Alginates

Alginates are naturally occurring linear anionic polysaccharides composed of β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). They are mostly found in the cell walls of brown seaweed. Alginates are mixtures of MG structures (MG-blocks) and M (M-blocks) and G (G-blocks) residues arranged in groups. The ratio of the two monomers can vary depending on the initial natural source [61]. Because alginates are non-toxic, biodegradable, and gel properly, they are widely utilized in biomedicine for a wide range of applications. Traditionally, they have been used in the food business as well as the medicinal, cosmetic, and agricultural sectors [62].

Alginate backbones with carboxyl and hydroxyl groups above them make them ideal mucoadhesive polymers. Through hydrogen bonding, the positive charges of mucin sialic acid and sulfate residues interact with the anionic charges of these groups. Alginates have a lot of hydrophilic groups in their structure, which allows for a lot of hydrogen bonding locations both inside and across polymers, which promotes mucus adherence. A modified PEG containing a functional maleimide end-group was covalently bonded to the alginate backbone. The two reactive carbons in maleimide groups allow them to establish covalent connections with the thiols in mucin. The hydroxyl or amine groups of mucins can also establish hydrogen bonds with maleimide carbonyl groups, greatly enhancing the alginate's mucoadhesive capabilities [63]. Chemical structure of alginate depicted in Figure 4 [64].

In order to effectively transport medication to mice with ulcerative colitis caused by dextran sulfate sodium, Zhang et al. synthesized astaxanthin-enriched colon targeted alginate microsphere. They developed astaxanthin-rich alginate microspheres using high-pressure spraying and the ionic gelation process; the majority of the particles have a diameter between 0.5 and 3.2 μm. Microspheres that are well-tolerated in the mouth, stomach, and small intestine release xanthin in the colon as a result of the gut microbiota fermenting. The administration of microspheres via oral gavage to DSS colitis model mice resulted in significant improvements in weight loss, oxidative damage, inflammation, colon mucosal integrity, and disease activity index. Additional confirmation of the treatment group’s lower histological score comes from H&E staining analysis [65].

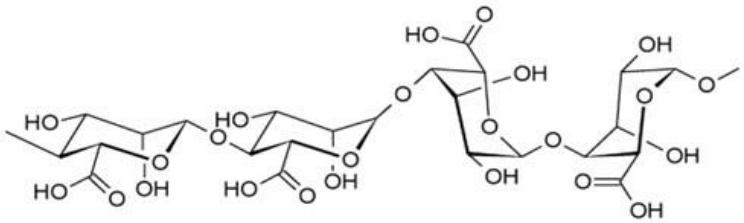

Figure 4. Chemical structure of Alginate [64]

Pectin

Pectins are linear, non-starch, negatively charged polysaccharides that dissolve in water and are

derived from the cell walls of plants. They have α-1,4 D-galacturonic acid and 1,2 D-rhamnose with D-galactose and D-arabinose chains as their backbone. Typically, they have high weights in the 50,000–150,000Da range. The presence or absence of methyl ester substituents in a molecule can vary depending on the plant origin and extraction technique. They are weak acids with a pKa of about 3.5 in aqueous environments. Pectins are inexpensive and non-toxic, which makes them ideal for use in the creation of medicinal formulations [66]. Chemical structure of pectin represented in Figure 5 [67].

Pectins may be broken down by the colonic microorganism pectinase, which makes them ideal for use in CDDS formulations. Pectins are insensitive to upper GIT enzymes found in the stomach and small intestine, such as protease and amylase [68].

Pectin indicate mucoadhesion by an electrostatic connection between the mucin molecule and pectin's carboxylic group, which forms a hydrogen bond with mucin [69].

It's interesting to note that Jorgensen et al.'s study on the impact of pectin's molecular weight on mucin layer penetration efficiency revealed that low molecular weight pectin penetrates the mucin layer more readily. Pectin with a low degree of esterification also exhibits stronger mucoadhesion activity than highly esterified pectin. Researchers looked at combining pectin with different polymers, like pectin-gellan gum beads, modified pectin-acrylate mixed carrier, and pectin-jackfruit seed starch beads, to increase the mucoadhesion activity of pectin [70].

Prezotti et al. developed gellan gum/pectin nanoparticles for the precise delivery of resveratrol to the colonic region via oral administration. They synthesized 330 nm-sized, spherically shaped nanoparticles with over 80% drug loading using the nebulization/ionotropic gelation process. Studies on drug release and permeability use triple co-culture models that secrete mucus and the caco-2 cell model. In stomach acid conditions, the mucoadhesive polymeric nanoparticles released 3% of their resveratrol in two hours, while in pH 6.8, they released 85% in thirty hours. 5.5% permeability was attained. According to this study, mucoadhesive pectin-based nanoparticles are a safe and effective delivery system for resveratrol that can be precisely delivered to the colon [71].

Wang et al. innovatively devised an oral colon-selective drug delivery system by utilizing a pectin/modified nano-carbon sphere nanocomposite gel film. To increase the targeted distribution of pectin-based oral colon products that contain 4-fluorouracil, 3-aminopropyltriethoxysilane modified nano-carbon sphere is inserted into the pectin Ca2+film. Their drug encapsulation efficiency (EE) ranged from 30 to 52%. In simulated gastric fluid (SGF), small intestinal fluid (SIF), and colon fluid (SCF), the composite fluids all displayed superior release rates, with values of 32, 22, and 635, respectively. The biocompatibility of the nanocomposite is confirmed by the in vitro cytotoxicity experiments. Thus, further research must be done before starting clinical trials [72].

Microparticles of amidated pectin were created by Deshmukh et al. to distribute sulfasalazine for inflammatory bowel disease (IBD) in a regulated manner. They created amidated pectin microparticles using the ionic gelation technique. In order to treat IBD, the microparticles placed in Eudragit S 100 coated hard gelatin capsules allow for pH- and time-dependent medication administration to the colon. The ideal formulation had a size of 463 nm, a zeta potential of -32, a yield of 91%, and an EE of 95%. At pH 6.8 and 7.4, the swelling index values are 0.88 and 0.98, respectively. During a 24-hour period, the drug releases 91% in simulated colonic fluid and 98% in rat cecal content. When given to the rabbit orally, the capsule crumbled at colonic pH 7.4 and released the medication from microparticles. The particles had a 3.3-year shelf life and demonstrated excellent stability. This study found that hard gelatin capsules coated with Eudragit S 100 and filled with amidated pectin microparticles may be a useful delivery strategy for the treatment of inflammatory bowel disease [73].

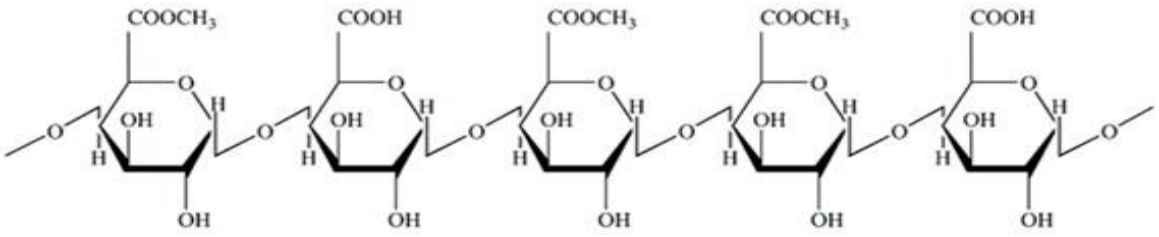

Figure 5. Chemical structure of Pectin [67]

Cellulose Derivatives

A cellulose derivative known as hydroxypropyl methylcellulose (HPMC) has hydroxypropyl and methoxyl group substituents joined to a cellulose backbone by ether bonds. The ether polymer HPMC is non-ionic, soluble in water, resistant to enzymes, and chemically stable at pH values between 3.0 and 11.0 [74].

Through hydrophobic/electrostatic interactions and hydrogen bonding, HPMC hydrophilic groups can form an association with the mucus layer. Mucin glycoproteins can interpenetrate the enlarged matrix to form a relaxed mesh and interact with the polymer, so swelling is essential for mucoadhesion to occur. The degree of crosslinking increases with an increase in HPMC content in a formulation, which limits the amount of HPMC chains available for the mucus layer to interpenetrate. A formulation's mucoadhesion is aided by the formation of additional pores in the enlarged matrix structure caused by a reduction in HPMC content. These pores allow mucin glycoproteins to interface with the matrix chains. Thus, it has been demonstrated that HPMC interacts with the mucosal layer but has inferior mucoadhesive qualities to other polymers [75,76].

Carboxymethyl cellulose (CMC) is a derivative of cellulose. It's a mucoadhesive polymer that dissolves in water. It is non-toxic, hydrophilic, bioadhesive, pH-sensitive, and gels readily. CMC is frequently utilized to deliver drugs. Because of their mucoadhesive responsive release properties, CMC hydrogels are more fascinating [77,78]. Chemical structure of CMC depicted in Figure 6 [79].

To deliver nucleolin specifically to colon adenocarcinomas, Nejabat et al. synthesized acetylated carboxymethylcellulose-coated mesoporous silica hydride nanoparticles. Hollow mesoporous silica nanoparticles (HMSNs) loaded with doxorubicin (DOX) and coated with acetylated carboxymethylcellulose (Ac-CMC) converted covalently with AS1411 aptamer. These nanoparticles improved blood circulation and released drugs in a regulated, sustained manner. The study conducted in vitro cytotoxicity and cellular uptake validates the targetability of AS1411 to nucleolin overexpressing MCF-7 and C26 cells. *In vivo* tumor inhibition demonstrated by the formulation was excellent [80].

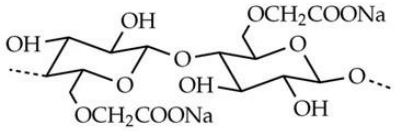

Figure 6. Chemical structure of Carboxymethyl Cellulose [79]

Poly (Acrylic Acid)

One of acrylic acid's derivatives is poly (acrylic acid), or PAA. As a homopolymer, PAA can generate a range of cross-linked and co-polymers. Being an anionic polymer, it could result in a negative charge if a proton is lost from the side chain of PAA. The deprotonated PAA exhibited the capacity to take in water and expand several times above its initial volume. Azobisisbutyronitrile (AIBN) and potassium persulfate can be used in free radical polymerization to create PAA. Because PAA is susceptible to mucoadhesion in its protonated form at colon pH, it is regarded as a mucoadhesive polymer. PAA's COOH and the sialic COOH of the mucin glycoprotein combine to form an H-bond. Viscosity was increased by this bond formation. Mucoadhesive colon drug delivery systems based on PAA have been fully investigated [81]. Chemical structure of PAA represented in Figure 7 [82].

Dey et al. used barley-grafted PAA to accomplish pH-sensitive delivery. In this investigation, 5-ASA was employed as a model medication for colon targeting. Given that barley is the fourth most widely grown food commodity in the world. Starch makes over 95% of the polysaccharides in barley. The most promising technique for changing polysaccharides is grafting. PAA grafted barley was created by Dey et al. with the use of a microwave. Anti-inflammatory medication 5-ASA is loaded, which is used to treat inflammatory bowel disease. Drug absorption rate is high in lower GIT; drug release follows the Fickian diffusion mechanism at both pH 1.2 and 7.2 [83].

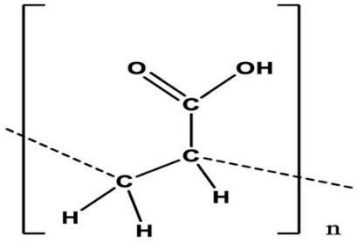

Figure 7. Chemical structure of Poly (Acrylic Acid) [82]

Current Chanllenges in Mucoadhesive Colon Drug Delivery

Mucoadhesive drug delivery systems encounter a number of difficulties, especially when it comes to colon-specific administration. Since last update, a few difficulties are as follows:

Variable Gastrointestinal Conditions: There are fluctuations in pH, enzyme activity, and transit time within the dynamic gastrointestinal environment. It can be difficult to design a mucoadhesive system that can tolerate these changes and release the medication in the colon efficiently.

Mucus Layer Variability: Individuals and various disease situations may have differences in the thickness and makeup of the mucus layer in the colon. The overall efficacy and mucoadhesive qualities of drug delivery systems may be impacted by this diversity.

Biodegradability and Biocompatibility: To prevent long-term negative effects, the materials employed in mucoadhesive systems must be both biocompatible and biodegradable. It is difficult to find materials that maintain excellent mucoadhesion while striking a compromise between these qualities.

Medication Stability: Some medications may degrade in the hostile gastrointestinal environment, which could have an impact on how effective they are. It is essential to guarantee the drug's stability during its passage through and release in the colon.

Optimal Release Kinetics: To optimize therapeutic efficacy, regulated and sustained medication release in the colon must be achieved. To guarantee the intended drug concentrations over an extended period of time, release kinetics must be carefully taken into account during the design of mucoadhesive systems.

Patient Compliance: Patients should find it convenient and agreeable to receive medication via mucoadhesive delivery methods. Patient compliance may be impacted by elements like dosage frequency, dosage form size, and simplicity of administration.

Regulatory Acceptance: For mucoadhesive drug delivery systems intended for colon-specific uses, regulatory approval necessitates proving safety, effectiveness, and uniformity. Complying with regulatory regulations makes the development process even more complex.

Scale-up Problems: Maintaining the uniformity and repeatability of mucoadhesive drug delivery systems can be difficult when moving from laboratory-scale production to large-scale manufacturing [78].

Through advances in materials science, formulation design, and targeted drug delivery systems, scientists and pharmaceutical companies are actively addressing these difficulties. Mucoadhesive colon medication delivery methods are becoming more dependable and successful as a result of developments in polymer chemistry, nanotechnology, and mucosal physiology.

RESULT AND DISCUSSION

Mucoadhesive polymers are essential for improving drug delivery methods, especially when it comes to administering drugs to the colon. These polymers have a number of benefits for colon-specific medication delivery because of their capacity to stick to mucosal surfaces. Mucoadhesive polymers enable focused and localized treatment by prolonging the duration of medication interaction with the intestinal mucosa due to their adhesive qualities. This is particularly important when treating disorders including colonic infections, colorectal cancer, and inflammatory bowel diseases. Both synthetic and natural polymers, such as alginate and chitosan, as well as derivatives of polyacrylic acid, are frequently

used in these systems.

Mucoadhesive polymers' capacity to deliver drugs to targeted sites while reducing systemic side effects is one of their main benefits. Moreover, the extended residence time promotes better bioavailability by enabling controlled and sustained medication release. Despite these advantages, there are also drawbacks, such as individual differences in mucosal conditions and the critical requirement to guarantee the biocompatibility of these polymers in order to avoid negative reactions. Mucoadhesive polymers are used in a wide range of medical applications, such as the precise delivery of antibiotics for colonic infections, the localized administration of chemotherapeutic agents for the treatment of colon cancer, and the targeted delivery of anti-inflammatory drugs for inflammatory bowel diseases.

In conclusion, mucoadhesive polymers present a viable approach to enhancing colon drug delivery, with enormous promise for the creation of efficient and well-tolerated treatments for a range of colonic illnesses.

AUTHOR CONTRIBUTIONS

Concept: A.D.G., T.C.; Design: A.D.G., T.C.; Control: A.D.G., T.C.; Sources: A.D.G., T.C.; Materials: A.D.G., T.C.; Data Collection and/or Processing: A.D.G., T.C.; Analysis and/or Interpretation: A.D.G., T.C.; Literature Review: A.D.G., T.C.; Manuscript Writing: A.D.G., T.C.; Critical Review: A.D.G., T.C.; Other: A.D.G., T.C.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

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DERLEME MAKALE / REVIEW ARTICLE

İNSAN PAPİLLOMA VİRÜSÜ (HPV) TEDAVİSİNDE YENİ YAKLAŞIMLAR: AKTİF HEKSOZ İLİŞKİLİ BİLEŞİK (AHCC®)

NEW APPROACHES IN HUMAN PAPILLOMAVIRUS (HPV) TREATMENT: ACTIVE HEXOSE-RELATED COMPOUND (AHCC®)

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ÖZ

Amaç: *İnsan papilloma virüsü (HPV) alt tiplerine bağlı olarak cilt ve mukoza zarlarında siğil ve kanser oluşumuna sebep olabilen cinsel yolla bulaşan en yaygın viral enfeksiyon olması sebebi ile ciddi bir halk sağlığı problemi oluşturmaktadır. Günümüzde HPV tedavisinde hastalığın eradikasyonunu sağlamayan, sadece dışa doğru büyüyen siğillerin uzaklaştırılmasını ve semptomların iyileştirilmesini amaçlayan seçenekler mevcuttur. Bu tedavilerin dışında immün sistemi destekleyici çeşitli doğal ürünlerin kullanımının da HPV tedavisinde faydalı olabileceği gösterilmiştir. Mantar ekstraktlarının bağışıklık sistemi üzerindeki kesin etkileri tam olarak aydınlatılamamış olsa da uzun yıllardan beri dünyanın farklı bölgelerinde çeşitli sağlık sorunları için kullanılmaktadır. Bu derlemede bir mantar ekstratı olan AHCC®'nin HPV enfeksiyonu üzerindeki etkilerine odaklanılmıştır.*
Sonuç ve Tartışma: *Yenilebilir bir mantar olan Lentinula edodes'in asetillenmiş α-1,4-glukanlar bakımından zenginleştirilmiş, standartlaştırılmış, kültürlenmiş bir özütü olan AHCC® sahip olduğu çeşitli farmakolojik etkileri nedeni ile HPV tedavisinde öne çıkan alternatif tedavi seçenekleri arasında yer almaktadır. Yapılan preklinik ve klinik çalışmalar, AHCC®'nin bağışıklık sistemini destekleyerek HPV tedavisi için umut veren yeni bir seçenek olabileceğini göstermektedir.*
Anahtar Kelimeler: *AHCC®, besin takviyeleri, HPV*

ABSTRACT

Objective: *Human papillomavirus (HPV) poses a serious public health problem as it is the most common sexually transmitted viral infection that can cause warts and cancer on the skin and mucous membranes, depending on its subtypes. Current treatment options only aim to remove warts and improve symptoms, without eradicating the disease. In addition to these treatments, it has been shown that the use of various natural products that support the immune system can be beneficial in the treatment of HPV. Although the pharmacological effects of mushroom extracts on the immune system have not been fully elucidated, they have been used for various health problems in different parts of the world for many years. This review focused on the effects of AHCC®, a mushroom extract, on HPV infection.*
Result and Discussion: *AHCC®, a standardized, cultured extract of the edible mushroom Lentinula edodes, enriched in acetylated α-1,4-glucans, is among the prominent alternative treatment options*

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in the treatment of HPV due to its pharmacological effects. Preclinical and clinical studies show that AHCC® may be a promising new option for HPV treatment by supporting the immune system.
Keywords: *AHCC, HPV, nutritional supplements*

GİRİŞ

İnsan papilloma virüsü (HPV), Papovaviridae familyasına ait yaklaşık 7.900 baz çifti içeren tek bir çift dairesel deoksiribonükleik asit (DNA) molekülünden oluşan ve beş cinse (alfa, beta, gama, mu ve nu papillomavirüs) ayrılan heterojen bir virüs grubudur [1-3]. Alfa cinsi en büyük grup olup siğillere neden olan türleri ve kanserojen potansiyele sahip virüsleri içermektedir [4-6]. Tropizmlerine dayanarak, HPV tipleri kutanöz ve mukozal HPV tipleri olmak üzere 2 ana gruba ayrılabilmektedir [7]. Alfa papilloma virüslerinin büyük çoğunluğu mukozal tropizm sergilemekle beraber deride yaygın siğillere sebep olan kutanöz tipler de içermektedir. Gama, mu ve nu cinsleri kutanöz tropizm sergilemekte ve siğillere neden olmaktadır [8]. Papillomavirüsler, konakçı türleriyle birlikte evrimleşmiştir ve 200'den fazla HPV genotipi bilinmektedir [1,5].

Ayrıca HPV tipleri onkojenik risk açısından düşük riskli HPV'ler HPV 6, 11, 40, 42, 43, 44, 54, 55 ve 62; olası yüksek riskli HPV'ler HPV 26, 53 ve 66 ve yüksek riskli HPV'ler HPV 16 başta olmak üzere HPV 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 68, 73 ve 82 şeklinde sınıflandırılmaktadır. Yüksek riskli HPV'ler, özellikle HPV 16 ve 18, yüksek dereceli servikal, anal displazi ve invaziv karsinom ile ilişkilidir. Düşük riskli HPV'ler ise erkeklerde ve kadınlarda anogenital kondilomlara ve laringeal papillomatoza yol açabilmektedir [9].

Günümüzde HPV tedavisi için profilaktik aşılama, siğillerin uzaklaştırılması için kullanılan topikal kremler ve klinik araştırma aşamasındaki ilaçlar da dahil olmak üzere çeşitli farmakolojik tedavi seçenekleri mevcuttur. Ancak enfeksiyonun vücuttan tamamen uzaklaştırılmasına aracılık etmeyen bu seçenekler sadece semptomların iyileştirilmesinde etkili olabilmektedir [2,3]. Bu nedenle, hastalığın eradikasyonunun sağlanabilmesi için bağışıklık sistemini destekleyici takviyelerin önemi dikkat çekmektedir. Kültürlenmiş *Lentinula edodes* miselyumunun tescilli, standartlaştırılmış bir özütü olan AHCC®, bu takviyeler arasında son zamanlarda üzerinde en çok çalışma yapılan bileşiktir. Çeşitli hayvan ve insan çalışmalarında, AHCC®'nin antioksidan, antikanser aktiviteleri ve bağışıklık sisteminin modülasyonu ile hem viral hem de bakteriyel enfeksiyonların riskini azaltma ve mevcut enfeksiyonların semptomlarını iyileştirme faydası olduğu gösterilmiştir [2]. Bu bilgiler doğrultusunda, bu çalışmada HPV enfeksiyonu, güncel tedavi seçenekleri ve son zamanlarda HPV'nin semptomları iyileştirmenin yanı sıra eradikasyonuna da yardımcı olabileceğini gösteren çalışmalarla ön plana çıkan AHCC® hakkında bilgiler derlenmiştir.

HPV Epidemiyolojisi

Cinsel olarak aktif kadınların yaklaşık %50-80'inin yaşamları boyunca HPV ile enfekte olabileceği öne sürülmektedir ve küresel HPV prevalansı %11.7 olarak tahmin edilmektedir [10]. Dünya genelinde cinsel yönden aktif erkeklerde yapılan bir başka araştırma, neredeyse her üç erkekten birinin en az bir genital HPV tipi ile enfekte olduğunu ve yaklaşık her beş erkekten birinin bir veya daha fazla yüksek riskli HPV tipleri ile enfekte olduğunu belirtmektedir. HPV prevalansının 15 yaşın üzerindeki erkeklerde yüksek olduğu ve 25-29 yaş arası en yüksek seviyeye ulaştığı görülmektedir. Bu veriler yaştan bağımsız olarak cinsel olarak aktif erkeklerin HPV genital enfeksiyonunun önemli bir kaynağı olduğu görüşünü desteklemektedir [11].

En yüksek HPV prevalanslarının görüldüğü konumlar arasında Güney Afrika (%17.4), Doğu Afrika (%33.6), Doğu Avrupa (%21.4), Batı Avrupa (%9.0), Doğu Avrupa (%21.4) ve Karayipler (%35.4) yer almaktadır. Dünya genelinde yürütülen bir çalışmada 12,7 milyon çeşitli kanser vakasında, HPV enfeksiyonunun servikal kanserlerin neredeyse %100'ü, anal kanal kanserlerinin %90-93'ü, orofaringeal kanserlerin %12-63'ü, vajinal kanserlerin %40-64'ü, vulvar kanserlerin %40-51'i ve penis kanserlerinin %36-40'ı ile ilişkili olduğunu gösterilmiştir. Ayrıca, kutanöz HPV tipleri melanom dışı deri kanserlerinin görülme riskinde artış ile ilişkilendirilmiştir [10].

HPV prevalansı, Türkiye'de popülasyona ve coğrafi bölgeye göre değişiklik göstermektedir [12]. Çukurova bölgesindeki kadınlardan alınan toplam 460 örnekten 24'ünde HPV DNA pozitifliği

görülmüştür ve bu bölgedeki kadınlarda genital HPV enfeksiyon prevalansı %5.2 bulunmuştur. HPV DNA pozitif 30 yaş ve üstü olan 24 kadında en sık görülen yüksek riskli HPV tipi HPV 16 (%33.3) olup bunu sırasıyla HPV 45 (%20.8), HPV 18 (%4.2) ve HPV 31 (%4.2) izlemiştir [13]. Sivasta yaşları 21 ile 67 arasında değişmekte olan 140 kadın üzerinde yapılan çalışmada kadınların % 6,4'ünde HPV DNA pozitifliğine rastlanmıştır ve HPV 6 (%25) en sık rastlanan genotip olurken ikinci sırada HPV 16 (% 16.6) gelmiştir [14]. Nisan 2014-Nisan 2021 yılları arasında Adana'da yapılan bir çalışmada ise toplam 2329 kadın hastanın verileri retrospektif olarak incelenmiştir ve 1046 kadında HPV DNA pozitifliği görülmüştür. Hastalarda en sık görülen HPV tipi HPV 16 (%14.2), daha sonra sırasıyla HPV 68 (%8.2), HPV 56 (%8.2), HPV 52 (%7.1), HPV 51 (%6.8), HPV 31 (%6.5), HPV 66 (%6.1), HPV 39 (%5.8) ve HPV 18 (%5.6) olarak saptanmıştır [15]. Ankara'da üçüncü basamak bir üniversite hastanesine Ocak 2017-Kasım 2020 tarihleri arasında jinekolojik semptomlarla başvuran 18-79 yaş aralığındaki kadınlardan HPV genotiplerinin taranması amacı ile toplam 4267 servikal sürüntü alınmıştır. Örneklerin %14.2'sinde (605/4267) HPV DNA pozitifliği görülmüştür ve HPV pozitif hastaların %2.4'ünde HPV 16 ve %0.7'sinde HPV 18 tespit edilmiştir. Örneklerin %8.8'inde diğer yüksek riskli genotipler bulunmuştur [16]. Ayrıca, 1 Ocak 2017 ile 1 Mart 2022 tarihleri arasında klinik muayene için başvuran ve serviks kanseri taraması yapılan hastaların retrospektif incelemesinin yapıldığı bir başka çalışmada, 529 vakanın %41.6'sı HPV pozitif tanısı aldığı belirtilmektedir [17].

Genel olarak ise Katalan Onkoloji Enstitüsü ve Uluslararası Kanser Araştırmaları Ajansı'nın HPV ve HPV'ye bağlı gelişen hastalıklar 2019 Türkiye raporuna göre Türkiye'de HPV 16 ve 18 prevalansı %4.2 ile %67.6 arasında değişkenlik göstermektedir [12].

HPV Enfeksiyonun Bulaş Yolları

HPV'nin öncelikli bulaş yolu olarak ciltten cilde veya ciltten mukozaya temas kabul edilmektedir [18]. HPV'nin bulaş yolları arasında cinsel temas, otoinokülasyon, anneden bebeğe dikey bulaş, fomitler ile bulaş ve inhalasyon ile bulaş yer almaktadır. Ancak HPV'nin en önemli bulaş şekli cinsel ilişkidir ve HPV cinsel aktivite esnasında skuamöz ya da mukozal epiteldeki aşınmalar veya hasarlarla bazal hücrelere ulaşması sonucu enfeksiyonlara sebep olmaktadır [10,12,19]. En sık vajinal, anal ve oral ilişki ile bulaş görülmesine rağmen cinsel ilişki olmadan sadece cilt temasıyla da bulaşma görülebilmektedir. Cinsel aktif yetişkinlerin %80–90'ına HPV bulaşının gerçekleştiği ve lezyon oluşumu düşük bir ihtimal olduğu için bulaş gerçekleşenlerin büyük bir kısmının enfeksiyonun farkında olamadığı belirtilmektedir [20]. Daha önce cinsel ilişkiye girmemiş kişilerde de genital siğil görülmüş olması ve virülansı tespit edilmemiş olsa da havlu, iç çamaşırı ve banyo yüzeylerinde HPV DNA'sının tespit edilmesi cinsel ilişki dışında fomit ile bulaşa işaret etmektedir [21]. Bir konakçı içinde otoinokülasyon, genital bölgeden parmaklara veya parmaklardan genital bölgeye bulaş şeklinde olmakta ve görece nadiren gerçekleşmektedir [22]. Ayrıca, amniyotik sıvı veya plasenta yoluyla veya doğal doğum sırasında maternal genital mukoza ile temas sonucu enfeksiyon bulaşı olasılığı da vurgulanmaktadır. Doğum sırasında anneden bebeğe bulaşan HPV enfeksiyonu bebekte laringeal ve konjuktival papillomlara yol açabilmektedir [18]. İnhalasyon ile bulaş her ne kadar tartışmalı kabul edilse de lazer ve elektrokoter tedavisinde ortaya çıkan dumandan kaynaklı olarak uzmanların nazofarenksinde HPV DNA'sı tespit edilmiştir. Bu olgular göz önünde bulundurulduğunda işlem sırasında dumanı uzaklaştırıcı sistemler, maske, eldiven, önlük ve gözlük kullanılması önerilmektedir [23,24].

HPV ile İlişkili Hastalıklar

HPV'nin rahim serviksinin tüm kanserlerinde nedensel rolü olabileceği kanıtlanmıştır. Vajina ve anüs kanserlerinin çoğu, vulva, penis ve orofarenks kanserlerinin bir kısmı da HPV ile ilişkilendirilmektedir. HPV 16 ve HPV 18'in serviks, vajina ve anüs kanserlerinin yaklaşık % 70'inden ve vulva, penis ve orofarenks kanserlerinin yaklaşık % 30-40'ından sorumlu olduğu bilinmektedir. HPV'ye bağlı açığa çıkabilecek diğer kanserler arasında melanom dışı cilt kanseri ve konjonktiva kanseri yer almaktadır. Hormonal kontraseptiflerin uzun süreli kullanımı, yüksek parite, sigara kullanımı, İnsan İmmun Yetmezlik Virüsü (HIV), *Chlamydia trachomatis*, herpes simpleks virüsü tip-2 gibi enfeksiyonlar ve bazı diyet eksiklikleri HPV'nin kansere ilerlemesine sebep olabilecek kofaktörler arasında yer almaktadır [25].

Düşük riskli tipler arasında onkojenik olmayan HPV tipleri, anogenital siğiller [9], bazı deri siğilleri [26] ve tekrarlayan solunum papillomatozisi ile ilişkilendirilirken, yüksek riskli onkojenik tipler belirtildiği gibi servikal, penil, anal, vajinal, vulvar ve orofaringeal kanserler ile ilişkilendirilmektedir [9]. Alfa cinsine ait HPV enfeksiyonları, serviks ve anüsteki skuamöz intraepitelyal lezyonların ve kanserlerin neredeyse tamamı ile açıkça ilişkilendirilmiştir. Bu kanserlerle ilişkili HPV tiplerinden en yaygın görülen HPV 16, kansere ilerleme açısından en yüksek riske sahiptir. Yüksek dereceli intraepitelyal neoplazinin bir formu olan intraepitelyal skuamöz hücreli karsinom Bowen hastalığı hem genital hem de ekstragenital formlara sahiptir ve el-ayak parmaklarında, avuç içlerinde ve genital mukozada oluşabilmektedir. Bu lezyonlardan HPV 16, 18, 31, 32 ve 34 dahil çok sayıda HPV tipi izole edilmiştir [27].

Korunma Yöntemleri

HPV enfeksiyonları çoğunlukla bağışıklık sistemi güçlü hastalar tarafından 2 yıl içinde vücuttan uzaklaştırılmaktadır. Ancak onkojenik veya yüksek riskli tiplerle kalıcı enfeksiyonlar prekanseröz lezyonlara ve kansere yol açabilmektedir [28]. Etkili birincil (HPV aşısı) ve ikincil (tarama programları) korunma yaklaşımları servikal kanser başta olmak üzere HPV ile ilişkili kanserler ve hastalıkların önlenmesinde önem taşımaktadır. HPV'de birincil korunma yöntemleri arasında tek eşli cinsel yaşam, ilk cinsel ilişki yaşının geciktirilmesi, bariyer yöntemlerin kullanımı gibi cinsel bulaş risk faktörlerinin ortadan kaldırılması ve profilaktik HPV aşı uygulamaları yer almaktadır [12,29]. Amerikan Gıda ve İlaç Dairesi (FDA) tarafından onaylanan iki değerlikli aşı (Cervarix®) HPV 16, 18'e karşı koruyucu, dört değerlikli aşı (Gardasil®) HPV 16, 18, 11, 6'ya karşı koruyucu, dokuz değerlikli aşı (Gardasil® 9) HPV 16, 18, 11, 6'ya ve HPV 31, 33, 45, 52 ve 58'e karşı koruyucu özelliktedir [28,30]. Tüm aşılar benzer düzeyde etkili kabul edilmektedir ve ciddi yan etkilere sebep olmamaktadır. Aşıların virüse maruziyetten önce protokole göre uygulanması durumunda neredeyse tam koruma sağladığı belirtilmektedir [5]. Kızlar için 9-26 yaş arası aşı yapılabilir ve 11-12. yaşlarda rutin olarak önerilmektedir. Erkeklerde ise dört değerlikli ve dokuz değerlikli HPV aşıları, 9-21 yaş arası yapılabilir ve 11-12. yaşlarda rutin olarak aşılama önerilmektedir. Genel olarak, aşılardan en yüksek yararı ilk cinsel aktiviteden önce uygulandıklarında elde edildiği için genç yaşta yapılmaları önerilmektedir [16]. Ancak İmmünizasyon Uygulamaları Danışma Komitesi tarafından Gardasil® aşısı için onaylanan yaş aralığı kadınlarda ve erkeklerde 9–26 yaş aralığından 9–45 yaş aralığına genişletilmiştir [12]. Ayrıca genital siğili olan, HPV testi pozitif olan, genital prekanser öyküsü olan kişilere de HPV aşısı uygulanabilmektedir. HPV aşılaması Türkiye'de henüz ulusal bağışıklama programında yer almamaktadır ancak son yıllarda kadınlarda aşılanma oranları artış göstermektedir [16]. Yapılan çalışmalarda, sünnetin diğer cinsel yolla bulaşan hastalıklar ile birlikte HPV enfeksiyonu için de koruyucu özellikte olduğu tespit edilmiştir [31,32]. Ayrıca sigara içenlerde HPV görülme sıklığının daha yüksek olması, genital siğilin vücuttan uzaklaştırılmasının daha güç olması ve tekrarlama olasılığının daha yüksek olması sebebiyle sigaranın bırakılması tavsiye edilmektedir [33,34].

İkincil korunma, tarama programlarının etkili uygulanması erken tanı ve tedaviye olanak sağlamaktadır. Sistematik ve nitelikli bir tarama programı altyapısı ve toplumda farkındalık oluşturulması HPV ile ilişkili rahatsızlıkların önlenmesinde önem taşımaktadır [29]. Ülkemizde, ulusal serviks kanseri tarama programı Sağlık Bakanlığı Kanser Dairesi Başkanlığı tarafından 2014 yılından itibaren yürütülmektedir. Bu program çerçevesinde 30-65 yaş arası kadınlar 5 yıl aralıklarla Kanser Randevu Sistemi ya da telefon aracılığıyla sağlık kuruluşlarına davet edilmekte ve rahim ağzı sürüntüsü alınarak HPV DNA testi yapılmaktadır [35,36]. Kişinin geçmiş tarama sonuçları Aile Hekimliği Bilgi Sistemi üzerinden kontrol edilerek kişiye uygun zaman için randevu verilmektedir [36]. Ulusal tarama programında HPV tipleri 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 ve 68 bakılmaktadır. Riskli grup HPV tespit edilen kadınların rahim ağzı sürüntülerinden Pap Smear alınıp sitolojik inceleme yapılmaktadır. HPV testi pozitif olan kadınlar sitoloji değerlendirilmesi yapıldıktan sonra gerekli görülürse kadın doğum uzmanlarına yönlendirilmekte ve bu şekilde lezyonların erken dönem tedavisi yapılabilmektedir [35].

Güncel HPV Tedavi Yöntemleri

HPV ile ilişkili servikal hastalıkların tam olarak tedavi edildiğini gösteren hiçbir veri yoktur.

Tedavinin birincil amacı semptomları iyileştirmek, siğillerin dönüşüm bölgesini ortadan kaldırmak ve gelecekteki muhtemel invaziv servikal kanser riskini azaltmaktır. HPV ile ilişkili hastalığın yönetimi ve tedavisi büyük ölçüde hastada mevcut olan HPV tiplerine, tercih edilen tedavi yöntemine ve hastanın genel sağlık durumuna bağlıdır [37]. HPV aşılamasının hastalığın önlenmesi ve yönetimi üzerine etkili olduğu bilinse de dış anogenital siğillerin topikal tedavisi hem görünür hem de görünmeyen (subklinik) lezyonlar için kaçınılmaz bir yöntem olarak kabul edilmektedir [38].

İmikimod makrofajları, interlökin-2, interferon-αlfa ve diğer sitokinlerinin salgılanmasını uyaran bir bağışıklık düzenleyicidir [37]. FDA tarafından HPV tedavisi için onaylanan İmikimod %5 krem büyük ölçüde anogenital siğillerin tedavisinde kullanılmaktadır. Tek kullanımlık saşeler halindeki İmikimod %5 kremin haftada üç kez uykudan önce doğrudan siğillere uygulanması ve 6-10 saat sonra sabun ve su ile yıkanıp vücuttan uzaklaştırılması gerekmektedir. Tedavinin siğil temizlenene kadar veya en fazla 16 hafta boyunca sürdürülmesi önerilmektedir [39]. Yapılan bir çalışmada dış anogenital siğilleri olan gebe kadınlarda İmikimod %5 kremin birinci basamak terapötik etkinliği ve güvenliği değerlendirilmiştir ve ciddi advers etki, fetal ve/veya neonatal anormallik gözlenmemiştir. Sonuçlar, gebelikte anogenital siğil tedavisinde İmikimod kullanımının umut verici ve güvenilir olduğunu düşündürmektedir [40].

Onkojenik olmayan HPV'lerin neden olduğu dış genital siğillerin tedavisi için önerilen tedaviler arasında antimitotik bir ajan olan Podofilotoksin de yer almaktadır. Podofilotoksin (%0.5) uygulamasının, dış genital siğillerin tedavisinde hem güvenli hem de etkili olduğu gösterilmiştir [37,41,42].

Sinekateşin, *Camellia sinensis* türlerinin yeşil çay yapraklarından elde edilen, kaspaz yolunu aktive eden ve telomerazı inhibe eden çeşitli immünomodülatör ve antiproliferatif özelliklere sahip bir bileşiktir. Yapılan çalışmalar, topikal olarak uygulanan Sinekateşin merhemin (%10 ve %15) dış anogenital siğilleri temizlemek ve siğil oluşumunu engellemek için etkili, iyi tolere edilen, kendi kendine uygulanabilen bir tedavi seçeneği olduğunu göstermektedir [37].

Gebelik sürecinde güvenle kullanılabileceği belirtilen %80-90 Triklorasetik asit çözeltisi, aşındırıcı özelliktedir ve tahta veya pamuk uçlu bir aplikatör ile doğrudan siğil yüzeyine uygulanması önerilmektedir. Ayrıca DNA sentezini bloke eden bir anti-metabolit olan 5-fluorourasil, yüzeysel bazal hücreli karsinom dahil olmak üzere neoplastik ve preneoplastik cilt rahatsızlıklarını tedavi etmek için %5'lik krem olarak kullanılmaktadır. Ancak diğer tedaviler başarısız olduğunda düşünülmesi önerilmektedir. İntralezyonel/topikal interferonun, anogenital siğiller tedavisinde sistemik kullanımına ilişkin kanıt yoktur ancak interferon-alfanın lokal uygulandığı çalışmaların bazılarında olumlu sonuçlar elde edilmiştir. Topikal veya enjekte edilen intralezyonel interferon tedavilerinin plaseboya göre üstün temizleme sağladıklarını gösteren çalışmalar mevcuttur [39].

Onkojenik HPV'lerin neden olduğu servikal prekanseröz lezyonların tedavisi için eksizyonel cerrahi yöntemler, kriyocerrahi (dondurma), koni biyopsi, konizasyon veya loop elektrocerrahi eksizyon prosedürü (LEEP) olarak da adlandırılan elektrocerrahi ve lazer tedavisi de hekimler tarafından uygulanabilecek tedavi seçenekleri arasında yer almaktadır. Alternatif olarak fotodinamik tedavi (fotosensitizer olarak topikal 5-aminolevulinik asit ve ardından ışınlama), bazı sınırlı dış genital siğil tedavileri için belirli durumlarda kullanılabilmektedir [37]. Cerrahi birincil tedavi olarak kullanılabilmektedir ve hastalar lokal anestezi altında tedavi edilebilmektedir. Dikkatli bir şekilde uygulandığında, basit cerrahi yaklaşımlar ile oldukça tatmin edici sonuçlar elde edilebilmektedir [39].

Bütün bu tedavi seçeneklerinin yanı sıra immün sistemi destekleyici çeşitli bitkisel ürünlerin kullanımının da HPV tedavisinde faydalı olabileceği gösterilmiştir. *Hamamelis virginiana* kabuğu [43], *Ficus religiosa* kabuğu [44], *Phyllanthus emblica* meyvesi [45], genistein [46], *Bryophyllum pinnatum* yaprağı [47], *Pinellia pedatisecta* rizomu [48] ile yapılmış çalışmalar mevcuttur [49]. AHCC®, sahip olduğu antienflamatuvar, antikanser ve antiviral aktivite gibi farmakolojik etkileri sebebiyle HPV tedavisinde dikkat çeken alternatifler arasında yer almaktadır.

AHCC®

AHCC®, 1987 yılında Tokyo Üniversitesi Farmasötik Bilimler Fakültesi'nde yüksek tansiyonu düzenlemek için doğal bir ürün olarak geliştirilmiştir [50,51]. AHCC®, Basidiomycete mantar ailesinden yenilebilir bir mantar olan *Lentinula edodes*'in asetillenmiş α-1,4-glukanlar bakımından

zenginleştirilmiş, standartlaştırılmış, kültürlenmiş bir özütüdür [52-55]. Polisakkarit, amino asit, lipit ve minerallerin karışımını içeren AHCC® besin takviyesi olarak kullanılmaktadır [52]. İçeriğindeki oligosakkaritlerin yaklaşık %20'sini oluşturan düşük moleküler kütleye (5 kDa) sahip α-1,4-glukan tipinin asetillenmiş formları, AHCC®'nin ana aktif bileşeni olarak kabul edilmektedir [56]. AHCC®'nin insan ve hayvanların bağışıklık hücreleri üzerindeki etkileri, *in vitro* ve *in vivo* çalışmalarda gösterilmiş olup bağışıklık sistemini modüle ederek enfeksiyonlara ve malignitelere karşı konağı savunmada takviyesinin faydalı olabileceği üzerine durulmaktadır [57]. Yapılan çalışmalarla, AHCC®'nin viral ve bakteriyel enfeksiyona karşı direnci, doğal öldürücü hücre aktivitesini, dendritik hücre fonksiyonu artırarak bağışıklık fonksiyonu üzerinde olumlu etkilere yol açtığı gösterilmiştir. Ayrıca antioksidan, antikanser, antiviral, antienflamatuar özelliklere sahip olduğunu, diyabet ve karaciğer hasarının başlamasını önlediğini ve bağışıklık tepkisini geliştirdiğini göstermiştir [53].

AHCC®'nin Antiviral Aktivitesi

Deney hayvanlarında yapılan çalışmalarda, insan eş değeri dozlarda AHCC® takviyesinin influenza virüsü enfeksiyonunun semptomlarını hafiflettiği, sağkalım oranını artırdığı, doğal öldürücü hücre aktivitesini uyardığı, virüsün akciğerlerden daha hızlı temizlenmesini sağladığı ve sonuç olarak iyileşmeyi hızlandırdığı gösterilmiştir. AHCC® takviyesinin bağışıklığı modüle ederek influenza virüsü enfeksiyonu da dahil olmak üzere geniş bir akut enfeksiyon yelpazesinde hastaların sağkalımını artırdığı bilinmektedir [57,58]. Ayrıca AHCC®'nin tek başına kuş gribini önleyici etki göstermediği fakat aşı ile kombinasyonun hastalığın önlenmesinde faydalı olabileceği sonucunu varılmıştır [59]. Başka bir çalışmada ise AHCC®'nin genç ve yaşlı farelerde Batı Nil Virüsü enfeksiyonuna karşı koruyucu konak bağışıklık tepkilerini artırdığını gösterilmiştir. AHCC® ile diyet takviyesinin, Batı Nil Virüsüne duyarlı kişiler için potansiyel olarak immünoterapötik olabileceği ortaya konmuştur [60].

Sonuç olarak, AHCC®'nin, çeşitli enfeksiyon etkenlerine karşı konakçı sağkalımının artırarak, enfeksiyon şiddetini azaltarak ve iyileşme süresini kısaltarak antiviral etki açığa çıkartabileceği ileri sürülmektedir [61].

AHCC®'nin Antienflamatuar Etkisi

Sıçanlarda trinitrobenzensülfonik asit kolit modelinde AHCC®'nin antienflamatuar etkisinin incelendiği bir çalışmada AHCC® takviyesinin kolon inflamasyonunu hafifleterek sıçan ağırlığını, gıda alımını, hasar skorunu, nekrozun uzamasını, kolon ağırlığını, kolon ağırlık/boy oranını, miyeloperoksidaz ve alkalin fosfataz aktivitelerini, glutatyon konsantrasyonunu ve proenflamatuar sitokin ve kemokinlerin (İnterlökin-1β, interlökin-1 reseptör antagonisti, tümör nekroz faktörü (TNF) ve monosit kemoatraktan protein-1) ekspresyonunu iyileştirmiştir. Kolon mikroflorası çalışmasında ise AHCC® ile tedavi edilen sıçanların daha yüksek aerobik ve laktik asit bakteri sayılarının yanı sıra daha yüksek bifidobakteri sayısına sahip olduğunu göstermektedir. Başka bir çalışmada AHCC'nin antienflamatuar etkisinin seviyesi sülfasalazin (200 mg/kg) ile benzerlik göstermiştir [62]. Çalışmaların sonuçları, AHCC®'nin muhtemelen prebiyotik etkisine bağlı olarak bağırsaklarda antienflamatuar etki açığa çıkardığı ve inflamatuar bağırsak hastaları için fonksiyonel gıdalar tasarlamak adına yararlı olabileceğini göstermektedir [56].

Trichinella ile enfeksiyon, bağırsak fazının başlangıcında tip 1 yardımcı T hücrelerinin (Th1) yanıtının indüklenmesi ve ardından parazitin atılması için gerekli olan baskın bir tip 2 yardımcı T hücrelerinin (Th2) yanıtının ortaya çıkması ile karakterize edilmektedir. Yapılan bir çalışmada, *Trichinella spiralis* enfeksiyonunun murin modelinde AHCC® hem Th1 hem de Th2 sitokinlerinin ekspresyon seviyelerini modüle etmiş ve histolojik hasar skorunu azaltarak immunmodülatör etki açığa çıkarmıştır. Ayrıca AHCC® ile tedavi edilen hayvanlarda *Trichinella spiralis*'in yetişkin sayısını azaltmıştır. Bu çalışmadan elde edilen sonuçlar, AHCC® tedavisi ile kombine edilen yarım doz albendazolün parazit yükü değerlendirmesini standart albendazol tedavisine benzer şekilde azalttığını göstermektedir. AHCC® ile tedavi edilen farelerde parazit atılımının daha erken gerçekleştiği ve yetişkin sayısının azaldığı gözlemlenmiştir [63].

AHCC®'nin Kanser Tedavisindeki Rolü

Gemsitabin ile kemoterapi uygulanan, pankreas veya safra yolları adenokarsinomu olan rezeke

edilemeyen yirmi-seksen yaş arası hastalarda yapılan çalışma, AHCC®'nin tat değişikliğini iyileştirerek kemoterapi sırasında beslenme durumunun korunmasına yardımcı olabileceğini ve kemoterapinin neden olduğu yan etkileri hafifletebileceğini göstermektedir [64].

Deney hayvanlarında yapılan bir çalışmada, AHCC®'nin oral olarak uygulanmasının sitozin arabinosidinin neden olduğu alopesinin önlenmesinde etkili olduğu gösterilmiştir. Sitozin arabinosidin ile tedavi edilen sıçanlarda saç foliküllerinde ciddi kayıplar görülürken, sitozin arabinosidin ve AHCC® ile tedavi edilen grupta foliküllerde hafif kayıplar görülmüştür. 6-merkaptopürin ve metotreksatın tedavisine eklenen AHCC®, farelerin vücut ağırlığını, eritrosit, lökositleri ve serum albümin seviyelerini önemli ölçüde artırmış, karaciğer hipertrofisini ve dejenerasyonunu iyileştirmiş, serum glutamik oksaloasetik transaminaz, serum glutamik piruvik transaminaz ve karaciğer ilaç metabolize edici enzimlerin aktivitelerini normalleştirmiştir. Sonuç olarak AHCC®'nin birlikte uygulanması, sitozin arabinosidin, 6-merkaptopürin ve metotreksat ile ilişkili yan etkileri önemli ölçüde azaltmıştır [65]. Ayrıca AHCC®'nin paklitaksel ve sisplatin ile tedavinin sebep olduğu hepatotoksisite, nefrotoksisite ve hematopoetik toksisiteyi iyileştirebileceği gösterilmiştir [66]. Dolayısıyla, AHCC®'nin kemoterapiye bağlı hepatotoksisite, nefrotoksisite, kemik iliği baskılanması ve genel mortaliteyi önemli ölçüde hafiflettiğine dair veriler kemoterapi gören kanser hastalarının yaşam kalitesinin ve refahının artmasına katkıda bulunabileceğine işaret etmektedir [67].

AHCC®'nin kemoterapiye bağlı yan etkileri azaltmanın yanı sıra antikanser aktivite gösterebileceğini ortaya atan çalışmalar bulunmaktadır. Baş ve boyun, karaciğer, akciğer, yumurtalık, kolorektal ve hematolojik dahil olmak üzere çeşitli kanserlerde immünomodülatör etkilere ve antikanser etkilere sahip olduğu gösterilmiştir. Ancak AHCC®'nin tamamlayıcı ve alternatif bir antikanser tedavi olarak geliştirilebilmesi için bu etkilerine aracılık eden mekanizmaların kapsamlı bir şekilde incelenmesi amacıyla daha fazla çalışmaya ihtiyaç duyulmaktadır [68].

AHCC®'nin HPV Tedavisindeki Rolü

Yapılan bir çalışmada, AHCC® ile günlük tedavinin HPV 16 ve HPV 18 ekspresyonu üzerindeki etkileri ve ksenograft fare modeli kullanılarak servikal tümör büyümesini önleyip önlemeyeceğini veya geciktirip geciktirmeyeceğini değerlendirilmiştir. Sürekli *in vitro* AHCC® maruziyet ile sürekli HPV baskılanması gözlenmiştir. *İn vivo* hayvan çalışmalarında ise, HPV ekspresyonu 90 gün boyunca günde bir kez 0,25 mL steril su içinde verilen 50 mg/kg AHCC® dozlamasıyla ortadan kaldırılmış ve tedaviden 30 gün sonra hiçbir HPV ekspresyonu tespit edilmemiştir. Buna ek olarak, AHCC® günlük tedavisi, tedavi edilmeyen kontrole kıyasla HPV 16 ve HPV 18 pozitif tümör büyümesinde %15.9'luk bir azalma ile ilişkilendirilmiştir. AHCC®, HPV negatif tümörlerinin büyüme oranını etkilemiştir. Sonuç olarak, bu veriler günlük AHCC® dozunun HPV 16 ve HPV 18 enfeksiyonlarını ortadan kaldırabileceğini ve HPV ile ilişkili servikal kanserin önlenmesinde bir rolü olabileceğini göstermektedir. Ayrıca AHCC®'nin rahim ağzı kanseri için birincil tedavi rejimlerine eklenmesi potansiyel olarak yanıt oranlarını artırabileceği ve nüksü önleyebileceği düşünülmektedir [69].

Melanom modeli geliştirilen farelerle yapılan bir çalışmada, AHCC® tedavisi sonrası antijenlere karşı özgüllüğü olan T lenfosit alt tiplerinden CD4+T ve CD8+T hücrelerinin aktivasyonunun ve çoğalmasının önemli ölçüde artığı gösterilmiştir. Bu durum, tümör antijenlerini hedef alan CD8+T hücrelerinin sayısında artışla beraber özellikle de tümör antijenlerine yanıt olarak üretilen interferon-gama seviyesinde yükselmeye sebep olmuştur. AHCC® uygulaması aynı zamanda öldürücü hücrelerin ve interlökin-12 seviyelerinin yükselmesine yol açmıştır. AHCC®'nin immünomodülatör aktivitesi ve enfeksiyöz patojenlere yanıt olarak bağışıklık üzerindeki olumlu etkisi nedeniyle kalıcı bir yanıtla inatçı, yüksek riskli HPV enfeksiyonlarını ortadan kaldırmada faydalı olduğu düşünülmektedir [70].

Randomize, çift-kör, plasebo kontrollü bir çalışmada 21 sağlıklı gönüllüye 4 hafta boyunca her gün 3g plasebo veya AHCC® takviyesi verilmiştir ve AHCC®'nin dendritik hücre sayısı ve fonksiyonu üzerine etkileri değerlendirilmiştir. AHCC® kullanımının doğuştan gelen bağışıklık sistemi ve adaptif bağışıklık sistemleri arasında haberci olarak çalışan immün hücrelerden olan dendritik hücrelerinin sayısında ve miyeloid dendritik hücrelerin fonksiyonunda kontrol grubuna göre önemli artışa sebep olduğu gözlenmiştir. Ancak interlökin-2, interlökin-4, interlökin-6, interlökin-10, interferon-gama ve doğal öldürücü hücrelerin seviyesinde gruplar arasında anlamlı farklılık görülmemiştir. Bu çalışmada, elde edilen verilerden yola çıkılarak AHCC®'nin bağışıklıkta spesifik etkisinin dendritik hücre sayısı

ve miyeloid dendritik hücrelerin fonksiyonları üzerindeki iyileştirici etkisi olduğu öne sürülmüş ve viral enfeksiyonun yanı sıra kanserin ilerlemesine karşı da koruma sağlamak için yararlı olabileceğine işaret edilmiştir [71].

Her biri kalıcı yüksek riskli HPV enfeksiyonu olan onar kadın hastadan oluşan iki pilot çalışma yapılmıştır. Birinci çalışmada hastalar günde bir kez 3g AHCC® takviyesini 5 hafta-6 ay arası kullanırken, ikinci çalışmadaki hastalar ise günde bir kez 1g AHCC® takviyesini 6-8 ay boyunca kullanmıştır. 3-6 aylık günde 3g AHCC® kullanımından sonra 6 hastadan 4'ünde (%66.7) ve 7 aylık günde 1g AHCC® kullanımından sonra 9 hastanın 4'ünde (%44) yüksek riskli HPV enfeksiyonunun temizlendiği doğrulanmıştır. HPV enfeksiyonu temizlenenlerde interferon-betanın 25 pg/mL'nın altına baskılandığı gözlenmiştir. İki pilot çalışmadan elde edilen ön veriler, AHCC® takviyesinin yüksek riskli HPV enfeksiyonlarının başarılı bir şekilde temizlenmesi için konakçı bağışıklık sistemini desteklediğini göstermiştir [72].

Randomize, çift-kör, plasebo kontrollü bir faz II çalışmasında (CTN:NCT02405533) 30 yaşın üzerinde, 2 yıldan uzun süredir kalıcı yüksek riskli HPV enfeksiyonu olduğu doğrulanmış 50 kadında AHCC®'nin HPV tedavisindeki rolü incelenmiştir. Bu çalışmada birincil sonlanım noktası AHCC® takviyesi alırken ve AHCC® takviyesinin tamamlanmasından sonra 3, 6 ve 12 ay boyunca sürdürülen HPV DNA negatif test sonuçlarıyla belirlenen kalıcı yüksek riskli HPV enfeksiyonunun temizlenmesini, plasebo uygulanan hastalar ile karşılaştırmalı bir şekilde değerlendirmek olarak belirlenmiştir. 12 ay sonunda HPV durumu ve bağışıklık göstergelerini değerlendirmek için çalışmanın körlüğü kaldırılmıştır. Hastalar 12 ay boyunca günde bir kez plasebo uygulanan (n=25) ve 6 ay boyunca günde bir kez aç karnına 3g AHCC® verilip ardından 6 ay plasebo uygulanan gruplara (n=25) randomize edilmiştir. 12 ay (6 ay AHCC® + 6 ay plasebo) sonrasında AHCC® takviyesi uygulanan grupta HPV DNA negatif olan kişiler, HPV DNA negatif kaldıklarını doğrulamak ve yanıtın kalıcılığını değerlendirmek için 6 ay daha izlenirken, HPV DNA pozitifler ise tedavi başarısızlığı veya yanıtsızlığı sebebiyle çalışmadan çıkarılmıştır. Plasebo uygulanan gruptan 12 ay sonra HPV pozitif olan hastalara (n=12) ise toksisitesi ve etkinliği hakkında daha fazla bilgi edinmek için körleme yapılmadan AHCC® uygulanmaya devam edilmiştir. AHCC® uygulanan gruptan 3 kişi çalışma merkezine gelemedikleri ve plasebo uygulanan gruptan ise 6 kişi 6 ay sonunda hala HPV DNA pozitif kalmaları gerekçesiyle ayrılmak istedikleri için kontrol randevularını tamamlamamış ve analiz dışı bırakılmıştır. Böylece, uyunç sağlayamayan 9 kişinin ayrılması ile çalışma 41 kişi ile tamamlanmıştır. AHCC® takviyesi alan 22 hastanın 14'ü (%63.6) 6 ay sonra HPV RNA/HPV DNA negatif ve 8'i (%36.3) HPV RNA/HPV DNA pozitif çıkmıştır. Takviye durdurulduktan 12 ay sonra 14 hastanın 9'u (%64.3) HPV RNA/HPV DNA negatif olarak belirlenmiştir. Plasebo uygulanan 19 hastanın ise 2'sinde (%10.5) 12. ayda HPV RNA/HPV DNA negatif çıkmıştır ve 12 ay izlem süresinin sonunda 17 hastada (%89.5) HPV RNA/HPV DNA pozitifliği devam etmiştir. Körleme yapılmadan plasebo grubundan 12 hastaya uygulanan 6 aylık AHCC takviyesi sonrasında hastaların %50'sinde (n=6) HPV RNA/HPV DNA negatif çıkmıştır. AHCC® takviyesi alan toplam 34 hastanın %58.8'i kalıcı HPV enfeksiyonun temizlenmesi için yapılan uygulamaya yanıt vermiştir. Hastalarda her 3 ayda bir uyunç, HPV RNA/HPV DNA testlerinin yanı sıra interferon-alfa, interferon-beta, interferon-gama, T lenfositleri ve doğal öldürücü hücre seviyelerini içeren bağışıklık göstergeleri değerlendirilmiştir. AHCC® takiyesi alan ve HPV enfeksiyonundan kalıcı olarak kurtulan kadınlarda interferon-gama ve T lenfosit seviyesinde artış gözlenmiştir. AHCC®'nin, enfeksiyon ile beraber aşırı uyarılmış interferon-beta seviyesini düşürerek, negatif geri bildirim mekanizması ile kronik viral kanalların temizlenmesi için gerekli interferon-gama ve T lenfositlerinin salınımını artırdığı belirtmektedir. Kronik olarak HPV ile enfekte bireylerde interferon-beta düzeyi 60.5±37.6 pg/ml iken AHCC® takviyesi alan kadınlarda interferon-betanın 20 pg/ml'nin altına kadar baskılanması, T lenfositleri ve interferon-gamadaki artış ve HPV enfeksiyonlarının kalıcı olarak temizlenmesi ile ilişkilendirilmiştir. Ayrıca, doğal öldürücü hücrelerin seviyesinde de artış gözlemlenmiştir. Çalışmadan elde edilen sonuçlar, günde bir kez 3 g AHCC®'nin, inatçı HPV enfeksiyonlarını ortadan kaldırmak için interferon-beta seviyesinde azalışla beraber interferon-gama ve T lenfosit seviyesinde yükselişe ve doğal öldürücü hücre sayısında artışa sebep olarak konakçı bağışıklık sistemini desteklemede etkili olduğunu ve önemli bir yan etki bildirilmeden iyi tolere edildiğini göstermiştir. Bu faz II çalışması ayrıca daha önce yapılan iki pilot çalışmada da ifade edilen 20 pg/ml'den düşük interferon-beta düzeylerinin yüksek riski HPV enfeksiyonlarının ortadan kaldırılmasıyla

korelasyonunu doğrulamıştır. Böylece hem HPV enfeksiyon durumuna hem de hedef interferon düzeyine göre takviye süresini optimize edilmesi ve kişiselleştirilmesi yönünde gelecekteki araştırmalara fırsat sunulmuştur [54].

AHCC®'nin, hastalıklarla mücadeleye yardımcı olmak için vücudun kendi bağışıklık sistemini kullanan bir tedavi olan immünoterapi olarak çalıştığı düşünülmektedir. İnsanlarda ve deney hayvanlarında yapılan çalışmalarda, AHCC®'nin vücudun enfeksiyonlara etkili bir şekilde yanıt vermesini ve tümörlerin çoğalmasını engellemesini sağlayan doğal öldürücü hücrelerin, dendritik hücrelerin ve sitokinlerin sayısını ve/veya aktivitesini, nitrik oksit salımını artırdığı gösterilmiştir. Ancak AHCC®'nin HPV eradikasyonunda kullandığı etki mekanizmalarının daha detaylı incelenmesi için yeni çalışmaların yapılmasına ihtiyaç vardır [71].

SONUÇ VE TARTIŞMA

Cinsel yolla bulaşan enfeksiyonlar arasında en yaygın görülen ve kanserle ilişkilendirilen ana virüs olan HPV, halk sağlığına yönelik dünyadaki en büyük tehditler arasında görülmektedir. HPV ilişkili hastalıkların önlenmesinde erken teşhis için tarama testleri, aşılar ve erken tedavi ön plana çıkmaktadır. Güncel tedavi seçenekleri, hastalığın eradikasyonu için yetersiz kalsa da özellikle erken başlanıldığında semptomların baskılanmasında ve olası kötü huylu hastalıkların gelişmesini önlemede başarılı olmaktadır. Bu tedavi seçeneklerinin yanı sıra çeşitli doğal bileşiklerin kullanımının da HPV tedavisinde faydalı olabileceği gösterilmiştir. Hastalığın eradikasyonunda dahi etkili olabileceği öne sürülen AHCC® aralarında en dikkat çekici bileşiktir ve üzerinde çok sayıda klinik ve klinik öncesi çalışma bulunmaktadır. AHCC® takviyesinin, HPV enfeksiyonlarının temizlenmesi için konakçı bağışıklık sistemini destekleme potansiyeline sahip olduğunu ve önemli bir yan etkiye sebep olmadan hastalar tarafından iyi tolere edilebildiğini gösteren çalışmalar bulunmaktadır. AHCC®'nin, HPV enfeksiyonu üzerindeki kalıcı sonuçlarını optimize etmek ve kullandığı etki mekanizmalarını aydınlatmak için daha fazla değerlendirme gerektirmektedir.

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REVIEW ARTICLE / DERLEME MAKALE

PETROSELINUM CRISPUM (MILL.) FUSS (PARSLEY), A FOOD AND MEDICINALLY IMPORTANT PLANT: A REVIEW OF RECENT STUDIES BETWEEN 2013-2023

PETROSELINUM CRISPUM (MILL.) FUSS (MAYDANOZ), GIDA VE TIBBİ OLARAK ÖNEMLİ BİR BİTKİ: 2013-2023 ARASINDAKİ SON ÇALIŞMALARIN DERLEMESİ

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ABSTRACT

Objective: *Petroselinum crispum (Mill.) Fuss is a bright green biennial medicinal and aromatic herb that grows almost all over the world. Today, it is one of the most commonly used culinary herbs. In addition to its use as food, it has been shown to possess broad pharmacological activities in several in vivo and in vitro studies. This study aimed to comprehensively summarize the current studies on the traditional use, phytochemical composition, pharmacological activities, clinical studies, toxicity, and drug interactions of parsley.*

Result and Discussion: *According to the literature data, parsley is used as a diuretic, carminative, emmenagogue and for the prevention and treatment of kidney stone formation, the treatment of conditions such as urinary tract infections and stomach disorders. Its phytochemical composition consists of flavonoids, coumarins, phenolic compounds, organic acids, carotenoids, vitamins, minerals, fixed oil, essential oil, and other compounds. Studies on P. crispum have shown that it has a wide range of pharmacological activities, including antioxidant, antibacterial, antifungal, antidiabetic, antihypertensive, antiplatelet, analgesic, antiinflammatory, antihepatotoxic, antinephrotoxic, anticancer, antiurolithiatic, wound healing, antiobesity, estrogenic and neuroprotective effects. This review comprehensively summarizes the scientific data of the last ten years (2013-2023) on P. crispum.*

Keywords: *Parsley, Petroselinum crispum, pharmacology, phytochemistry, traditional*

ÖZ

Amaç: *Petroselinum crispum (Mill.) Fuss, neredeyse dünyanın her yerinde yetişen, iki yıllık, parlak yeşil renkli, tıbbi ve aromatik bir bitkidir. Günümüzde en çok kullanılan mutfak bitkilerinden biridir. Gıda olarak kullanımının yanı sıra çeşitli in vivo ve in vitro çalışmalarda geniş farmakolojik aktivitelere sahip olduğu gösterilmiştir. Bu çalışmada maydanozun geleneksel kullanımı, fitokimyasal bileşimi, farmakolojik aktiviteleri, klinik çalışmaları, toksisitesi ve ilaç etkileşimleri üzerine yapılan güncel çalışmaların kapsamlı bir şekilde özetlenmesi amaçlanmıştır.*

Sonuç ve Tartışma: *Literatür verilerine göre maydanoz diüretik, karminatif, emenagog olarak, böbrek taşı oluşumunun önlenmesi ve tedavisi, idrar yolu enfeksiyonları ve mide rahatsızlıkları gibi rahatsızlıkların tedavisi için kullanılmaktadır. Fitokimyasal bileşimi flavonoitler, kumarinler, fenolik bileşikler, organik asitler, karotenoitler, vitaminler, mineraller, sabit yağ, uçucu yağ ve diğer bileşiklerden oluşmaktadır. P. crispum üzerinde yapılan çalışmalar, antioksidan, antibakteriyel,*

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antifungal, antidiyabetik, antihipertansif, antiplatelet, analjezik, antiinflamatuar, antihepatotoksik, antinefrotoksik, antikanser, antiürolitiyatik, yara iyileştirici, antiobezite, östrojenik ve nöroprotektif etkiler dahil olmak üzere çok çeşitli farmakolojik aktivitelere sahip olduğunu göstermiştir. Bu derleme, P. crispum'a ilişkin son on yıla (2013-2023) ait bilimsel verileri kapsamlı bir şekilde özetlemektedir.

Anahtar Kelimeler: *Farmakoloji, fitokimya, geleneksel, maydanoz, Petroselinum crispum*

INTRODUCTION

Petroselinum crispum (Mill.) Fuss (parsley) is a shiny green biennial medicinal and aromatic herb belonging to the Apiaceae (Umbelliferae) family [1,2]. It is native to the Mediterranean region and today it has been cultivated in many parts of the world [1]. *P. crispum* is called heung choi (Chinese), peterselie (Dutch), persil (French), petersilie, petersil, peterwurz (root) (German), maintanos, makedonisi, petroselino (Greek), ajmood (Hindi), prezzemolo (Italian), salsa (Portuguese), petrushka (Russian), perejil (Spanish), and maydanoz (Turkish) [3].

It is a glabrous hairless, biennial, bright green plant. It forms a tripennate rosette and a cylindrical, corrugated, and hollow taproot in the first year. It grows with yellow to yellowish-green flowers and flat-topped umbels in the second year. The stem is erect, striated, cylindrical, and can grow up to 50-80 cm long. Leaves are triangular-ovate in outline, 3-10×2-7 cm, glabrous, straight or curled. Inflorescence paniculate-corymbose, umbels long-peduncled. Flowers opening between August and September, are 6-8 small, yellow-green, and hermaphrodite. The fruit is a schizocarp, 2-3 mm long, oval, and divided into 2 mericarps. Parsley seeds are pear-shaped, brown colored, mericarps 2.5-3×0.5 mm, slightly arcuate at maturity [4].

There are two species belonging to the genus *Petroselinum* in the world, *P. crispum* (garden parsley) and *P. segetum* W.D.J.Koch (corn parsley). *P. crispum* is native to Algeria, Greece, Morocco, Yugoslavia and cultivated throughout Türkiye [5,6]. *P. segetum* is native to Belgium, France, Great Britain, Italy, Netherlands, Portugal, Spain [7]. There are three principal varieties of *P. crispum*: *P. crispum* var. *neapolitanum*, *P. crispum* var. *crispum*, and *P. crispum* var. *tuberosum*. The curly-leaf *P. crispum* var. *crispum* is tougher than the Italian or flat-leaved *P. crispum* var. *neapolitanum*. *P. crispum* var. *tuberosum*, Hamburg's parsley/turnip root parsley, has much thicker roots than the other species [8].

Besides being used as food, parsley has been used against diseases such as urinary tract infections, menstrual pain, diabetes, hemorrhoids and as a diuretic, to pass kidney stones and to lower blood pressure. In the literature, flavonoids, coumarins, phenolic compounds, vitamins, minerals, fixed oil, and essential oil were reported in various parts of *P. crispum*. The plant has antioxidant, antibacterial, antifungal, antidiabetic, antihypertensive, antiplatelet, analgesic, antiinflammatory, antihyperuricemic, antihepatotoxic, antinephrotoxic, anticancer, wound healing, antiobesity, estrogenic, and neuroprotective activities [4,9].

Parsley is an important culinary herb that has been used frequently since ancient times and exhibits important pharmacological activities. For this reason, the number of studies on it is increasing day by day. This review aims to summarize current studies on traditional uses, phytochemical composition, pharmacological activities, clinical studies, drug interactions and toxicity of *P. crispum*. Studies conducted on the species between 2013 and 2023 using various scientific databases were discussed. Some studies with very similar results and using the same methods were not included. As a result 106 references in English and Turkish have been included.

Traditional Uses

It is known that parsley has been cultivated as a medicinal plant in the Mediterranean region for about 2000 years [4]. It is used in traditional medicine in many parts of the World. The traditional uses of the plant in different countries are shown in Table 1. The use of the leaves, aerial parts, petioles, and roots for various purposes has been reported. It is used for menstrual pain, urinary tract infections, diuretic effect, to pass kidney stones, to lower blood pressure, diabetes, and various stomach ailments.

Table 1. Traditional use of parsley in different countries

Country	Part used	Preparations	Use	References
Algeria	Fresh aerial parts	Eat and/or add to salad daily	For bladder infection, kidney inflammation, prostate enlargement, cancers, stone disease	[10]
	Seed	Infusion	For pyelonephritis	
Bosnia and Herzegovina	Leaves	Infusion	For urinary tract infections	[11]
Brazil	Leaf	Infusion	For uterus cleansing, infection and menstrual cramps	[12]
Catalonia	Root	Ointment, liniment	For irritation; As antiecchymotic, antierythematous, external antiseptic	[13]
	Aerial parts	Ointment, bath	As antiecchymotic, cosmetic (hair)	
Morocco	Leaf	Internal, external	Food, hypertension, hair	[14]
	Whole plant, leaf, aerial parts, stem, seed, root	Infusion, maceration, decoction, oil, juice	For kidney stones, renal colic, renal detoxification	[15]
			For renal pain, diuretic, kidney inflammation, polycystic kidney disease	
Serbia	Leaves and roots	Infusion, spice	In urinary tract diseases and infections; to relieve edema	[16]
Türkiye	Aerial parts	Freshly internal	As diuretic	[17]
	Leaves and petiols	Infusion	For menstrual cramps, stomach pain, gastritis, ulcer, diabetes, cystitis, to increase breast milk production; as laxative	[18]
	Leaf, petiole	Infusion, decoction, crude, raw	For liver steatosis, cholesterol, urinary tract infection, edema, kidney stone, stomach disorder, gall bladder disorder, kidney disorder, eye diseases, inappetence; as anti-inflammatory, diuretic, expectorant	[19]
	Leaf	Decoction	For urinary tract infection, to lower cholesterol; as antiinflammatory	[20]
	Aerial parts	Fresh, crushed and mixed with lemon		

Phytochemical Composition

The most important secondary metabolites of the phytochemical composition of parsley are flavonoids. It contains especially flavones and flavonols and their glycosides [9]. In addition, coumarins (especially furanocoumarins) have been detected [21]. In some studies, coumaroyl-derived compounds whose structure was not fully determined were detected [22,23]. There are also compounds such as phenolic compounds, organic acids, carotenoids, carbohydrates, phenylpropanoids and fatty acids. The compounds that make up the phytochemical composition of parsley, plant part, extraction method and detection method of the compounds are given in Table 2. It has been determined that its leaves and roots contain vitamins (A, B, C, K, thiamine, niacin, folate) and minerals (Fe, Mg, Na, K, Zn) [24-26]. There are studies to determine the composition of the essential oil obtained from different parts of *P. crispum* and are presented in Table 3. Studies conducted to determine essential oil content have shown that essential oil yield is between 0.08-9.63%. Differences in essential oil yield and composition may have occurred depending on geographical conditions, collection season, and methods used for extraction [27].

Table 2. Phytochemical composition of *P. crispum*

Compound	Plant part/Extract	Detection Method	References
Flavonoids			
Flavones and Flavone Glycosides			
Apigenin	Fresh plant/Ethanol:water 80:20% v/v; L./Ultrasound-Assisted Extraction	Ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), ultra-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS), high-performance liquid chromatography-photodiode array detector (HPLC-PDA)	[28,29]
Apigenin 7-glucoside (cosmosiin)	Fresh L./Ethanol:water 80:20% v/v	HPLC-electrospray ionisation tandem mass spectroscopy (HPLC-ESI-MS)	[22]
Apigenin-7-*O*-glucuronide	Fresh plant/70% ethanol	Resin, polyamide chromatography, HPLC, and antioxidant index evaluation methods	[30]
Apigenin 7-apiosylglucoside (apiin)	Fresh L./Ethanol:water 80:20% v/v; ethanol; L., St./80% methanol; L./Methanol:water 80:20% v/v; Fresh AP./By decoction using water	HPLC-ESI-MS, HPLC, LC-MS-QTof, HPLC-diode array detection (DAD)-MS/MS, HPLC-DAD-MS/MS	[22,23,25,31,32]
Apigenin-acetylapiosylglucoside (acetyl-apiin)	L./Methanol:water 80:20% v/v	HPLC-DAD-MS/MS	[25]
Apigenin-*O*-hexosyl-hexoside	L./Dry powder	UPLC/MS	[33]
Apigenin-*O*-pentosyl-hexoside	L./Dry powder	UPLC/MS	[33]
Apigenin-*O*-acetyl-pentosyl-hexoside	L./Dry powder; Fresh L./Ethanol:water 80:20% v/v	UPLC/MS, HPLC-ESI-MS	[22,33]
Apigenin-6,8-di-C-glucoside (vicenin 2)	L., St./80% methanol	LC-MS-QTof	[31]
Apigenin 7-malonylapiosylglucoside	L./Methanol:water 80:20% v/v	HPLC-DAD-MS/MS	[25]
Luteolin	L./Ultrasound-Assisted Extraction	UPLC-QTOF-MS, HPLC-PDA	[29]
Luteolin-di-glucoside	L., St./80% methanol	LC-MS-QTof	[31]
Luteolin 4'-methyl ether apiosylglucoside	Fresh AP./By decoction using water	HPLC-DAD-MS/MS	[23]
Diosmetin-7-*O*-glucoside	Fresh plant/70% ethanol	Resin, polyamide chromatography, HPLC, and antioxidant index evaluation methods	[30]
Diosmetin 7-apiosylglucoside	Fresh L./Ethanol:water 80:20% v/v; Fresh plant/Ethanol:water 80:20% v/v; L., St./80% methanol; L./Methanol:water 80:20% v/v	HPLC-ESI-MS, UHPLC-MS/MS, LC-MS-QTof, HPLC-DAD-MS/MS	[22,25,28,31]
Diosmetin-*O*-pentosyl-hexoside (isomer I)	L./Dry powder	UPLC/MS	[33]
Diosmetin-*O*-pentosyl-hexoside (isomer II)	L./Dry powder	UPLC/MS	[33]
Diosmetin-*O*-acetyl-pentosyl-hexoside (isomer I)	L./Dry powder	UPLC/MS	[33]
Diosmetin-*O*-acetyl-pentosyl-hexoside (isomer II)	L./Dry powder	UPLC/MS	[33]
Diosmetin-acetylapiosylglucoside	L./Methanol:water 80:20% v/v	HPLC-DAD-MS/MS	[25]

Table 2 (*continue*). Phytochemical composition of *P. crispum*

Compound	Plant part/Extract	Detection Method	References
Scutellarin	Fresh plant/70% ethanol	Resin, polyamide chromatography, HPLC, and antioxidant index evaluation methods	[30]
Chrysoeriol-7-*O*-malonylapiosylglucoside B	Fresh L./Ethanol:water 80:20% v/v	HPLC-ESI-MS	[22]
Flavonols and Flavonol Glycosides			
Kaempferol	L. fixed oil/Hexane	Gas chromatography-mass spectrometry (GC-MS)	[34]
Kaempferol-7-*O*-glucoside	Fresh plant/70% ethanol	Resin, polyamide chromatography, HPLC, and antioxidant index evaluation methods	[30]
Kaempferol-3-*O*-[6”-malonyl-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopiranoside]	L./Methanol:water 80:20% v/v	HPLC-DAD-MS/MS	[25]
Quercetin	AP/Ethanol:water 70:30% v/v	HPLC-DAD	[35]
Quercetin-*O*-pentosyl-hexoside	Fresh L./Ethanol:water 80:20% v/v	HPLC-ESI-MS	[22]
Quercetin-3-*O*-glucoside (isoquercitrin)	Fresh plant/Ethanol:water 80:20% v/v	UHPLC-MS/MS	[28]
3’-Methoxyquercetin dihexoside	Fresh AP./Extract prepared by decoction using water	HPLC-DAD-MS/MS	[23]
Isorhamnetin-di-*O*-hexoside	L./Dry powder; Leaves and stems/80% methanol	UPLC/MS, LC-MS-QTof	[31,33]
Isorhamnetin-3-*O*-glucoside	L., St./80% methanol	LC-MS-QTof	[31]
Isorhamnetin-3-*O*-galactoside	L., St./80% methanol	LC-MS-QTof	[31]
Isorhamnetin-3-*O*-hexoside	L., St./80% methanol	LC-MS-QTof	[31]
Isorhamnetin-di-pentosyl-rhamnoside	L., St./80% methanol	LC-MS-QTof	[31]
Myricetin	AP./Ethanol:water 70:30% v/v extract; AP./70% ethanol	HPLC-DAD, Liquid chromatography tandem mass spectrometry (LC-MS/MS)	[35,36]
Flavanols			
Catechin	L./Dry powder	UPLC/MS	[33]
Flavanones and Flavanone Glycosides			
Naringenin	AP./Ethanol:water 70:30% v/v	HPLC-DAD	[35]
Naringin	AP./70% ethanol	LC-MS/MS	[36]
Phenolic acids			
Hydroxybenzoic acid derivatives			
Gallic acid	AP./Ethanol:water 70:30% v/v	HPLC-DAD	[35]
Protocatechuic acid	Fresh plant/Ethanol:water 80:20% v/v	UHPLC-MS/MS	[28]
Vanillic acid	L. fixed oil/Hexane	GC-MS	[34]
Hydroxycinnamic acid derivatives			
p-Coumaric acid	Fresh L./Ethanol:water 80:20% v/v; Fresh plant/Ethanol:water 80:20% v/v	HPLC-ESI-MS, UHPLC-MS/MS	[22,28]
p-Coumaric acid 4-*O*-hexoside	Fresh L./Ethanol:water 80:20% v/v; Fresh plant/Ethanol:water 80:20% v/v	HPLC-ESI-MS, UHPLC-MS/MS	[22,28]
p-Coumaroyl-hexoside	L., St./80% methanol	LC-MS-QTof	[31]
o-Coumaric acid (melilotosid)	Fresh AP./Extract prepared by decoction using water	HPLC-DAD-MS/MS	[23]
Chlorogenic acid	Fresh plant/Ethanol:water 80:20% v/v	UHPLC-MS/MS	[28]

Table 2 (*continue*). Phytochemical composition of *P. crispum*

Compound	Plant part/Extract	Detection Method	References
Caffeic acid	Fresh plant/Ethanol:water 80:20% v/v; L./Ultrasound-Assisted Extraction	UHPLC-MS/MS, UPLC-QTOF-MS, HPLC-PDA	[28,29]
Ferulic acid	AP./Ethanol:water 70:30% v/v	HPLC-DAD	[35]
Phenolic compounds			
Cinnamic acid	AP./Ethanol:water 70:30% v/v	HPLC-DAD	[35]
trans-Cinnamic acid	L. fixed oil/Hexane	GC-MS	[34]
Hydroxytyrosol	AP./Ethanol:water 70:30% v/v	HPLC-DAD	[35]
Arbutin	AP./70% ethanol	LC-MS/MS	[36]
Furocoumarins			
Oxypeucedanin	L./Dichlorometan subfraction	Column chromatography (normal phase silica gel, Sephadex LH-20), preparative thin layer chromatography (TLC)	[21]
Oxypeucedanin hydrate	L./Dichlorometan subfraction	Column chromatography (normal phase silica gel, Sephadex LH-20), preparative TLC	[21]
Pabulenol	L./Dichlorometan subfraction	Column chromatography (normal phase silica gel, Sephadex LH-20), preparative TLC, HPLC	[21]
Organic acids			
Citric acid	Fresh L./Ethanol:water 80:20% v/v; Fresh AP./By decoction using water	HPLC-ESI-MS HPLC-DAD-MS/MS	[22,23]
Malic acid	Fresh L./Ethanol:water 80:20% v/v; AP./70% ethanol	HPLC-ESI-MS LC-MS/MS	[22,36]
Carotenoids			
All-*trans*-lutein	L./Aceton, transferred to petroleum ether/diethyl ether mixture (1:1 v/v), saponified with 10% (w/v) methanolic KOH	HPLC-DAD-MS/MS	[25]
All-*trans*-*β*-carotene	L./Aceton, transferred to petroleum ether/diethyl ether mixture (1:1 v/v), saponified with 10% (w/v) methanolic KOH	HPLC-DAD-MS/MS	[25]
Carbohydrates			
D-Glucose	AP./70% ethanol	LC-MS/MS	[36]
D-Mannitol	AP./70% ethanol	LC-MS/MS	[36]
Talose	AP./70% ethanol	LC-MS/MS	[36]
Saccarose	Fresh AP./By decoction using water	HPLC-DAD-MS/MS	[23]
Monoterpenes			
Limonene	Fresh L./Ethanol	HPLC	[32]
Phenylpropanoids			
Eugenol	Fresh L./Ethanol	HPLC	[32]
Apiol, myristicin, elemicin, 3-methoxy-γ-asarone	Seed-Fixed oil/Petroleum ether	GC	[37]
Fatty Acids			
13-docosenoic acid methyl ester, (*Z*), *cis*-13-docosenoic acid, *cis*-11-eicosenoic acid methyl ester, 11-octadecenoic acid (stearate), methyl ester, hexadecanoic acid (palmitate) methyl ester, 15-tetracosenoic acid methyl ester (*Z*), cyclopentanone, 3,4-bis(methylene) and stigmastan-3-ol, 5-chloro-acetate	Fresh L./Cold maceration with methanol	GC-MS	[38]

Table 2 (*continue*). Phytochemical composition of *P. crispum*

Compound	Plant part/Extract	Detection Method	References
Palmitic acid, oleic acid, stearic acid	Seed-Fixed oil/Petroleum ether	GC	[37]
Palmitic acid, linoleic acid, *α*-linolenic acid, stearic acid, oleic acid	L. fixed oil/Hexane	GC-MS	[34]
Petroselinic acid, linoleic acid, lauric acid, myristic acid, palmitic acid	S./Methanol	GC-MS	[39]
Tocopherols			
(β+γ)-Tocopherol	L. fixed oil/Hexane	GC-MS	[34]
γ-tocopherol, *α*-tocopherol, *α*-tocotrienol, *γ*-tocotrienol	S./Methanol	GC-MS	[39]
Sterols			
Stigmasterol+campesterol, *β*-sitosterol	L. fixed oil/Hexane	GC-MS	[34]
β-sitosterol, Δ-5-stigmasterol, Δ-7-stigmasterol, compesterol	S./Methanol	GC-MS	[39]
Other Compounds			
Oleuropein	AP./70% ethanol	LC-MS/MS	[36]
N-(2'-phenylethyl)-hexanamide	L./Dichlorometan subfraction	Column chromatography (normal phase silica gel, Sephadex LH-20), Preparative TLC	[21]

L.: Leaves; AP.: Aerial parts; S.: Seed, St.: Stem

Table 3. Some studies on the components of essential oil obtained from *P. crispum*

	Part used / place of assembly	Essential oil extraction method	Essential oil yield (%)	Main ingredient(s) (%)	References
1	Seed/ Hatay, Türkiye	Hydrodistillation	2.52 (w/w)	3-Methoxy-*γ*-asarone (34.2), apiol (27.5), myristicine (23.8), *α*-pinene (2.5), *β*-pinene (2.4), *β*-Phellandrene (1.3)	[37]
2	Aerial parts/ Parana, Brazil	Hydrodistillation	0.2 v/w	Apiol (50.3), myristicine (14), *β*-phellandrene (14.6)	[40]
	Dried leaf/ Estonia		2.9 mg/g	*p*-Menta-1,3,8-triene (40), *β*-phellandrene (15.1), myristicine (13.1), myrcene (6.5)	
	Fresh root/ Estonia		0.42 mg/g	Apiol (34.5), myristicine (28.8), terpinolene (13.2), *β*-phellandrene (4.6)	
3	Fresh leaf/ Giza, Egypt	Hydrodistillation	-	*α*-Pinene (26.6), myristicin (20.3), apiol (13.2), 1-allyl-2,3,4,5-tetramethoxybenzene (11.6), *β*-pinene (10.5)	[41]
4	Seed/ Draa-Tafilalet, Morocco	Hydrodistillation	2.01 v/w	Apiol (23.5), *α*-pinene (19.0), myristicine (17.2), allyltetramethoxybenzene (4.3)	[42]
5	Leaves/ Al-Kharj, Kingdom of Saudi Arabia	Hydrodistillation	0.08 w/w	Myristicin (41.2), sabinene (9.3), *β*-myrcene (6.0), benzene, (2-methyl-1-propenyl) (5.3), *p*-mentha-1,5,8-triene (4.2), *β*-Caryophyllene (4.0)	[43]
6	Chopped fruit, seed/ Serbia	Hydrodistillation	9.63 w/w	Myristicin (35.8), apiol (24.4), 6-methoxyelemicin (17.4), *α*-pinene (8.2), *β*-pinene (6.0), elemicin (5.5)	[44]
7	Fresh aerial parts/ Mauritius	Hydrodistillation	0.09 w/w	Myristicin (40.3), 1,3,8-*p*-dimenthatriene (17.9), *β*-phellandrene (15.0), myrcene (4.2) *α,p*-dimethylstyrene (3.7), terpinolene (2.6), limonene (2.5)	[45]
8	Leaf/ New Delhi, India	Hydrodistillation	-	Carvacrol (48.5), *d*-limonene (20.8), cuminaldehyde (15.8)	[46]

Table 3 (*continue*). Some studies on the components of essential oil obtained from *P. crispum*

	Part used / place of assembly	Essential oil extraction method	Essential oil yield (%)	Main ingredient(s) (%)	References
9	Aerial parts/ Mauritius	Hydrodistillation	-	Myristicin (40.3), 1,3,8-*p*-dimenthatriene (17.9), *β*-phellandrene (15.0), myrcene (4.2), *α,p*-dimethylstyrene (3.7), terpinolene (2.6), limonene (2.5)	[47]
10	Seeds/ Peru	Steam distillation	0.106	1,3,8-*ρ*-Menthatriene (22.6), apiole (22.4), *β*-phellandrene (15.0), 6-methoxy-elemicin (7.0), myrcene (5.9)	[48]
11	Aerial parts/ Milan, Italy	Steam distillation	-	*α*-pinene (30.8), *β*-pinene (19.4), Limonene+*β*-phellandrene (13.3), myristicin (9.5), terpinolene (5.4), *p*-cymenene (4.9)	[49]

Pharmacological Activities

Antioxidant Activity

It has been observed that research on *P. crispum* is mostly focused on its antioxidant activity. Different extracts of leaves and stems of parsley (hexane, dichloromethane, ethyl acetate, methanol and water) were prepared. The antioxidant activity of dichloromethane extract, which had the highest phenolic content, was evaluated. It was found that the extract showed antioxidant activity for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (IC_{50}= 3310.0±80.5 µg/ml). In mouse fibroblasts (3T3-L1) applied with 400 µg/ml extract, the extract was determined to have a protective activity against cancer by exhibiting 50.9% protection against H_2O_2-induced DNA damage detected by the Comet assay. In addition, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was found to inhibit H_2O_2-induced MCF-7 (estrogen-sensitive breast cancer cell line) cell migration (41%±4%) [50]. The antioxidant activity of the hydroethanolic extract and polyphenolic fraction prepared from the aerial parts of *P. sativum* was evaluated by DPPH, Ferric ion reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) methods. While the DPPH radical scavenging activity of the hydroethanolic extract was higher than that of the polyphenol extract, the polyphenol extract was found to be more effective in the FRAP assay. The activities are lower than standard compounds. In the TAC assay, hydro-ethanolic extract (175.2±6.360 mg ascorbic acid equivalent (AAE)/g) showed higher antioxidant capacity than polyphenolic extract (148.2±13.86 mg AAE/g) [36].

The results of the analyzes performed to determine the antioxidant activity of the hydroalcoholic extract of fresh leaves are as follows: total phenolic content (TPC)= 5.12±0.03 mg GAE/g, total flavonoid content (TFC)= 14.73±1.01 mg quercetin equivalent/g, 13.07±0.64 %, oxygen radical antioxidant capacity (ORAC)= 54.76±1.12 µM trolox equivalent (TE)/g, *β*-carotene/linoleic acid assay= 12.14±2.89% [22]. The antioxidant activity of fixed oil obtained from *P. crispum* leaves was examined by different methods. It was determined as TPC= 40.81±0.7 mg gallic acid equivalent (GAE)/100 g, DPPH= 13.31±1.29 mg AAE/100 g, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)= 788.39±42.61 mM TE/100 g, FRAP= 2104.38±109.64 mM TE/100 g [34]. Apiin was isolated as the main component of the aqueous extract from the leaves of curly leaf parsley. The extract was determined to contain great phenolic (12.49±1.70 mg/g GAE) and also TFC (15.05±2.20 mg quercetin equivalent). The extract showed high antioxidant activity by the FRAP assay (189.8 mM Fe(II) per mg dry extract of the plant) and DPPH (EC_{50}= 15.50 mg/ml) methods. With the *in vitro* analysis performed on the *Saccharomyces cerevisiae* cells, extract had low toxicity. It has been reported that apiin has a more potent antioxidant effect and lipid peroxidation (0.01 and 0.1 mM) than apigenin under oxidative stress in a cell viability assay (0.1 mM) in yeast cells. It was concluded that the extract and apiin showed antioxidant activity in the eukaryotic cellular model [51].

The antioxidant activity of the ethanol extract of green parts and seeds of *P. crispum* was investigated by *in vitro* methods. The TPC was determined to be in parsley green parts (0.92±0.4) and seeds (0.62±0.01 g GAE/100 g). In the primary oxidation of sunflower oil experiment, the peroxide

value was measured at 7-day intervals for 28 days and it was observed that parsley seed significantly reduced the peroxide value than butylated hydroxytoluene (BHT), more than its leaves. In the P-anisidine experiment, it was determined that parsley green parts and seeds had a significant inhibitory effect on 2-alkene formation in sunflower oil after 7 days. It has been determined that the thiobarbituric acid (TBA) method reduces malondialdehyde (MDA) formation in sunflower oil better than BHT with parsley seeds and green parsley. DPPH radical scavenging activity was found to be high in parsley seeds (91.97±4.38%) (BHT= 90.73±2.69%) and green parts of parsley (88.91±1.41%), respectively, at a concentration of 1000 µg/ml. It is possible to consider parsley as a food additive owing to its antioxidant activity [52]. The TPC of the methanol extract of *P. crispum* seeds was found to be 11.59±0.20 mg GAE/g DW, DPPH and ABTS radical scavenging activity, IC_{50}= 368.78±5.69 and 439.35±2.91 µg/ml, respectively [39]. *P. crispum* samples were exposed to processes such as roasting (150-180°C), baking (200°C) or boiling (100°C) for 10, 20, and 30 min, or no treatment was applied. The TPC and antioxidant activity (DPPH and total reducing power) of the samples were compared. It was determined that the samples had higher phenolic content after heating and the highest reduction potential was found in the samples heated for 10 minutes. It has been reported that the TPC and DPPH results show a strong correlation [53]. 40, 60, and 80% ethanol extracts were prepared from *P. crispum* by microwave-assisted extraction method, and total phenolic content and antioxidant activity were investigated. It was shown that the TPC determined by the Folin-Ciocalteu method was 600.33 mg GAE/100 g dry weight (DW), and the 40% ethanol extract had the highest content (747.73±21.32 mg GAE/100 g DW) compared to other extracts. It was revealed that the antioxidant activity determined by the DPPH method was 7.60 mM TE/100 g DW, and the 40% ethanol extract was higher (5.51% 8.42±1.11 mM TE/100 g DW) [54].

The antioxidant activity of the essential oil obtained from fresh leaves of *P. crispum* was investigated with DPPH and Ferric Chloride Assay. Although the activity is lower than ascorbic acid, it was determined that it showed 68.42%±0.27% inhibition and 0.517±0.01 absorbance at 5 mg/ml, respectively. Myristicin, the main component of the essential oil, was found to interact with antioxidant (PDB: 3NM8 and 1HD2) receptors *in silico* [43]. The antioxidant activity of the essential oil obtained by hydrodistillation from chopped fruits and seeds of *P. crispum* was examined with DPPH and ABTS radical scavenging activity. The antioxidant activity of the essential oil was found to be 1.52±0.9029 µM TE/g in the DPPH experiment at a concentration of 20 mg/ml and 24.95±0.3825 µM TE/g at 10 mg/ml in the ABTS assay [44]. The activity of essential oil obtained from *P. crispum* by hydrodistillation was measured by different methods. DPPH (53.91±1.19 mg TE/g EO), ABTS (75.58±1.76 mg TE/g EO), radical scavenging activity, cupric ion reducing antioxidant capacity (CUPRAC) (147.48±4.08 mg TE/g EO), FRAP (110.64±1.44 mg TE/g EO) and the TPC were found to be 18.46±0.55 mg GAE/g EO [55].

Antimicrobial Activity

There are studies on the antimicrobial activity of different extracts and essential oils obtained from the different parts of *P. crispum*. In a study conducted the inhibition of the leaves of *P. crispum* on bacteria isolated from patients with burning infection were investigated using the agar well diffusion method. 250 mg/ml of hot aqueous extract shows an efficient inhibition against *Pseudomonas aeruginosa* reproduction. Inhibition zone diameter (29.667 mm) was significantly different compared to nitrofurantoin (positive control) ($p<0.05$). Based on the results of this study, *P. crispum* has a good antibacterial against *P. aeruginosa, Staphylococcus aureus* and *Staphylococcus pyogenes* associated with skin infections [56]. In another study, the antibacterial activity of silver nanoparticles and the extract prepared from the aqueous extract of *P. crispum* leaves using different methods (autoclave or heater) was examined according to the well diffusion method. As a result, it was determined that the clean area created by the nanoparticles prepared with autoclave and heater against *Escherichia coli* and *S. aureus* was 16±1, 17±1 and 12±1, 14±1 mm, respectively. It was determined that the particle prepared by autoclave showed the highest antibacterial activity, and the extract alone was not effective [57]. It has been determined that the cold methanol maceration extract of *P. crispum* leaves, which contains carbohydrates, steroids, and saponins shows low-spectrum antibacterial effects against some pathogenic bacteria and is more effective against gram-negative bacteria than gram-positive bacteria [38]. The effect of apiin detected in the ethanol extract prepared from the leaves of *P. crispum* on SARS-CoV-2 was

evaluated *in silico*. It has been shown that the binding affinity of apiin to the nucleocapsid N-terminal RNA binding domain of SARS-CoV-2 is better than remdesivir [32].

The antibacterial effect of the essential oil obtained from the aerial parts of parsley was evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. It was determined that the essential oil inhibited the growth of all bacteria with MIC values between 0.04 and 1.00 mg/ml, and killed all bacteria with MBC values between 0.15 and 10.00 mg/ml. According to results the most resistant bacteria were found to be *Enterobacter cloacae* and *E. coli* (MBC values of 10.00 mg/ml). The most sensitive bacteria were *P. aeruginosa, Salmonella enterica*, and *S. aureus*. The MBC value of essential oil against *S. aureus* was close to ampicillin and 1.7 times lower than streptomycin ($p \leq 0.05$) [40]. The antifungal activity of the essential oil obtained by hydrodistillation from the aerial parts of *P. crispum* was examined. In experiments using the Microdilution Broth Susceptibility Assay, it was found to show MIC values of 4 mg/ml (fungistatic) against *Candida albicans* and 2 mg/ml (fungicidal) against *C. tropicalis* [45]. The antimicrobial activity of the essential oil obtained from the aerial part of *P. crispum* was determined by the agar disc diffusion method. Its effect against *Aspergillus flavus* (MIC= 0.25%, MBC= 0.5%), *A. ochraceus* (MIC= 0.125%, MBC= 0.5%), *Geotrichum candidum* (MIC= 0.25%, MBC= 0.5%), *Mucor circinelloides* (MIC= 2%, MBC= 4%) and *Penicillium roqueforti* (MIC= 1%, MBC= 2%) has been detected [49]. The antifungal activity of the essential oil extracted from the aerial parts of parsley was evaluated by determining the minimum fungicidal concentration (MFC) values. *P. ochrochloron* and *Trichoderma viride* were found to have low concentrations of MICs compared to ketoconazole. As a fungicide, parsley essential oil was efficient for all fungi, especially *P. funiculosum* (MFC= 1.25 mg/ml) and *T. viride* (MFC= 2.50 mg/ml). However, in general, the fungicide amounts of the essential oil against fungi (*A. niger, A. fumigatus, A. ochraceus, A. versicolor, P. ochrochloron, P. funiculosum, P. verrucosum, T. viride*) were 5-62.5 times higher than those of the positive controls ($p \leq 0.05$) [40]. The antimicrobial activity of the essential oil obtained from the fresh leaves of *P. crispum* was determined by the agar diffusion method. At 20 mg/ml, the inhibition zone and MIC values were found to be *C. albicans* (19.4±0.08 mm, 1.25 mg/ml), *S. aureus* (17.86±0.09 mm, 2.5 mg/ml), *Bacillus subtilis* (15.73±0.04 mm, 2.5 mg/ml), *Klebsiella pneumoniae* (9.43±0.09 mm, 5 mg/ml) and *E. coli* (8.67±0.12 mm, <5 mg/ml), respectively. The interaction of myristicin, the main component of the essential oil, with antibacterial receptors (PDB: 1AJ6 and 1JIJ) *in silico* experiments has been shown to support *in vitro* study and has been reported to be responsible for the activity [43]. It was determined that the nanoemulsion carrying the essential oil obtained by hydrodistillation from *P. crispum* leaves completely inhibited the growth of *A. flavus* and some other storage fungi at 1.0 µl/ml and the production of Aflatoxin B1 at 0.75 µl/ml. The mechanism of antifungal action has been reported to be by reducing cellular ergosterol and subsequent release of cellular components [46].

The antimicrobial activity of the hydroethanolic extract and polyphenolic fraction prepared from the aerial parts of *P. sativum* against *P. aeruginosa, S. aureus*, and *C. albicans* was determined by agar well diffusion and broth dilution methods. It was found that the polyphenolic fraction (inhibition diameters and MIC values: *P. aeruginosa*: 14.00±0.33 mm, 6.25 mg/ml, *S. aureus*: 9±0.16 mm, 3.125 mg/ml and *C. albicans*: 13±0.57 mm, 6.25 mg/ml) showed better activity than the hydroethanol extract (inhibition diameters and MIC values: *P. aeruginosa*: 14.00±0.33 mm, 6.25 mg/ml, *S. aureus*: 9±0.16 mm, 3.125 mg/ml and *C. albicans*: 13±0.57 mm, 6.25 mg/ml), being lower than the standard substances [36].

The antimicrobial activity of 40, 60, and 80% ethanol extracts prepared from *P. crispum* by microwave-assisted extraction method was examined by disc-diffusion method. The 60% ethanol extract was found to show higher activity against *E. coli* (3.00±1.41 mm) and *C. albicans* (2.00±0.00 mm) than other extracts [54]. The activity of aqueous and ethanol extracts of parsley against bacteria isolated from children with urinary tract infection was examined by disc diffusion method. It was observed that the inhibition zones of the ethanol extract for the bacteria studied except *Miroccoccus* were between 2-22 mm. It was determined that the aqueous extract was not effective against *Micrococcus, P. aeruginosa, K. oxytoca*, and others it created an inhibition zone between 2-21 mm [58]. The antimicrobial activity of the ethanol extract of green parts and seeds of *P. crispum* against pathogenic bacteria, yeast and food-born fungi was determined by the agar well diffusion method. It

was observed that the seeds formed more inhibition zones than the green parts. The inhibitions of seeds and green parts were determined as *C. tropicalis* (25, 22 mm), *S. typhi* (23, 20 mm), *S. aureus, A. flavus* (18, 16 mm), *Mucor* sp. (19, 19 mm) and *Emericella nidulais* (17, 15 mm), respectively [52]. *P. crispum* samples were exposed to processes such as roasting (150-180 °C), baking (200 °C) or boiling (100 °C) for 10, 20, and 30 min, or no treatment was applied. The antibacterial effect of the samples was examined against *E. faecalis*, *B. subtilis*, *E. coli,* and *K. pneumoniae*. Fresh samples heated for 10 minutes were found to be effective against *K. pneumoniae*. It was observed that the processed sample heated for 10 minutes was effective against *B. subtilis* [53].

Antiinflammatory Activity

The antiinflammatory activity of the hydroethanolic extract and polyphenolic fraction of the aerial parts of *P. sativum* was examined *in vivo* by the paw edema method. Rats were administered indomethacin (positive control), 500 and 1000 mg/kg *P. sativum* hydroethanolic extract, or 220 mg/kg polyphenolic fraction. At the end of 3 and 6 hours, it was determined that 25%, 65.33%, and 38% inhibition occurred with 500 and 1000 mg/kg extract, 74%, 48% and 81% inhibition occurred with the fraction. Indomethacin exhibited 50% and 86.67% inhibition, respectively [35].

In another study, the antiinflammatory activity of the essential oil obtained from fresh leaves of *P. crispum* was examined by albumin and trypsin analysis. It was determined that inhibition with egg albumin provided 22.2-90.4% inhibition at 5-1000 ppm, while ibuprofen varied between 32.8-91.7%. It has been reported that inflammation caused by trypsin is inhibited by 8.4-74.7% with essential oil at 5-200 ppm and by 74.7-76.5% with ibuprofen. The interaction of the main component of the essential oil, myristicin, with anti-inflammatory (3N8Y and 3LN1) receptors was examined *in silico* and it was suggested that it may be responsible for the activity [43]. Thangavelu et al. [59] examined the cytotoxicity of methanol, petroleum ether, and aqueous extracts (500 µg/ml) prepared from *P. crispum* leaves on A549 cells by MTT assay. The IC_{50} value of the methanol extract was found to be 1521.4 mg/ml. It was determined that cell migration was significantly accelerated after application and the extract had a high antiinflammatory effect.

Antidiabetic Activity

The antidiabetic activity of *P. crispum* was determined by evaluating its effect on enzymes such as α-amylase and α-glucosidase. In a screening study, it was determined that the methanol extract of *P. crispum* inhibited α-amylase significantly (37.48±0.33%) at a dose of 2 mg/ml. It has been found that the correlation of this activity with the free radical scavenging effect of the extract is relatively weak [60]. *P. crispum* samples were exposed to processes such as roasting (150-180 °C), baking (200 °C) or boiling (100 °C) for 10, 20, and 30 min, or no treatment was applied. α-amylase enzyme inhibition of the samples was examined and it was noted that fresh (83.8%) and processed (75.4%) samples heated for 10 minutes showed the best activity [53]. It was determined that the essential oil obtained from fresh *P. crispum* by hydrodistillation inhibited α-amylase enzyme (1.35±0.05 mM acarbose equivalent/g EO) but did not inhibit α-glucosidase [55].

Albino mice with streptozotocin-induced gestational diabetes were administered aqueous extract of *P. crispum* (1 ml/150 g/bw) from the 7th to the 19th day of the experiment. Developmental changes such as thin skin, thin muscles, absence of eyelids, and kyphosis occurred in the fetuses of diabetic mice. More normal morphological development was observed in the group given parsley. It has been determined that parsley reduces the harms of hyperglycemia [61].

Analgesic Activity

The aqueous extract of *P. crispum* was evaluated for analgesic activity at doses of 2, 5, and 10 g/kg using hot plate and acetic acid-based writhing methods. The extract also showed significant ($p<0.01$) analgesic activity in both methods, and additionally enhanced morphine and aspirin-induced analgesia [62]. The analgesic activity of the hydroethanolic extract and polyphenolic fraction prepared from the aerial parts of *P. sativum* was investigated by abdominal writhing and formalin tests in rats. The acetic acid method was used in the abdominal writhing test and hydro-ethanolic extract (1000 mg/kg, 38.96%) and polyphenols (200 mg/kg, 29.23%) showed a significant decrease. In the formalin

test, in the first stage, 1000 mg/kg decreased the response time by 33.32% and 500 mg/kg decreased the response time by 25.86%. In the second stage, hydroethanolic (500 mg/kg, 28.55%; 1000 mg/kg, 37.35%) and polyphenol extracts, and polyphenols (200 mg/kg, 30%) revealed a significant decrease in response time. Tramadol used as a standard, was found to be more effective [36].

Activity on the Gastrointestinal System

In a study evaluating the activity of parsley on gastric damage caused by oxidative stress, aqueous extract of parsley (28 g/kg) was given to rats with gastric damage and it was examined microscopically and biochemically after 7 days. Histopathologically significant difference was observed in rats with gastric stress given parsley ($p<0.05$), standard diet ($p<0.05$), and lansoprazole ($p<0.05$). It was determined that MDA levels decreased in the groups given extract and lansoprazole. Parsley was found to increase the average glutathione (GSH) level, catalase (CAT), and superoxide dismutase (SOD) activities [63].

The effect of carbinol extract of *P. crispum* seeds (500 μg/ml) on iron absorption was examined *in vitro*. The extract enabled 8.24% of the iron applied to Caco-2 cells to be absorbed by the cells and showed lower permeability (2.01×10^{7} cm/s). It was concluded that parsley is a good source of chlorophyll and iron and increases the absorption of iron from human intestinal cells [64].

Antiplatelet Activity

The antiplatelet activity of the aqueous extract from the aerial parts (major component is apiin) of its flat leaf was investigated by *in vivo* and *ex vivo* experiments. It was found that the extract, when administered intravenously (25 mg/kg body weight-bw) and orally (125 mg/kg bw) five minutes before thrombosis induction, inhibited venous thrombosis formation by 98.2% and 76.2%, respectively. 15 or 25 mg/kg extract was found to increase carotid artery occlusion time by 37.0±6.44 minutes (150%) and more than 60 minutes (240%), respectively, when administered orally 60 minutes before induction of thrombosis. Parsley has been reported to be a potential drug candidate for the treatment of thromboembolic disease [23].

Antiurolithiatic Activity

The antiurolithiatic effect of ethanol extract was investigated *in vivo* by administering (500 mg/kg) to the rats. Subsequently, the kidneys of the rats were removed and evaluated histopathologically. Calcium oxalate crystals were found to be significantly ($p<0.001$) low in both histological sections and urine samples after treatment with parsley. It was concluded that parsley acts by decreasing urinary calcium and protein excretion, as well as increasing urinary pH and diuresis [65].

The effect of the hydroalcoholic extract prepared from aerial parts of *P. crispum* on calcium oxalate urolithiasis was investigated *in vitro*. It has been shown to cause a decrease in the size of calcium oxalate crystals with a significant decrease in the mean diagonal (4.41 μm, $p<0.01$). It was found to significantly inhibit ($p<0.05$) calcium oxalate aggregation after 60 minutes compared to the negative control at 10 mg/ml. It has been reported that its effect may be due to polyphenols such as flavonoids. In addition, it was found that it did not show significant activity in acetylcholine (Ach)-induced contraction (*ex vivo*) in the rat ileum [66].

Antihyperuricemic Activity

The hypouricemic effect of *P. crispum* was investigated on mice at the biochemical, molecular, and cellular levels. 7 g/kg of parsley leaf aqueous extract was administered orally to hyperuricemic and control mice for 10 days, either separately or in combination. It was found that the giving parsley remarkably decreased serum blood urea nitrogen and uric acid levels, increased CAT levels, decreased MDA, GSH, glutathione peroxidase (GPx), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-10 in hyperuricemic mice. The mRNA expression of urate transporters and uric acid excretion genes in kidney tissues were evaluated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and it was determined that regulate urate excretion associated genes (URAT1, GLUT-9, OAT-1 and OAT-3). It was found that the alterations in transforming growth factor beta 1 (TGF-β1) immunoreactivity were effectively normalized by the administration of the extract [67].

The antihyperuricemic effect of parsley was investigated *in vivo*. The leaves of *P. crispum* (3.5, 7, and 10.5 g/kg/day) were administered to oxonate-induced hyperuricemic rats for 7 days. As a result, it was determined that the serum uric acid level decreased the most when 7 g/kg/day parsley was given. It was determined that liver lesion scores decreased with all three doses, and kidney lesions decreased with a dose of 7 g/kg/day. It has been reported that parsley reduces serum uric acid levels and has the potential to improve kidney and liver damage caused by hyperuricemia [68].

Anticancer Activity

The ethanol extract (PSE) and the seed oil (PSO) of *P. crispum* were investigated by MTT assay, neutral red uptake and microscopic examination in MCF-7 cells by exposing the cells to 10 to 1000 µg/ml PSE and PSO for 24 hours. Cell viability was found by the MTT assay. PSE was found to be 81%, 57%, 33%, 8% and 5% at 50, 100, 250, 500, and 1000 µg/ml concentrations, respectively, while 90%, 78%, 62%, and 8% for PSO at 100, 250, 500, and 1000 µg/ml concentrations. PSE doses of 50 µg/ml and above and PSO concentrations of 100 µg/ml and above were determined to be cytotoxic in MCF-7 cells. It was found that 250, 500 and 1000 µg/ml doses changed the cellular morphology of MCF-7 cells in a associated with concentration, and PSE was more effective than PSO [69].

The activity of the root extract on MCF-7 and MCF-12A cell lines with lactate dehydrogenase (LDH) cytotoxicity analysis, DNA synthesis with bromodeoxyuridine (BrdU) proliferation analysis, and metabolic activity with MTT cell viability assay in the dose range of 0.01-100 µg/ml, were evaluated. In LDH analysis, no significant cytotoxicity was observed in either cell line, but a better result was obtained at 500 µg/ml dose. BrdU showed notable DNA synthesis inhibition of up to 80% at 10, 50, 100 and 500 µg/ml in the proliferation assay. According to MTT analysis, 63% and 75% inhibition of metabolic activity in MCF-7 and MCF-12A were reported at only 500 µg/ml. It has been noted that parsley shows antiproliferative activity in both cell lines [70].

The anticancer effect of aqueous and methanol extracts of the aerial parts of *P. crispum* against human glioblastoma cells U87MG was evaluated. The adhesion test was performed on various protein matrices of the extracts at doses of 10, 20, 50, and 100 µg/ml. It was found that the methanol extract specifically inhibited the adhesion of human glioblastoma cells U87MG to fibrinogen, fibronectin, and non-specific substrate Poly-L-Lysine (PLL), and the IC_{50} value was 19.4±0.15 and 23.86±0.92 and 20.25±0.59 mg/ml, respectively. When the activity of the samples on tumor cell proliferation was examined, it was found that only methanol extract could entirely reduce cell proliferation at a concentration of 1 mg/ml after four days of incubation [71].

The hydroalcoholic extract of the seeds of parsley was evaluated against A375 human melanoma cells and dendritic cells. It was reported that parsley extract showed significant apoptotic potential. It was determined that parsley extract had a cell growth inhibition of 24.9-2.9% ($p<0.0001$) after 60 µg/ml and 72 hours of incubation. It was observed that the amount of caspase 3 protein increased significantly ($p<0.001$) with 30 µg/ml parsley extract and decreased at 60 µg/ml [72].

The different concentrations (3.25-200 mg/ml) of silver nanoparticles prepared from methanol extract of *P. crispum* seeds (AgNPs@PCS) were examined by MTT assay against MCF-7 cell line. The IC_{50} value was found to be 200 mg/ml after 24 hours of exposure [73].

Antihepatotoxic Activity

Oils [*α*-pinene (26.6%) and myristicin (20.3%)] obtained from *P. crispum* leaves by hydrodistillation were administered to rats with carbon tetrachloride (CCl_4)-induced hepatotoxicity. It was found that increased serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase levels decreased ($p<0.05$) and increased SOD and GSH activity. It has been observed that 0.5 ml of peppermint oil is effective in reducing MDA. In rats with CCl_4-induced hepatotoxicity, urea level was seen to be remarkably reduced by parsley oil administration [41].

The effect of the aqueous extract prepared from the leaves of *P. crispum* against oxidative liver damage caused by bile obstruction was evaluated *in vivo*. The extract was administered orally at a dose of 2 g/kg to rats with ligated bile ducts for 28 days. AST, ALT, bilirubin levels in serum, as well as SOD, GSH, MDA, Na^+/K^+-ATPase and 8-hydroxyguanosine for evaluation of oxidative stress,

myeloperoxidase for inflammation, caspase-3 for apoptosis, TGF-β and hydroxyproline for fibrosis were investigated. It has been determined that the extract reduces increased ALT, AST and bilirubin levels and improves oxidative damage parameters. It has been shown that *P. crispum* extract is protective against bile obstruction-induced hepatic damage and fibrosis in rats owing to its antioxidant and anti-inflammatory effects [74].

The effect of the hydroalcoholic extract prepared from *P. crispum* leaves against lead (Pb)-induced liver damage was examined *in vivo*. The extract was administered to rats together with Pb at doses of 100 or 200 mg/kg via oral gavage for 21 days. It was determined that Pb-increased liver enzymes, MDA, TAC, CAT, and SOD activities decreased, Bax and TNF-α gene expressions related to apoptosis increased and B-cell lymphoma gene-2 (Bcl-2) gene expression decreased. It was determined that oxidative and apoptosis changes were significantly improved by administration of parsley extract. It was concluded that parsley has a direct protective effect on the damaged liver by regulating the expression of genes related to antioxidant activity and apoptosis [75].

The protective effect of the hydroethanolic extract of the aerial parts of *P. crispum* on paracetamol-induced hepatotoxicity was examined *in vivo*. Rats were given extract (200 mg/kg) and paracetamol (200 mg/kg) for 15 days. It has been observed that the extract significantly reduces ALT, AST, ALP, and LDH levels in the liver, which are increased by paracetamol. In addition, histopathological examinations revealed less congestion and mononuclear cell infiltration in the liver tissue [76].

Antinephrotoxic Activity

The *in vitro* antinephrotoxic potential of the seeds of *P. crispum* against CCl_4-induced oxidative damage in mammalian kidney (Vero) cells was investigated. Antiradical experiments of the tested extracts showed that the DPPH radical scavenging effect of *P. crispum* extract had equal potential as BHT. Treatment with the extract greatly reduced the number of CCl_4-induced necrotic cell populations, with higher ($p<0.05$) potency than ketosteril (25.56%). Treatment with extract suppressed CCl_4-induced toxicity by inhibiting major necrotic mediators [77].

In a study on the effects of 5% aqueous extract of *P. crispum* (administered *ad libitum*), on diuretic activities, electrolyte composition, antioxidant capacities, and their effects on the kidney by histopathological consultation of the kidney tissue after their application were evaluated. It was determined that lipid peroxidation decreased, GSH levels increased, and the activities of antioxidant enzymes (GPx, SOD, and CAT) in kidney tissue were recovered with oral parsley administration. It has been shown that the best diuretic effect, electrolyte excretion, DPPH radical scavenging effect is provided by parsley extract. Parsley has been suggested for the avoidance of kidney diseases [78].

The ameliorating effect of ethanol extract (500 mg/kg bw) of *P. crispum* leaves and stems on the toxicity of orellanin in rat kidney was examined. It was observed that there was a decrease in body weight, relative kidney weight, and an increase in creatinine, uric acid, and urea levels in the group given orellanin. Additionally, it was determined that cystatin C levels increased while GPx activity decreased. In histopathological examination, it was determined that the toxicity caused by orellanin, especially in the kidney cortex, nephron, and proximal tubules, was improved by parsley [79].

The protective effect of the hydroethanolic extract of the aerial parts of *P. crispum* on paracetamol-induced nephrotoxicity and proteinuria was examined *in vivo*. The extract (200 mg/kg) was administered to rats receiving paracetamol by oral gavage for 15 days. It was determined that the increase in blood urea, creatinine, and triglyceride levels was inhibited by the extract. It was observed that the extract reduced urinary protein excretion compared to control groups and increased urinary creatinine and urea excretion compared to paracetamol control groups [76].

The protective effects of ethanol extract of *P. crispum* seeds (5 mg/kg) on the kidneys of pregnant rats aborted using prostaglandin were examined. The extract was applied for 18 days and MDA, total antioxidant status, creatinine, and urea levels were monitored, and histopathological examination was performed. As a result, it was reported that parsley reduced the dysfunction caused by prostaglandin-induced abortion in rat kidneys and was useful in reducing the progression of prostaglandin-induced edema [80].

Neuroprotective Activity

Mice poisoned with cadmium (Cd) were administered parsley juice at two doses (5 g/kg/day and 10 g/kg/day) via gastric intubation. Cd has been shown to cause behavioral abnormalities, histopathological and biochemical disorders in mice. It was determined that especially low dose (5 g/kg/day) fruit juice significantly improved Cd-related behavioral changes. It was found to reduce the increase of lipid peroxidation and normalize the Cd effect on reduced peroxidase and GSH activities in the brain of treated mice, reducing neuronal aberrations in the brain [81].

The activity of *P. crispum* extract against morphine-induced damage in the prefrontal cortex of rat brain was evaluated. The extract was giving rats intraperitoneally (*i.p.*) at doses of 50, 100, and 150 mg/kg alone or in combination with morphine. In the group given morphine, the density of neurons and neuronal dendritic spines, total antioxidant capacity significantly decreased, and nitric oxide (NO) levels have been increased ($p<0.05$). It was observed that these effects have been reversed ($p<0.05$) at all doses in the groups given the extract alone or in combination with morphine. It has been determined that *P. crispum* provides protection against morphine induced oxidative stress via its antioxidant effect [82].

The neuroprotective effect of the aqueous extract of *P. crispum* leaves on oxidative damage that may occur in the brain of rats with bile duct ligation induced biliary cirrhosis was examined. The extract (2 g/kg) was administered orally for 28 days. It was determined that lipid peroxidation, sialic acid, and NO levels decreased and GSH levels and CAT activities increased significantly in the extract-administered group. It was determined that there was no significant change in total protein, GST, SOD, and boron levels. It was observed that histological findings also supported the results of biochemical analysis. It has been found that parsley is effective in regressing the oxidant damage caused by cirrhosis in brain tissues [83].

Şener et al. [84] investigated the protective effect of the aqueous extract of *P. crispum* leaves (2 g/kg, oral, 14 days) on the brains of rats with scopolamine-induced Alzheimer's disease using the novel object recognition test and Morris water maze test methods. It was observed that the M1 receptor expression in the hippocampus and frontal cortex, Bcl-2/Bcl-2 associated x protein (Bax) ratio, and GSH levels decreased with scopolamine, and the increased MDA levels, caspase-3/9 expressions, and acetylcholinesterase (AChE) activity were reversed by the extract. It has been determined that parsley has a healing effect on spatial and recognition memory, M1 receptor expression, apoptosis, oxidative stress, and increased AChE activity. In another study, the protective effects of the aqueous extract of *P. crispum* leaves (2 g/kg) against damage to the lens tissues of the scopolamine-induced experimental Alzheimer's model were investigated. Reduced GSH, SOD, CAT, GPx), glutathione reductase, glutathione-S-transferase parameters, which were found to decrease with scopolamine, were shown to increase significantly again with the extract. It was determined that lipid peroxidation, NO, and advanced oxidation protein products, which increased with scopolamine, decreased with the extract. As a result, it was concluded that it can reduce oxidative damage and protect lens tissue against oxidative damage due to Alzheimer's disease, owing to its rich content of phenolics and flavonoids [85].

Activity on Some Hormones and Reproductive Functions

The effect of parsley leaf essential oil against the detrimental effects of CCl_4 on the thyroid gland and testicles of mice was investigated. Mice that received *i.p.* 3 ml/kg CCl_4 twice weekly for 4 weeks had a decrease in CAT, SOD activities ($p<0.05$), and an increase in MDA levels in testes and thyroid glands ($p<0.05$). In addition, it was determined that luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid hormones (thyroid stimulating hormone (TSH), free triiodothyronine (fT_3), total triiodothyronine (T_3), free thyroxine (fT_4), and total thyroxine (T_4)) decreased significantly. Parsley essential oil was applied at 0.5 ml/kg/day. The essential oil has been found to reduce testicular and thyroid oxidative stress significantly. It has also been found to increase LH, FSH, fT_3, T_3, fT_4 and T_4 [86].

The *in vivo* estrogenic effect of the hydroethanolic extract and polyphenolic fraction of the aerial parts of *P. sativum* was examined. Female rats were administered 308.33 mg/kg clomiphene citrate (positive control), 500 and 1000 mg/kg *P. sativum* hydroethanolic extract, or 220 mg/kg polyphenolic fraction for 4 weeks. As a result, it was determined that there was no change in ovarian weights with the

extracts, and uterine weights increased by approximately 30%. It was determined that 1000 mg/kg hydroalcoholic extract, clomiphene citrate, and polyphenolic fraction decreased ovarian cholesterol by 56%, 50%, and 40%, respectively. It was found that 1000 mg/kg hydroethanolic extract increased uterine protein levels by 73% and polyphenolic fraction by 65%. 1000 mg/kg hydroalcoholic extract increased serum estradiol by 43%, 220 mg/kg polyphenolic fraction increased by 31%, and clomiphene citrate increased by 64%. It was concluded that parsley has an estrogenic effect owing to its components such as ferulic acid, gallic acid, and quercetin [35].

Wound Healing Activity

The burn wound healing activity of hydroethanolic extract and polyphenolic fraction prepared from the aerial parts of *P. sativum* in vaseline (10% w/w) was examined in rats. It was determined that ointments prepared from hydroethanolic extract and polyphenolic fraction produced high wound shrinkage of 97.17% and 94.98%, respectively, in 25 days. It was determined that the hydroethanolic extract provided complete wound healing on the 20th day compared to the polyphenol fraction and positive control (Madecassol®) [36].

In another study, the wound healing activity of the ointment obtained by mixing the ethyl acetate extract rich in phenolic compounds prepared from the aerial parts of *P. crispum* with vaseline was investigated *in vivo*. Ointment (13% and 22% w/w) was applied topically to wounds in mice twice a day for 10 days. It was observed that *P. crispum* ointment caused a significant reduction in wound size ($p \leq 0.001$), an increase in epithelialization and angiogenesis scores ($p \leq 0.05$) and an increase in collagen scores at 22% w/w concentration compared to vaseline. It was found to increase collagen III more ($p \leq 0.05$) compared to *β*-sitosterol. It has also been found to significantly increase epidermal endothelial growth factor. As a result, it was concluded that parsley phenolic extract was more effective than vaseline and accelerated wound healing at a level comparable to *β*-sitosterol [87]. The wound healing activity of methanol, petroleum ether, aqueous extracts (500 µg/ml) prepared from *P. crispum* leaves on A549 cells was examined *in vitro* by Scratch test. It was observed that the scratch area in the wound was covered 7.46% faster with *P. crispum* compared to the control [59].

The wound healing activity of the ointment prepared by mixing AgNPs@PCS with vaseline, prepared from the methanol extract of *P. crispum* seeds, was evaluated in rats. It has been determined that the ointment prevents inflammation in the wound area, increases the number of fibroblast cells and, as a result, accelerates wound healing. In the *in vivo* examination, it was observed that the wound closure percentage was higher on the 7th and 14th days than in the control group (vaseline) and the wound was completely closed after 21 days. It has been reported that nanoparticles have the potential to be used clinically [73].

Antiobesity Activity

The TPC and antioxidant capacity (DPPH method) of extracts of *P. crispum* var. *neopolitanum* leaves prepared by different methods such as boiling, blanching, and microwaving were compared. Boiled parsley was observed to have the highest values and was administered orally to rats fed a high-fat diet at a dose of 200 mg/kg for 8 weeks. It was found to significantly reduce body weight, adipose tissue, fasting blood glucose, triglycerides, low density lipoprotein, very low density lipoprotein, liver lipids, creatinine, and urea levels and increase high density lipoprotein, and liver GSH levels compared to the positive control. It has been determined that the antioxidant capacity of parsley increases by boiling and is effective for obesity [88].

Other Activities

Apart from the activities mentioned, there are studies in the literature on the hypolipidemic [89], antianemic [90], antiosteoporotic [91], antihypertensive [2], antidepressant, anxiolytic [92] anti-acne, anti-aging [45], antifatique [30], prebiotic effect [93], enzyme inhibitory (adenosine deaminase, neurominidase, xanthine oxidase, acetylcholinesterase, tyrosinase) [55,94] activities and nutraceutical potential [33,95-97] of parsley.

Clinical Studies

In the study conducted on 37 patients with urinary tract infections, mostly women, general urinary examination and abdominal ultrasonography were performed 14 days after the capsules containing 500 mg of leaf and stem powder were given to the patients twice a day for 10 days. It is observed that the frequency of 11/17 ($p=0.011$) and 17/20 emergencies ($p<0.0001$) improved, 100% of dysuria and suprapubic pain ($p<0.0001$) were cured, and 23/31 of the patients reported improvement in low back pain ($p<0.0001$). The total symptom score showed a significant decrease from baseline scores of 5.94±0.14 ($p=0.0313$) at the second evaluation. It was stated that no remarkable side effects were observed in the patients [98].

In a double-blind, randomized clinical trial, powdered *P. crispum* (infusion of 2.5 g in a glass of water (125 cc)) was applied topically to patients with melasma (54 patients) in a medical center in Iran. At the end of eight weeks, the severity of the disease was evaluated using the melasma area and severity index (MASI). It was determined that 4% hydroquinone ($p=0.000$) and parsley ($p=0.002$) in the control group reduced the severity of melasma and the efficacy of the two groups was similar ($p=0.858$). Moreover, the total cost in the hydroquinone group was found to be approximately 10.5 times higher than the parsley group [99].

Toxicity

Studies have mostly reported that *P. crispum* is safe in wide dose ranges and does not show toxicity. In a study, the acute toxicity of ethanol extract of leaves and stems (5, 50, 300, and 2000 mg/kg bw) was examined *in vivo*. No behavioral changes or lethal effects were observed in rats after 24 hours [79]. When the ethanol extract of its leaves was examined *in vivo* and histopathologically in rats, the hematological data were analyzed using bromocresol green and Berthelot method. It was found to have hepatotoxic and nephrotoxic effects when used continuously at doses of 1000 mg/kg or more and showed no significant toxicity when taken at lower concentrations [1]. The toxicity of the hydroethanolic extract and polyphenolic fraction of the aerial parts of *P. sativum* was investigated *in vivo*. Rats were given clomiphene citrate (positive control), 500 or 1000 mg/kg hydroethanolic extract for 4 weeks. It was determined that the extract had no toxic effects on liver and kidney tissues [35]. In the studies conducted, no oral toxicity was observed with the aqueous extract of *P. crispum* leaves on hematological, biochemical parameters, liver and kidney histology. It is suggested that the LD_{50} value in wistar rats is greater than 5000 mg/kg [100].

Median cytotoxic concentration (CC_{50}) values in extracts (hexane, chloroform, ethyl acetate, methanol, ethanol, water) of leaves and stems of *P. crispum* in healthy kidney epithelial (Vero) cells derived from the African monkey cells at a concentration of 100 µL (5-640 µg/ml) were found to be 82.7±8.1 for chloroform, 105.3±4.5 for ethyl acetate and 159.3±8.0 µg/ml for hexane. No cytotoxicity was detected in ethanol, methanol, and aqueous extract [101]. It was determined that the carbinol extract of *P. crispum* seeds at different concentrations (62.5-1000 µl) did not show toxicity in Caco-2 cells by MTT assay [64].

In an ethnobotanical study conducted in Morocco, its leaves were reported to have hypotensive effects [14]. Apiol, the main component of parsley leaf and seed oil, has been reported to be used as an abortifacient [102].

Drug Interactions

Since parsley has a diuretic effect, care should be taken to avoid excessive fluid loss, dehydration and hypotension, as the effect may be increased when used together with diuretic drugs [103]. *In silico* studies, it has been reported that rutin, kaempferol, apigenin, elemicin, myristicin, estragol, caffeic acid, 4-terpinol found in *P. crispum* have the potential to interact with antihypertensive drugs [104].

The enzyme-inducing or inhibitory activity of powdered parsley (200 mg) for simvastatin (80 mg) was evaluated at the level of the metabolic enzyme cytochrome P-450. C_{max} (mean plasma maximum concentration) and $AUC_{0-\infty}$ (area under the concentration-time curve) of simvastatin were found to increase by 2 and 2.2 fold, respectively, when given with parsley ($p<0.01$). A decrease in the clearance of simvastatin ($p<0.01$) and an increase in $t_{1/2}$ between 3.2 and 6.12 hours was observed. Since parsley

can be a potential inhibitor of the enzyme that metabolizes simvastatin, it has been reported that it should be used with caution [105].

A 19-year-old female patient was immunosuppressed by sirolimus administration after renal transplantation. At the control of the patient taking 1.5 mg of sirolimus twice daily, it was observed that the blood level of the drug was high (14.8 ng/ml). At the previous control, conditions that could increase the medicine level, which ranged from 2-4 ng/ml, were excluded. A more detailed history of the patient revealed that he had been drinking about 30 g of parsley juice for seven days to lose weight and improve her health. It was observed that the medicine level returned to the normal range (4.6 ng/ml) after one week of stopping the parsley juice [106].

RESULT AND DISCUSSION

Parsley, which originated in the Mediterranean Region and is now cultivated almost all over the world, has been used worldwide for many years. It is commonly used by adding it to the meals and salads prepared in the daily diet for a healthy life. Besides its use as a food, it is used for therapeutic purposes for various conditions such as urinary tract infections, stomach disorders, menstrual pain, and some dermatological disorders in folk medicine. Its phytochemical composition includes flavonoids, coumarins, phenolic compounds, carotenoids, carbohydrates, vitamins and minerals, essential oil, and other constituents. Studies conducted on extracts of parsley and its isolated constituents show that the plant has a wide range of pharmacological activities, owing to flavonoids and phenolic compounds, especially antioxidant effects. As a result, numerous preclinical studies have demonstrated that parsley has significant therapeutic potential. Despite this data, the number of clinical studies conducted on the species is quite limited. Its effects need to be demonstrated through clinical studies and dose-toxicity studies need to be conducted for different diseases.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

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DERLEME MAKALE / REVIEW ARTICLE

OKÜLER İLAÇ TAŞIYICI SİSTEM OLARAK LİPİT BAZLI NANOPARTİKÜLLER

LIPID-BASED NANOPARTICLES AS OCULAR DRUG DELIVERY SYSTEM

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ÖZ

Amaç: *Bu derleme kapsamında, oküler kullanımdaki katı lipit nanopartikül (KLN) ve nanoyapılı lipit taşıyıcı (NLT) sistemlerine, bu formülasyonların sterilizasyonuna ve tasarımla kalite (QbD) hakkında yapılan son araştırmaları tartışmak amaçlanmıştır.*
Sonuç ve Tartışma: *Göze ilaç taşınması son yıllardaki gelişmelere rağmen hala karmaşık bir sorun olarak devem etmekte ve etkili ilaç taşınabilmesi için yenilikçi yaklaşımlara ihtiyaç duyulan bir alandır. Bu derlemede, yenilikçi yaklaşımlardan olan KLN'ler ve NLT'lerin oftalmik ilaç uygulamalarında sağladıkları üstünlükler güncel literatür örnekleriyle tartışılmıştır. KLN'ler oda sıcaklığında katı halde bulunan lipitlerin genellikle eritilmesi ya da çeşitli solvanlarda çözündürülmesi ile hazırlanan lipit partiküllerdir. NLT'ler ise yapısında katı lipitlerle birlikte oda sıcaklığında sıvı halde bulunan lipitleri de içermektedir. Oküler ilaç uygulamalarında kritik aşamalardan biri de sterilizasyon basamağıdır. Uygun sterilizasyon işleminin seçiminde, kullanılan lipitlerin erime dereceleri, serbest radikal oluşturma eğilimleri ve partikül büyüklükleri göz önünde bulundurulmalıdır. Sonuç olarak, KLN ve NLT'ler hem biyouyumluluk hem de etkililik anlamında oküler tedaviler için umut vadeden ilaç taşıyıcı sistemlerdir. Derleme kapsamında incelenen literatür çalışmaları da bu çıkarımı desteklemektedir. Ancak saklama süresince karşılaşılabilen stabilite sorunları ve tekrarlanabilir büyük ölçekte üretim konusunda yaşanan sıkıntılar nedeniyle klinik tedavide yeterince kullanılamamaktadır. Bu sorunların çözümü aşamasında QbD'nin etkili olacağı düşünülmektedir.*
Anahtar Kelimeler: *Göz, katı lipid nanopartiküller, nano yapılı lipit taşıyıcılar, QbD, sterilizasyon*

ABSTRACT

Objective: *Within the scope of this review, it aims to discuss the latest research on solid lipid nanoparticle (KLN) and nanostructured lipid carrier (NLT) systems for ocular use, sterilization of these formulations, and quality by design (QbD).*
Result and Discussion: *Despite the developments in recent years, drug delivery to the eye remains a complex problem and is an area where innovative approaches are needed for effective drug delivery. This review discusses the advantages of KLNs and NLTs, innovative approaches in ophthalmic drug applications, with examples from current literature. KLNs are lipid particles prepared by melting solid lipids at room temperature or dissolving them in various solvents. NLTs,*

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on the other hand, contain solid lipids in their structure, as well as lipids that are liquid at room temperature. One of the critical stages in ocular drug applications is the sterilization step. In choosing the appropriate sterilization process, the melting degrees, free radical formation tendencies, and particle sizes of the lipids used should be considered. In conclusion, KLNs and NLTs are promising drug delivery systems for ocular treatments in terms of biocompatibility and efficacy. The studies examined within the scope of the review also support this inference. However, it cannot be used adequately in clinical treatment due to stability problems that may be encountered during storage and difficulties in reproducible large-scale production. It is thought that QbD will be effective in solving these problems.
Keywords: *Eye, nanostructured lipid carriers, QbD, solid lipid nanoparticles, sterilization*

GİRİŞ

Dünya Sağlık Örgütü (DSÖ); sağlıksız beslenme, sigara içme, dijital cihazların aşırı kullanımı gibi yaşam tarzı değişiklileri ve diyabet, kalp-damar problemleri gibi hastalıkların artması sonucu ilerleyen yıllarda oküler hastalıklarda ciddi artışlar olabileceğini belirtmektedir. Mevcut yaşam tarzları ve sürekli yaşlanan nüfus da göz önüne alınınca, 2030 yılına kadar glokom (76 milyondan 95.4 milyona) ve yaşa bağlı makula dejenerasyonu (195.6 milyondan 243.3 milyona) gibi kronik oküler hastalıklarda ciddi bir artış olacağı öngörülmektedir. Ayrıca, yakın zamanda "Küresel Hastalık Yükü Çalışması" kapsamında yapılan bir analizde, 2050 yılına kadar yaklaşık 474 milyon insanın orta ila şiddetli görme bozukluğuna sahip olacağı ve bunların 61 milyonunun tamamen görme kaybı yaşayacağı öngörülmektedir [1].

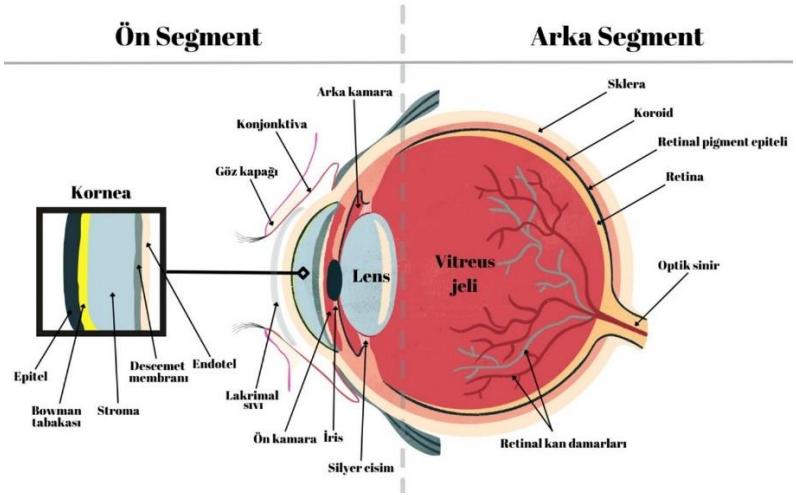
Yıllardır farklı oküler hastalıkları tedavi etmek için çeşitli ilaç sınıfları üzerinde çalışmalar sürdürülmektedir. Bununla birlikte, zorlu bir süreç olan oküler hastalıkların tedavisinde istenilen tedavi etkinliğinin sağlanmasında sorunlar yaşanmaktadır. Uygun tedaviyi sağlamakta başlıca zorluk, eşsiz bir yapı ile donatılmış olan oküler anatomi ve fizyolojiden kaynaklanmaktadır. Bu sebeple oküler hastalıkların tedavisinde farklı dozaj şekilleri kullanılmaktadır. Oküler olarak en çok tercih edilen uygulama şekli topikal damlalar olup özellikle de ön segment hastalıklarında sıklıkla değerlendirilmektedirler [2]. Oküler damlalar; yüksek hasta uyuncuna, düşük maliyetlere ve kolay üretim süreçlerine sahip sistemlerdir. Ancak formülasyonların viskozitelerindeki düşüklüğe bağlı olarak oküler temas süresinin kısalması ve uygulama hacmine rağmen oküler yüzeyde kalan düşük ilaç miktarı bu formülasyonların oküler biyoyararlanımını %5'e kadar düşürmektedir [3]. Bu problemlerin önüne geçebilmek ve oküler kalış süresini artırmak amacıyla jeller, merhemler gibi yarı katı ilaç şekilleri geliştirilmiştir. Ancak bu sistemlerin de arka segment hastalıklarının tedavisinde etkili olamadığı ve hasta uyuncunu azalttığı, dolayısıyla da hala iyileştirilmeye ihtiyaç duydukları bir gerçektir. Belirtilen sebeplerden ötürü, geleneksel oküler formülasyonların teknolojik özelliklerinin geliştirilmesi ve bu kapsamda yeni ilaç taşıyıcı sistemlerin araştırılması, başarılı tedavi sağlamanın bir gerekliliğidir [4].

Geçtiğimiz yıllarda, nanoteknolojideki ilerlemeler, oküler taşıyıcı sistemler açısından da umut vadetmektedir. Etkin maddelerin, nanotaşıyıcılar (20-1000 nm) içinde enkapsüle edilmesi, ilacın çözünürlüğünde ve stabilitesinde artış sağlamaktadır. Bu sayede hedeflenen oküler bölgelerde biyoyararlanım artışı da elde edilir [5]. Özellikle katı lipit nanopartiküller (KLN) ve nanoyapılı lipit taşıyıcılar (NLT) gibi lipit kaynaklı nanotaşıyıcılar, doğal lipitler kullanılarak hazırlanabildikleri için yüksek oranda biyouyum göstermektedir. Ayrıca, lipit matrisleri yüzey aktif maddelerle birlikte kullanıldığında, hidrofobik bileşiklerin daha iyi çözünmesine ve korunmasına olanak tanımaktadır. Bununla birlikte, lipit nanopartiküllerin etkili bir şekilde geliştirilmesi hala istenilen seviyelere ulaşmamıştır [6]. Etkili formülasyon geliştirmek amacıyla tasarımla kalite (QbD), yani klasik ampirik metodolojiler yerine sistemsel yaklaşımların uygulanmasını öneren sistemler ile çalışmalar yoğunlaşmıştır [7]. QbD, gıda veya kimya endüstrilerinde ürün optimizasyonu ve süreç iyileştirme amacıyla yaygın olarak uygulanmaktadır. Bununla birlikte, katı dozaj formlarından lipozomlara, polimerik misellerden nanopartiküllere kadar birçok ilaç taşıyıcı sistemde kullanımı da giderek artmaktadır [8].

Bu derleme kapsamında, oküler kullanımdaki KLN ve NLT sistemlerine, bu formülasyonların sterilizasyonuna ve QbD hakkındaki son araştırmalara odaklanılmıştır.

Oküler Anatomi ve İlaç Uygulama Yolları

Göz, benzersiz bir anatomi ve fizyolojiye sahip olan, görmeden sorumlu organdır. Yapısal olarak iki kısımdan oluşmaktadır. Ön segment (anterior segment) kornea, konjonktiva, iris gibi dokuları içerirken; arka segment (posterior segment) retina, sklera ve optik sinirleri içinde barındırmaktadır (Şekil 1). Bu iki farklı segment ve bu segmentlerde bulunan dokularda gelişen problemlere bağlı olarak çeşitli ilaç uygulama yolları mevcuttur. Topikal ilaç uygulama genellikle keratit, konjonktivit veya glokom gibi gözün ön segmentini etkileyen hastalıklarda tercih edilmektedir. Göz damlaları, kullanım kolaylığı, yüksek hasta uyuncu ve düşük maliyeti ile oküler preparatların %70'ini oluşturmaktadır. Oküler yüzey, sırasıyla harici bir lipidik katman ve ağırlıklı olarak sulu ve mukoza yapısına sahip iki ardışık hidrofilik katman sunan 7-9 µl lakrimal sıvı ile kaplıdır. Tek bir formülasyon damlasının uygulanması, göz kırpma refleksini etkinleştirir ve bu sebeple damlalar nazolakrimal drenaj ve gözyaşı döngüsü yoluyla hızla uzaklaştırılmaktadır. Ayrıca, kornea dışında göz yüzeyini ve göz kapaklarının iç yüzeyini kaplayan konjonktiva aracılığıyla gerçekleşen özgül olmayan emilim, transkorneal emilim için göz yüzeyinde kalan formülasyonun ciddi şekilde azalmasına sebep olmaktadır [9].



Şekil 1. Oküler anatominin şematik gösterimi

Kornea, lipofilik bir dış epitel ve daha kalın bir hidrofilik stroma ile ayrılmış iç endotel içeren oldukça farklılaşmış bir dokudur. Bu hidrofobik yapıdaki değişim ve epitel içindeki hücreler arası sıkı bağlantıların yoğunluğu, ilaç difüzyonunu engellemektedir. Yukarıda belirtilen engellerden ötürü başlangıçta uygulanan dozun ancak %5'ten azı ön segmentteki aköz hümöre ulaşabilmektedir (Şekil 1). Bu bölgedeki aköz hümör dolaşımı, uveal dönüşüm gibi çeşitli konvektif akışlar nedeniyle ilacın biyoyararlanımı daha da azalmaktadır [10]. Oküler hastalıklarda tercih edilen bir diğer ilaç uygulama şekli sistemik ilaç uygulamasıdır. Ancak, ön segmente ulaşması amacıyla sistemik ilaç uygulanması yapılamamakta olup sadece bazı arka segment hastalıklarında tercih edilmektedir. Diğer yandan arka kısımda bulunan iki farklı kan-retina engeli de ilacın verimli bir şekilde istenilen bölgeye ulaşmasına engel olmaktadır. İlk bariyer olan dış kan-retina engeli, temel olarak retinal pigmentli epitelden oluşur. Bu tek tabaka melanin açısından zengindir, pencereli koryokapillarise sıkıca bağlıdır ve ilacın retinaya dağılımını sınırlar. İç kan-retina engeli olarak bilinen ikinci bariyer ise retinaya gömülü ince kılcal damarlardan oluşur ve paraselüler yoldan ilaç erişimini engelleyen sıkı bir şekilde kapalı endotel tabakadan meydana gelmektedir. Bu sınırlamalara rağmen, mevcut klinik uygulamada fotodinamik tedavi gibi intravenöz uygulama yapılan bazı tedavi seçenekleri kullanılmaktadır [11].

İntravitreal enjeksiyonlar doğrudan gözün arka segmentini hedef almakta olup diyabetik retinopati ve yaşa bağlı makula dejenerasyonu gibi dejeneratif hastalıkların hasar verdiği retina bölgesini tedavi etmeyi amaçlamaktadır. Hem düşük moleküler ağırlıklı bileşikler (örn. kortikosteroidler) hem de biyolojik moleküller (örn. bevacizumab, ranibizumab, pegaptanib) gibi yüksek moleküler ağırlıklı bileşikler intravitreal enjeksiyonla uygulanmaktadır. İşlemin invazif olmasından dolayı endoftalmi,

katarakt veya retina dekolmanı gibi çok sayıda yan etki rapor edilmiştir [12].

Farklı uygulamalar değerlendirildiğinde, oküler biyoyararlanımı ve hasta uyuncunu artıran, etkinliği yüksek, girişimselliği ve yan etkisi düşük sistemlerin geliştirilmesinin bir zorunluluk olduğu görülmektedir. Yukarıda belirtilen engeller ve uygulama yollarının eksiklikleri dikkate alınarak geliştirilecek yeni ilaç taşıyıcı sistemler sayesinde göz yüzeyindeki etkin maddenin uzaklaştırılmasının azaltılabileceği ve göz yüzeyinde kalış süresinin önemli ölçüde artırılabileceği düşünülmektedir [2].

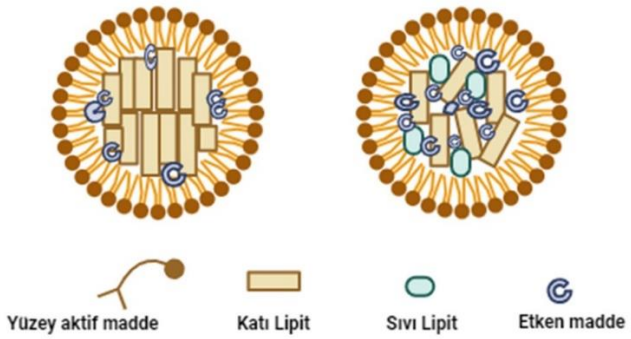
Oftalmolojide Lipit Nanopartiküllerin Uygulanabilirliği

Nanoemülsiyonlar, lipozomlar, niozomlar, kübozomlar ve lipit nanopartikülleri gibi lipit bazlı ilaç taşıyıcı sistemler; biyouyumlulukları, uygun partikül boyutları, biyobozunurlukları ve tolere edilebilirlikleri nedeniyle araştırmacılar tarafından yoğun bir ilgi çekmektedir. Bahsedilen tüm lipit bazlı nanotaşıyıcıların oftalmolojideki kullanılabilirliklerini özetleyen bir inceleme burada sunulmaktadır [13]. Başlangıçta üstün fiziksel stabiliteleri, düşük maliyetli üretim malzemelerine ve süreçlerine sahip olmalarıyla lipozomlara alternatif olarak ortaya çıkan ve aynı zamanda toksik bozunma ürünleri bulunmamasından dolayı polimerik nanopartiküller yerine değerlendirilen KLN'ler, çeşitli uygulama yollarına yönelik geliştirilerek dermal, oküler, pulmoner, parenteral ve oral ilaç taşıyıcı sistemler olarak araştırılmıştır [14]. Lipit nanotaşıyıcıların bir başka özelliği de birden fazla terapötik ajanı enkapsüle edebilmesidir. Bu durum, sinerjik bir etkiye ve artan terapötik performansa sahip olan, ikili veya çoklu ilaç lipit nanopartiküllerinin hazırlanmasını sağlar. Oftalmolojide ise bilhassa KLN'ler ve ikinci nesil olan NLT'ler, ilaç deposu görevi üstlenen formülasyonlar olarak sürekli ilaç salımı sağlamaları ve yapılarına katılan iyonik olmayan maddeler sayesinde kornea geçirgenliğini arttırma yeteneklerinden dolayı özellikle faydalı kabul edilir [15]. Ayrıca, kornea epitel hücreleri arasındaki sıkı kavşakları (tight junctions) açarak, paraselüler ilaç geçişini kolaylaştırarak ve ilaç geçişini sınırlandıran P-glikoprotein aktivitesini inhibe ederek oküler biyoyararlanımın yükseltilmesine katkıda bulunabilir.

Oküler ilaç taşıyıcı sistemler göz önüne alındığında, ortalama nanopartikül boyutu (Z ortalama değeri) polidispersite indeksi (PDI) ve zeta potansiyeli, deneysel tasarımlar ile sistemin kalitesini artırmak için düzenlenmesi gereken hedef parametrelerin başında gelmektedir. Genel olarak, ≤200 nm'lik partiküllerin oküler bariyerlerden yeterli geçirgenlik ve hareketlilik sağladığı kabul edilirken, 20 nm civarındaki küçük partiküllerin, oküler yüzeyden hızlı bir şekilde temizlendiği tespit edilmiştir. Bu nedenle dar parçacık boyutu dağılımına (PDI < 0.2) ve 200 nm'nin altında Z-ortalama değerine sahip sistemler hedeflenmektedir. Zeta potansiyeli ise, nanopartiküllerin stabilitesini ve topaklaşma davranışını etkileyen elektrostatik kuvvetlerin derecesidir. Kolloidal stabilite ile ilgili olarak, yüksek mutlak değerler (yaklaşık ±20 mV) ilgi çekicidir, çünkü daha düşük değerler, partiküller arasındaki çekici kuvvetler tarafından aşılabilir ve bu durumda formülasyonlarda kararsızlığa yol açabilir [16-19].

Lipit Nanopartiküllerin Yapısal Özellikleri ve Oküler Tedavideki Son Gelişmeler

Yapısal bileşenlerine göre lipit nanopartiküller, KLN'ler (ortam ve fizyolojik koşullar altında katı haldeki lipitlerden oluşan) ve NLT'ler (bileşimlerinde ayrıca sıvı lipitler içeren) olarak iki farklı grup altında incelenebilir. Her iki durumda da lipit bileşenler, yüzey aktif maddelerle stabilize edilmiş sulu bir ortamda disperse olur [20]. Spesifik yapıları ve türleri Şekil 2'de gösterilmektedir.



Şekil 2. Katı lipit nanopartiküller ve nanoyapılı lipit taşıyıcıların şematik gösterimi

Lipit Nanopartiküllerin Hazırlanma Yöntemleri

Literatür incelendiğinde lipit nanopartiküllerin hazırlanmasında farklı yöntemlerin olduğu tespit edilmiştir. Başlıca KLN hazırlama yöntemleri arasında homojenizasyon, ultrasonikasyon, emülsiyon oluşturma-çözücü buharlaştırma, püskürtmeli kurutma, süperkritik sıvı kullanma ve çift emülsiyon oluşturma sayılabilir [21]. NLT hazırlama yöntemleri arasında da yüksek basınçlı homojenizasyon, mikroemülsiyon, sonikasyon, çözücü difüzyonu, emülsiyon oluşturma-çözücü buharlaştırma, çözücü enjeksiyonu/yer değiştirmesi ve ters faz yer alır [22].

Katı Lipit Nanopartiküller

KLN'ler genellikle 50 ila 1000 nm arasında değişen, küresel şekildeki kolloidal sistemlerdir. Hem hidrofilik hem de hidrofobik ilaçlar için taşıyıcı olarak başarılı şekilde değerlendirilmektedir. Hazırlanmalarında en sık kullanılan katı lipitler arasında trigliseritler (tristearin (Dynasan 118), tripalmitin (Dynasan 116), trimiristin (Dynasan 114)), monogliseritlerin, digliseritlerin ve trigliseritlerin karışımları (gliseril behenat (Compritol 888 ATO), gliseril palmitostearat (Precirol ATO 5)), mumlar (balmumu, karnauba mumu), yağ asitleri (laurik/stearik/miristik asit) ve karşılık gelen yağ alkolleri yer alır [23]. Çeşitli çalışmalarda bildirildiği üzere, lipitlerin kimyasal yapısının fizikokimyasal özellikleri ve nanopartiküllerin taşıma süreci üzerinde önemli bir etkisi vardır. Boonme ve ark. yaptıkları bir çalışmada, farklı lipitlerin (gliseril trimiristat, gliseril tripalmitat, gliseril tristearat, stearik asit, gliseril monostearat) mikroemülsiyon tekniği ile elde edilen KLN'lerin özelliklerine etkisini araştırmıştır. Seçilen lipitler, yağ asitleri zincirlerindeki C atomlarının sayısı ve polariteleri bakımından farklılık göstermektedir. Elde edilen sonuçlara göre, lipit polaritesi, mikroemülsiyon elde etme kapasitesini etkilemektedir. İncelenen üç formülasyonda (gliseril monostearat, stearik asit ve gliseril trimiristat içeren) bu etki rapor edilmiştir. Bu durum, gliseril tripalmitat/gliseril tristearat yapısında polar fonksiyonel grupların bulunmaması ve bunların uzun (C-16/C-18) zincirleriyle ilişkili olabilir. Bu da yüzey aktif madde arayüzeyindeki hidrofobik bölgeye nüfuz edemeyen büyük moleküler hacimlere yol açabilir. Yağ asidinde kalan karbon atomu sayısı aynı zamanda nanopartikül boyutunu da etkilemektedir. En küçük çap, daha kısa karbon zincirinin (C-14 ve C-18) yüzey aktif maddenin arayüze nüfuz etmesini kolaylaştırmasının bir sonucu olarak gliseril trimiristat bazlı formülasyonda gözlenmiştir.

Katı bir lipit veya lipit karışımının uygun seçimi, nanotaşıyıcıların fizikokimyasal özelliklerinin (boyut, etken madde yükleme kapasitesi) yanı sıra ilaç salımını ve saklama stabilitesini de etkilediği için önemli bir husustur. Formülasyon çalışmaları sırasında dikkate alınması gereken önemli konular arasında ilacın lipit matrisindeki çözünürlüğü, ilaç/lipit uyumluluğu ve lipitlerin kristal davranışı yer alır. Nanopartiküller içindeki yapısal oluşuma ve ilaç konumuna bağlı olarak, Şekil 2'de gösterildiği gibi iki tip KLN tanımlanabilir.

Homojen matris modeli, esas olarak yüksek basınçlı homojenizasyon yöntemiyle üretilen, lipit matris içinde (çözünmüş veya amorf kümeler halinde) tekdüze şekilde yerleşmiş bir ilaçla karakterizedir. Homojen matris parçacıkları, ilacın toplu lipit içinde karıştırılmasından (soğuk teknik uygulandığında) veya sıcak homojenizasyon durumunda soğutulmuş sıvı damlacıklarının kristalleştirilmesinden kaynaklanır. İkincisi, çözücü gerektirmediğinden yüksek oranda lipofilik olan ilaçlar için uygundur [24].

İlaç açısından zengin kabuk modeli; soğutma aşamasındaki faz ayrımı ve migrasyondan kaynaklı ilacın nanopartiküllerin dış kabuğunda lokalize olmasıyla meydana gelir. Hızlı soğutma, merkezdeki lipidin çökelmesine neden olurken, kalan sıvı lipitteki ilaç konsantrasyonu artarak dış kabuğu oluşturur. Bu model, hızlı ilaç salımı ile karakterizedir. İlaç açısından zengin çekirdek modeli; erimiş lipitteki yüksek ilaç konsantrasyonuyla karakterizedir. Bu durum, ilacın aşırı doygunluğuna ve lipidin yeniden kristalleşmeden evvel soğutma fazı sırasında çökmesine yol açar. Daha fazla soğutma, lipidin yeniden kristalleşmesine ve ilaç açısından zengin çekirdeği kaplayan bir membranın oluşumuna yol açar [25].

Lipit bileşenlerine ek olarak, bir KLN formülasyonu ayrıca lipitlerin sulu ortam içinde dağılımını kolaylaştıran ve her iki karışmayan faz arasındaki arayüzey gerilimini azaltarak sistemi stabilize eden yüzey aktif maddeleri de barındırır. Genel olarak yüzey aktif maddeler formülasyon bileşimine ağırlık/ağırlık olarak %5'e kadar dahil edilir ve bunların seçimi; hidrofilik-lipofilik denge (HLB değeri),

KLN'lerin uygulanma yolu, güvenlik profili ve diğer yardımcı maddelerle uyumluluk gibi çeşitli değerlendirmelere dayanır. Oftalmik uygulamalara yönelik KLN'lerde en sık tercih edilen yüzey aktif maddeler; polioksietilen sorbitan yağ asidi esterleri (Polisorbatlar/Tweenler), polioksietilen/polioksipropilen blok kopolimerleri (Poloksamerler/Pluronik) ve anyonik-katyonik muadillerine kıyasla üstün güvenlik profillerine sahip olan amfoterik moleküllerdir (soya lesitini) [23]. Silva ve ark. 2019'da yaptıkları bir çalışmada, katyonik yüzey aktif maddeler setiltrimetilamonyum bromür (CTAB) ve dimetildioktadesilamonyum bromür (DDAB) içeren KLN'lerin sitotoksisitesini farklı kökenli beş insan hücre hattında değerlendirmiştir. Elde edilen sonuçlara göre, CTAB içeren KLN'ler, DDAB içerenlere kıyasla yüksek sitotoksisite sergilemiştir. Bu durum ilk olarak çalışılan konsantrasyonun CTAB'ın kritik misel konsantrasyonuna daha yakın olmasıyla alakalı olup ikinci olarak hücre lizisi ile ilgilidir [26]. Oküler olarak araştırılmış KLN formülasyonları ile ilgili olarak yapılan çalışmaların bazıları derleme kapsamında anlatılmıştır.

Yapılan bir çalışmada Liang ve ark. fungal keratitte kullanılmak üzere Ekonazol yüklü tripalmitin, tween 80, gliserol bazlı KLN'leri mikroemülsiyon yöntemi ile üretmişlerdir. Yapılan *in vitro* karakterizayon çalışmaları sonucunda KLN'lerin partikül boyutu 19 nm, zeta potansiyeli -2 mV ve etken madde yükleme etkinliği %94 olarak bulunmuştur. Ayrıca formülasyonların *in vitro* salım çalışmasında 96 saate kadar kontrollü salım gösterdiği tespit edilmiştir [27]. Yapılan başka bir çalışmada Nair ve ark. endoftalmi tedavisinde kullanılmak üzere klaritromisin yüklü stearik asit, tween 80 ve transcutol P bazlı KLN'ler yüksek hızlı karıştırma ve ultrasonikasyon yöntemi ile hazırlanmıştır. *İn vitro* karakterizasyon çalışması sonucunda, KLN'lerin partikül boyutunun 157 nm, zeta potansiyelinin -17 mV ve etken madde yükleme etkinliğinin %81 olduğu tespit edilmiştir. *İn vitro* salım çalışmaları sonuucunda formülasyonların 8 saat boyunca geciktirilmiş salım yaptığı tespit edilmiş ve etken madde çözeltisine kıyasla kornea geçişinin daha iyi olduğu belirlenmiştir [28]. Yapılan bir başka çalışmada Bonaccorso ve ark. sorafenib yüklü softisan 100 (Hidrojenlenmiş Coco-Gliseritler), suppocire NB (C10–C18 Trigliseritler), tween 80, tegin O, DOTAP, DDAB bazlı KLN'leri ters faz ısıtma yöntemi ile hazırlamışlardır. Yapılan *in vitro* karakterizasyon çalışmaları sonucunda KLN'lerin partikül boyutu 127 nm, zeta potansiyeli 20 mV ve etken madde yükleme etkinliği %75 olarak hesaplanmıştır. *İn vitro* salım sonuçları incelenediğinde 72 saatte etken maddenin %25'inin salındığı tespit edilmiştir, ayrıca fiziksel stabilite çalışmaları sonucunda formülasyonların stabil olduğu tespit edilmiştir. Hücre kültüründe yapılan sitotoksisite çalışmalarında ise formülasyonların biyouyumlu olduğu tespit edilmiştir [29].

Eid ve ark. yürüttükleri bir çalışmada, PEGilasyon ve kitosan kaplamanın ofloksasin yüklü KLN'lerin oküler biyoyararlanımı üzerindeki etkisini araştırmıştır. Formülasyon bileşimine kitosan yerine PEG stearat ilave edilince mukoadezyon üzerinde orta düzeyde bir etki ve daha yüksek transkorneal geçirgenlik elde edilmiştir. Geliştirilen PEGlenmiş kitosan kaplı KLN'ler, tavşanların gözlerindeki ilaç konsantrasyonunu saf ilaca kıyasla iki ila üç kat artırarak ofloksasinin oküler biyoyararlanımını yükseltmiştir [30]. Dang ve ark. da bir çalışmalarında PEGilasyon yaklaşımından faydalanıp PEGlenmiş KLN yüklü kontakt lens tasarlamıştır. Bulgulara göre, artmış bir latanoprost yükleme kapasitesi, PEGlenmemiş KLN'lere kıyasla daha küçük partikül boyutu ve 96 saate kadar sürekli ilaç salımı sağlanmıştır [31]. Yapılan başka bir çalışmada Wang ve ark. glokom tedavisinde kullanılmak üzere metazolamid yüklü fosfolipidler (Lipoid S100), gliseril monostearat, tween 80, PEG400 bazlı KLN'leri emülsiyon solvan uçurma yöntemiyle hazırlamış ve kitosan ile kaplama yapmışlardır. Yapılan *in vitro* salım çalışmasında formülasyonların uzatılmış salım yaptıkları tespit edilmiştir. Ayrıca yapılan *in vivo* çalışmalarda metazolamid yüklü KLN'lerin göz içi basıncını düşürdüğü tespit edilmiştir [32].

Nanotaşıyıcılara ve bir aracı sisteme (yarı katı formülasyonlar, *in situ*/ jeller, kontakt lens) dayalı hibrit ilaç taşıyıcı platformların geliştirilmesi, her iki sistemin olumlu özelliklerinden faydalandığı için oküler taşıma amaçları için üstünlük sağlar. Sun ve Hu yaptıkları çalışmada, uygun jelleşme ve reolojik özelliklere (jelleşme sıcaklığı 32°C, psödoplastik davranış) sahip, serbest ilaca kıyasla geliştirilmiş farmakodinamik etkileri olan ve sürekli ilaç salımı sergileyen, ısıya duyarlı olarak yerinde jelleşen takrolimus yüklü KLN'ler geliştirmiştir [33]. Yapılan başka bir çalışmada Ghada ve ark. alerjik konjonktivitte kullanılmak üzere mizolastin yüklü KLN formülasyonlarını sıcak homojenizayon/ultrasonikasyon yöntemiyle hazırlamışlar ve üretimi takiben mizolastin yüklü KLN'leri sodyum aljinat bazlı hidrojellerin içerisine yüklemişlerdir. KLN formülasyonları üzerinde yapılan *in*

vitro karakterizasyon çalışmaları sonucunda partikül büyüklüklerinin 202 nm, zeta potansiyellerinin -22 mV ve etken madde yükleme etkinliklerinin %86 olduğu tespit edilmiştir. Yapılan *in vitro* salım çalışmalarında ise 48 saat süre ile mizolastin salımı gerçekleştiği tespit edilmiştir. Ayrıca *in vivo* çalışmalarda tavşan gözlerinde alerjik durumların tetiklenmesini takiben mizolastin KLN yüklü hidrojeller uygulanmış ve alerjik reaksiyonu ortadan kalktığı tespit edilmiştir, bununla birlikte yapılan immünohistokimyasal çalışmalarda formülasyonların oküler yüzeyde TNF-α seviyesini ciddi anlamda azalttığı tespit edilmiştir [34].

Nanoyapılı Lipit Taşıyıcılar

NLT, mükemmel şekilde düzenlenmiş kristal yapıları nedeniyle düşük etken madde enkapsülasyon etkinlikleri ve depolama sırasında etken madde salım eğilimi gibi KLN'lerle ilişkili sınırlamaların üstesinden gelmek için geliştirilmiştir [20]. NLT formülasyonlarına sıvı lipit(ler)in eklenmesi ile birlikte daha düzensiz bir kristal yapı oluşur, bu sayede hem etken madde yüklenmesi için ekstra alan sağlanmakta hem de lipit matrisinin kristallik derecesi azaldığından ilacın saklama sırasında salım sergilemesi önlenmektedir. Sıvı lipit(ler), NLT formülasyonlarına toplam lipit miktarının en fazla %30'una kadar dahil edilmektedir. Bu kapsamda araştırmacılar formülasyonlara sıklıkla hint/zeytin/argan yağı, oleik asit, miglyol® 812 (orta zincirli trigliseritler), propilen glikol dikaprilokaprat-Labrafac™ PG (Gattefosse, Saint-Priest, Fransa), Labrasol® (Gattefosse, Saint-Priest, Fransa) veya kaprilokaproil makrogol-8 gliseritleri sıvı lipit olarak eklemektedir [35].

Katı lipitlerin seçimi NLT'lerin partikül boyutunu etkilemektedir. Apostolou ve ark. göre [36], precirol ATO 5 (Gattefosse, Saint-Priest, Fransa), compritol 888 ATO (Gattefosse, Saint-Priest, Fransa) veya dynasan 118 (IOI Oleo GmbH, Hamburg, Almanya) gibi katı lipitler içeren NLT'lerin gliseril monostearat bazlı nanotaşıyıcılarla karşılaştırıldığında daha büyük parçacık boyutları sergilemektedir. Bu durum, lipitlerin daha yüksek moleküler ağırlığının, daha karmaşık bir yapının oluşmasına yol açması ve moleküller arasında toplanma eğilimi göstermesi ve bunun da nanopartikül çapının artmasıyla sonuçlanması ile açıklanabileceği düşünülmektedir. Ayrıca sıvı lipitlerin seçimi de partikül boyutunu etkilemektedir, yapılan çalışmalarda Mygliol® 812 içeren NLT'lerin (IOI Oleo GmbH, Hamburg, Almanya), oleik asit veya Capryol 90 içerenlerle (Gattefosse, Saint-Priest, Fransa) karşılaştırıldığında genellikle daha büyük partikül boyutuna sahip olduğu tespit edilmiştir [37].

NLT'ler hazırlama yöntemlerine, lipit matris yapısına ve ilacın konumuna bağlı olarak üç modele ayrılabilir. Kusurlu tip (imperfect type), yapısal olarak farklı lipitlerin harmanlanmasıyla elde edilmektedir. Bu sayede düzensiz lipit matrisinin oluşmasına neden olmaktadır. Seçilen lipitler, genellikle daha fazla miktarda katı lipit ile karıştırılmış küçük bir sıvı yağ fraksiyonu, yağ asidi kökeni, karbon zinciri uzunluğu veya doygunluk derecesi bakımından farklılık gösterebilmektedir. Bu tip NLT, lipit matrisindeki kusurlarla orantılı olarak ilişkili olan yüksek etken madde yükleme kapasitesi ile karakterize edilmektedir [38]. Amorf tip NLT'ler, formülasyona hidroksioktakozanil hidroksistearat ve izopropil miristat gibi spesifik lipitlerin eklenmesiyle oluşturulmaktadır. Bu lipitler, kristal olmayan (amorf) bir matrisin oluşumuna katkıda bulunur ve bu sayede katı lipit kristalizasyonunun bir sonucu olarak etken madde salımını sınırlar. Çoklu tip NLT'ler, genellikle sıcak homojenleştirme tekniğiyle elde edilen, katı bir lipit matrisi içindeki katı içinde yağ, su içinde yağ gibi çok sayıda bölümden oluşan, nanotaşıyıcılarıdır. Çoklu tip NLT'ler, lipofilik ilaçların katı lipitlerdekilere kıyasla sıvı lipitlerdeki üstün çözünürlüğünden dolayı yüksek etken madde yükleme kapasiteleriyle karakterize edilmektedir. Ayrıca katı matris, ilaç salımını sınırlayan ve salım sürecini kontrol eden bir bariyer fonksiyonu sergilemektedir. Bununla birlikte formülasyondaki sıvı lipit miktarının daha fazla olması, faz ayrılmasına ve soğutma fazı üzerine nano boyutlu damlacıkların oluşmasına yol açabilmektedir [39]. Oküler olarak araştırılmış NLT formülasyonları ile ilgili olarak yapılan çalışmaların bazıları derleme kapsamında anlatılmıştır.

Yapılan bir çalışmada Varela ve ark. keratokonus tedavisinde kullanılmak üzere; gliserol monostearat 40-55, soya lesitini, Compritol 888 ATO, kolesterol, kapryol 90, miglyol 812 N, kolliphor P 407, kolliphor P 188, α-Tokoferol-PEG kullanarak laktoferrin yüklü NLT formülasyonu geliştirmiştir. Üretimi takiben yapılan ölçümlerde partikül büyüklüğünün 119 nm olduğu, zeta potansiyelinin 17 mV, enkapsülasyon etkinliğinin %75 olduğu tespit edilmiştir. Yapılan stabilite çalışmalarında NLT'lerin 3 ay süre ile stabil olduğu belirlenmiştir. Ayrıca *in vivo* çalışmalarda NLT'lerin oküler tolere

edilebilirliğinin yüksek olduğu tespit edilmiştir [40]. Yapılan başka bir çalışmada Kumari ve ark. [41], kuru göz tedavisinde kullanılmak üzere deksametazon yüklü Labrafac™ Lipophile WL 1349 (Gattefosse, Saint-Priest, Fransa), kolesterol, tween 80 içeren NLT üretmişlerdir. HCEC hücre hattında ve domuz korneasında yapılan *ex vivo* geçiş çalışmalarında formülasyonların serbest deksametazona kıyasla daha etkin bir geçiş sağladığı tespit edilmiştir. Aynı zamanda yapılan *in vivo* etkinlik çalışmalarında deksametazon içeren NLT formülasyonlarının kuru göze bağlı olarak artan sitokinlerin (IL-6, TNF-α) seviyesini serbest ilaca kıyasla ciddi anlamda azalttığı belirlenmiştir. Diğer bir çalışmada Öner ve ark. loteprednol etabonat yüklü precirol® ATO 5 ve oleik asit bazlı KLN, NLT ve mikroemülsiyonları QbD ile üretmişlerdir. Yapılan salım çalışmalarında kontrollü salım yapan formülasyonların salımlarında difüzyon ve erozyonun etkili olduğu belirlenmiştir. Ayrıca, ELISA test sonuçları, formülasyonların IL-1 ve IL-6 düzeylerini önemli ölçüde azalttığını göstermiştir [42]. Zingale ve ark., diyabetik retinopatide kullanılmak üzere diosmin yüklü kompritol 888 ATO, miglyol 812, lutrol F68 içeren NLT'ler yüksek hızlı homejenizasyon yöntemiyle üretilmiştir. Formülasyonların yaklaşık 60 gün süre ile stabil kaldığı tespit edilmiştir. ARPE 19 hücre hattında yapılan çalışmada formülasyonların biyouyumlu olduğu belirlenmiştir. Ayrıca NLT'ler retinal inflamasyon modeli üzerinde *in vitro* olarak değerlendirilmiş ve formülasyonların çeşitli konsantrasyonlarda sitoprotektif etkisi olduğu tespit edilmiştir [43]. Başka bir çalışmada Chen ve ark., brinzolamid ve latanoprost yüklü captex 200P (propylene glycol dicaprate), soya lesitin, capmul®, MCM C10 (glyceryl monocaprate), tween 80, Transkutol P, stearilamin ve captex 200P bazlı NLT formülasyonlarını sıcak mikroemülsiyon yöntemiyle hazırlamışlardır. Yapılan kornea geçiş çalışmasında 24 saatin sonunda brinzolamid ve latanoprostun sırasıyla %82 ve %84 oranında geçtiği tespit edilmiştir. Ayrıca yapılan *in vivo* çalışma sonucunda formülasyonların lazer ile indüklenmiş glokomda göz içi basıncını etkili bir şekilde düşürdüğü bildirilmiştir [44]. Li ve ark., korneal neovaskülarizasyonda kullanmak amacıyla, dasatinip yüklü gliserin monostearat, miglyol 812 N, solutol HS 15, gelucire 44/14, soya lesitin bazlı NLT formülasyonlarını üretmişlerdir. *İn vitro* karakterizasyon çalışmaları sonucunda partikül boyutlarının 78 nm olduğu, zeta potansiyelinin -29 mV olduğu ve enkapsülasyon etkinliğinin %97 olduğu tespit edilmiştir. Çözünürlük çalışmalarında NLT içine yükleme ile birlikte dasatinibin çözünürlüğünün 1200 kat arttığı gösterilmiştir. Ayrıca *in vivo* çalışmalar sonucunda farelerde geliştirilen korneal neovaskülarizasyonu ortadan kaldırdığı bildirilmiştir [45].

KLN'lere benzer şekilde NLT'lerin yüzeyi, mukoadhezifliği, sürekli ilaç salımını ve penetrasyon artırmak için katyonik maddeler (örn. kitosan) kullanılarak modifiye edilebilmektedir. Kitosan (trimetil kitosan) türevleri ve kitin (kitosan oligosakkarit) nötr pH'da (gözyaşı sıvısı dahil) yüksek çözünürlük sergiledikleri için nanopartikül yüzey kaplama amacıyla kullanılmaktadır. Modifiye kitosan, doğal kitosanla karşılaştırıldığında üstün güvenlik profilleri sunarken aynı zamanda kitosana ait tüm üstün özelliklerini de (biyolojik olarak parçalanabilirlik, muko yapışması, penetrasyonu arttırıcı özellikler vb.) korumaktadır [46].

Mukoadezif NLT örneklerinde, oküler yüzeydeki siyalik asit kalıntılarını spesifik olarak hedefleyerek korneada kalma süresini artırmak için kondroitin sülfata bağlı (3-aminom etilfenil) boronik asit ile işlevselleştirme yapılan çalışmalar bulunmaktadır. Bu sayede, özellikle kuru göz hastalığına ilişkin tedavi etkinliği artırılmıştır [47]. Abdelhakeem ve ark., merkezi seröz korioretinopatinin tedavisi için yüzeyi modifiye edilmiş eplerenon yüklü NLT'leri geliştirmişlerdir. Çalışma kapsamında üç farklı kaplama polimerinin (hiyalüronik asit, kitosan oligosakarit laktat ve hidrojenlenmiş kollajen) nanotaşıyıcıların özellikleri üzerindeki etkisi değerlendirilmiştir. Formülasyonlar içerisinde en yüksek eplerenon yükleme etkinliğine sahip formülasyonlar hiyalüronik asit kaplı NLT'ler olarak rapor edilmiştir. Sahip olduğu yüksek viskozite, diğer NLT modellerine kıyasla hiyalüronik asitle modifiye edilmiş NLT'lerin uzun süreli ilaç salımına sebep olmuştur. Draize testinde seçilen optimal formülasyonların (hiyalüronik asit/kitosan oligosakkarit laktat kaplı) oküler tolere edilebilirliğinin yüksek olduğu gösterilmiştir [48].

NLT'ler aynı zamanda hibrit ilaç taşıyıcı sistemlerin bir bileşeni olarak denenmekte olup son zamanlarda *in situ* jeller ile beraber kullanıldığı çalışmalar bulunmaktadır. Abdolmonem ve ark., COVID 19'la ilişkili oküler semptomları önlemek amacıyla loratadin yüklü compritol 888 ATO®, labrasol® ve span® 60 bazlı NLT'leri sıcakta eriyen emülsifikasyon yöntemiyle üretmişlerdir. Daha sonraki aşamada formülasyona jel oluşturmak amacıyla hidroksipropil metilselüloz (HPMC)

eklenmiştir. *İn vitro* karakterizasyon çalışmaları incelendiğinde, partikül boyutunun 156 nm, zeta potansiyelinin -40 mV ve etken madde yükleme etkinliğinin %94 olduğu tespit edilmiştir. Tavşanlar üzerinde yapılan draize testi sonuçlarında formülasyonların oküler tolere edilebilir olduğu tespit edilmiştir [49]. Yu ve ark., yapmış oldukları iki farklı çalışmada pH ve ısıya duyarlı *in situ* jellere baicalin NLT'leri ve quercetin NLT'lerini yüklemişlerdir. Bu iki hibrit sistemin, uzun süreli ilaç salımı ve uzatılmış kornea temas süresi sağladığı tespit edilmiştir, ayrıca göz damlalarına kıyasla daha iyi transkorneal penetrasyon gösterdiği bildirilmiştir [50].

KLN ve NLT'lerin Sterilizasyonu

Formülasyonların benzerlikleri nedeniyle KLN ve NLT'ler, yüksek basınçlı homojenizasyon (sıcak/soğuk seçeneği), yüksek hızlı homojenizasyon ve/veya ultrasonikasyon, solvent emülsifikasyonu/buharlaştırma, mikroemülsiyon, faz ters çevirme tekniği ve solvent enjeksiyon yöntemi gibi benzer yöntemlerle hazırlanabilmektedir. Bununla birlikte, oküler uygulamalar için büyük önem taşıyan durum, üretim sonrası adımlardan biri olan formülasyonların sterilizasyonudur. Oküler uygulamaya yönelik hazırlanan KLN ve NLT'lerin sterilizasyonu için sıcaklık ile sterilizasyonu (otoklavlama), filtrasyon ve gama ışınlaması gibi farklı teknikler kullanılmaktadır. Spesifik yöntemin seçimi için, kullanılan ilacın ısı stabilitesi, formülasyon bileşenleri (lipitlerin erime noktası, yüzey aktif maddelerin seçimi), nanopartikül boyutu ve steril filtrasyon durumunda çözeltinin viskozitesi gibi çeşitli hususlar dikkate alınmalıdır. Otoklavlama, oküler lipit nanopartiküllerinin sterilizasyonu için en sık kullanılan tekniktir, bununla birlikte nanotaşıyıcıların fizikokimyasal özellikleri üzerindeki etkisi konusunda tartışmalı mevcuttur. Bazı raporlara göre, geliştirilen lipit nanotaşıyıcıların sterilizasyon öncesi ve sonrası partikül boyutunda veya enkapsülasyon etkinliğinde önemli bir değişiklik yokken bazı raporlara göre partikül boyutunda artışları olabileceği belirtilmektedir [51]. Otoklav ile görülebilecek bir diğer problemde katı lipitlerin 121°C'de erimesine ve bir Y/S emülsiyonunun oluşumuna yol açmasıdır [51]. Gama ışıması da NLT ve KLN'lerin sterilizasyonu için kullanılmaktadır. Youshia ve ark., otoklavlama ve gama ışınlaması yoluyla sterilizasyonun metazolamid yüklü katyonik NLT'lerin fizikokimyasal parametreleri üzerindeki etkisini araştırmışlardır. Sonuçlara göre, ısıyla sterilizasyona tabi tutulan NLT'lerin önemli ölçüde daha düşük enkapsülasyon etkinliği ve zeta potansiyeli değerlerine sahip olduğu tespit edilmiştir. Bununla birlikte, partikül boyutunda ve partikül büyüklüğü dağılımında bir artış gözlemlenmiştir. Aksine, sterilizasyon için gama radyasyonu uygulandığında partikül boyutunda, partikül büyüklüğü dağılımında ve metazolamidin enkapsülasyon etkinliğinde önemli bir değişikliğin olmadığı tespit edilmiştir [52]. Ancak bu yöntemin ana sınırlamalarından biri serbest radikallerin oluşmasıdır, bu nedenle bileşenlerin kimyasal stabilitesini değerlendirmek için sonraki çalışmaların yapılması gerekmektedir. Ek olarak radyasyonun olumsuz etkilerini azaltmak için uygulanan dozun ayarlanması, numunelerin liyofilizasyonu ve uygun (γ radyasyona dayanıklı) yardımcı maddelerin kullanılması gibi farklı stratejilerin uygulanması gerekmektedir. Filtrasyon ile süzme de NLT ve KLN'lerin sterilizasyonunda kullanılan başka bir yöntemdir. Yapılan bir çalışmada, farklı tipte membranların (polipropilen, polietilen sülfon, poliviniliden florür; gözenek boyutu 0.22 μm) sorafenib yüklü KLN'lerin filtrasyon fizibilitesi üzerindeki etkisini araştırmışlardır. Elde edilen sonuçlar, polipropilen ve polietilen sülfon filtrelerin, KLN'lerin geçişini sağlayan poliviniliden florür membrandan farklı olarak, nanopartikülleri membran içerisinde tutarak filtrasyon sürecini kısıtladığını göstermiştir [29].

Tasarımla Kalite (QbD)

Son yıllardaki güçlü araştırma çabalarına rağmen, nanopartiküler sistemleri içeren ürünlerin pazardaki yerinin göreceli olarak düşük bulunmasının nedeni, bu ürünlerin kalite ve güvenliğinin üretim aşamasında yeterince kontrol edilememesinden kaynaklandığı düşünülmektedir. Nanoterapötiklerin üretimi sırasında, üretim sürecindeki küçük değişiklikler, nanopartikül popülasyonu için gereken kalite özelliklerinden önemli ölçüde sapmalara neden olabilmektedir. Buna ek olarak, yeterli düzenleyici ve güvenlik yönergelerinin bulunmaması, üreticilerin bu alandaki çalışmaları kısıtlamaktadır [53].

Tasarımla kalite (QbD), esnek olmayan üretim adımları ve bulk ile hem ara hem de nihai ürünler üzerinde kapsamlı testler yoluyla nihai ürün yeterliliğini sağlamaktadır. Klasik formülasyon ve kalite kontrol yöntemleriyle hazırlanan ürünlerde, pazar onayından sonra yapılacak herhangi bir değişiklik,

kapsamlı düzenleyici ilaveler gerektirir. Bu da prosesin kolay gelişimini ve uyarlanabilirliğini engellemektedir. Bu nedenle, kronik oküler hastalıkların prevalansında beklenen artışla birlikte, nanoteknolojiye dayalı değerli adayların pazara erişimini kolaylaştırmak için yeni yaklaşımlara ihtiyaç duyulmaktadır. Son yıllarda Avrupa İlaç Ajansı (EMA) ve ABD Gıda ve İlaç İdaresi (FDA) gibi düzenleyici kurumlar, klasik kalite güvence yöntemlerinde bir değişiklik hareketi başlatmışlardır ve tasarımla kalite, QbD, olarak adlandırılan bir yönteme doğru ilerlemişlerdir. Bu terim, Uluslararası Uyumlaştırma Konferansı kılavuzlarında önceden tanımlanmış hedeflerle başlayan, bilime ve kalite risk yönetimine dayanan, ürün ve süreç anlayışını ve süreç kontrolünü vurgulayan sistematik bir gelişim yaklaşımı olarak tanımlanmıştır.

Geleneksel sistemlerin aksine QbD, üretim değişkenleri ile kritik, hasta odaklı kaliteli ürün özellikleri arasındaki ilişkilerin ortaya çıkarılmasına olanak tanımaktadır. Üretim tutarlı ve sağlam kalır ancak aynı zamanda değişikliklere karşı da esnek hale gelir. Aslında, süreç kontrolü ve olası değişkenlik kaynaklarının kapsamlı tanımlanması gerçek kalite güvencesi olduğundan, son ürün testi neredeyse ikincil hale gelmektedir. Sonuç olarak, sürekli test gerekmeyebileceğinden toplu sürüm daha hızlı olabilmektedir. QbD'yi takip eden ürün araştırması ve geliştirme, hedef ürünün (kalite hedef ürün profili (QTPP) olarak da tanımlanır) ve kritik kalite özelliklerinin (CQA'lar) net bir tanımıyla başlar. Bu nedenle hastayla ilgili özelliklerin tanımlanması ve bunların formülasyon niteliklerine dönüştürülmesi son derece önemlidir. QbD perspektifinin ilk araştırma aşamalarında da erken benimsenmesi, hasta odaklı başarılı bir ürüne yönelik ilk adımları atılmasını sağlamaktadır. Bulk materyalin kritik malzeme özelliklerinin (CMA'lar) ve kritik süreç parametrelerinin (CPP'ler) tanımlanması, uygun ayarlama yoluyla istenen ürüne yol açabilecek üretim sürecinin temel parametrelerinin kontrol edilmesine olanak tanımaktadır. Tüm olası değişkenlik kaynakları kontrol altında olmalıdır. Sürecin başındaki ve sonundaki risk değerlendirme (RA) faaliyeti, olası tehlikelerin ve bunlarla ilişkili risklerin sistematik olarak tanımlanmasına olanak sağlamaktadır [54].

QbD yaklaşımları araştırmanın etkinliğini artırarak hem üreticilere hem de düzenleyici kurumlara fayda sağlamaktadır. QbD'nin, araştırma süresinin ve maliyetinin azalmasına ve karmaşık nanopartiküllü sistemlerin pazar onayını kolaylaştırabilecek üretim sürecinin daha geniş ve daha sağlam bir şekilde anlaşılmasına olanak sağlayacağı düşünülmektedir. Sonuç olarak, QbD yaklaşımlarının erken adaptasyonu, yıllarca süren araştırmaların yenilikçi pazarlanan nanoterapötiklere etkili bir şekilde çevrilmesine yol açabilir [55].

Rathod ve ark. yapmış oldukları bir çalışmada, Plackett-Burman deneysel tasarımı yardımı ile ibuprofen yüklü NLT'ler üretmişlerdir. Yedi üretim parametresinin (yüzey aktif madde türü ve konsantrasyonu, lipit konsantrasyonu, homojenizasyon hızı ve süresi gibi), üç yanıt (partikül ortalama boyutu, PDI ve zeta potansiyeli) üzerindeki etkileri değerlendirmek amacıyla 12 farklı formülasyon üretmişlerdir. Kullanılan deney tasarımı modeli sayesinde sadece 12 formülasyon ile yedi faktörün bu üç değişkenin üzerindeki etkisi tespit edilmiş bu sayede 2 ila 8°C arasında saklandığında 1 ay boyunca stabil ve 12 saat boyunca sürekli salım yapabilen NLT'ler elde edilmiştir [56]. Gonzalez ve ark. yapmış oldukları bir çalışmada, flurbiprofen yüklü NLT'leri, toplam lipit, sıvı lipid konsantrasyonu, yüzey aktif madde ve flurbiprofen konsantrasyonu, partikül boyutu, polidispersite indeksi ve enkapsülasyon etkinliği gibi değişkenlerden üzerinde deneysel tasarım yolu ile üretmişlerdir. Sonuçlar, artan yağ (ağırlıkça %) miktarının ardından daha küçük boyutta partiküller elde edilebildiğini göstermiştir fakat üretimde tekrarlanabilirlik sorunları nedeniyle sıvı-katı lipit oranının ağırlıkça %30'u aşmaması gerektiğini göstermiştir. Optimum NLT formülasyonun, dar bir boyut dağılımı (0.156) ve yüksek enkapsülasyon etkinliği (~%90) ile oftalmik uygulama için uygun bir ortalama boyut (228.3 nm) gösterdiği tespit edilmiştir. *İn vitro* salım çalışmaları sonunda, NLT'lerin kontrollü flurbiprofen salım yaptığı ve oküler dokularda toksisite göstermediği tespit edilmiştir [57]. Kiss ve ark. yapmış oldukları başka bir çalışmada, deksametazon yüklü NLT'leri yüzey aktif madde, partikül boyutu, polidispersite indeksi ve enkapsülasyon etkinliği gibi değişkenler üzerinde deneysel tasarım yolu ile üretmişlerdir. Optimum NLT formülasyonun, dar bir boyut dağılımı (0.34) ve yüksek enkapsülasyon etkinliği (~%86) ile oftalmik uygulama için uygun bir ortalama boyut (200.73 nm) gösterdiği tespit edilmiştir [58]. Aytekin ve ark., optimum NLT formülasyonun tespit edilmesi amacıyla her bir grup formülasyon için faktöriyel tasarım gerçekleştirmişlerdir. Formülasyonda kullanılan lipid yüzdesi ve sürfaktan miktarı bağımsız değişkenler olarak kullanılmış, partikül büyüklüğü, partikül dağılım indeksi (PDI) ve zeta

potansiyel değerleri de optimizasyon parametreleri olarak değerlendirilmiştir. Buna göre partikül boyutu ve PDI değeri minimumda tutulmak istenmiş olup, zeta değeri pozitif yüklü nanopartikül grubu için olabildiğince yüksek tutulmaya çalışılmıştır. Bu sonuçlara göre lipid yüzdesinin partikül büyüklüğü ile doğrudan ilişkili olduğu bulunmuş ve partikül büyüklüğünü düşük tutmak amacıyla tüm formülasyonlar için en düşük değer olan %10 değeri ile devam edilmiştir. Sürfaktan miktarındaki artış da partikül büyüklüğünü azaltmış olsa da *in vitro* çalışmalarda partikül büyüklüğünün küçülmesine bağlı olarak bir üstünlük sağlanamamıştır. Bu sebeple yüzey aktif ajan kullanımına bağlı olarak olası toksisite riskini en aza indirmek amacıyla düşük sürfaktan konsantrasyonu olan %2 ile devam edilme kararı alınmıştır. Bu sayede deney tasarımı kullanılarak optimum formülasyon *in vitro* çalışmalarla belirlenmiş *in vivo* çalışmalarda hayvan grubu azaltılarak çalışmada daha az sayıda tavşan ve rat kullanılması sağlanmıştır [20].

SONUÇ VE TARTIŞMA

KLN'ler ve NLT'ler, bu derlemede özetlenen bulgularla da doğrulandığı gibi, etkili oküler ilaç taşıyıcı sistemler olarak önemli bir potansiyele sahiptir. Formülasyonlar hazırlanırken kullanılan lipit bileşenlerin biyouyumlu, biyobozunur olmaları ile birlikte kontrollü ve uzatılmış salım sağlama ve kornea geçişini artırma gibi özellikleri sayesinde etkin ve biyoyararlanımı artırılmış formülasyonların elde edilmesine olanak sağlamaktadır. Oküler toksisite, oftalmik formülasyonların geliştirilmesi sırasında dikkate alınması gereken bir diğer kritik konudur. İncelenen makalelerden elde edilen bulgulara göre, KLN ve NLT'lerin herhangi bir düzeyde toksisite göstermediği belirlenmiştir (*in vitro* veya *in vivo* çalışmalara dayanarak). Bununla birlikte hem KLN'lerin hem de NLT'lerin yüzeyi, farmakokinetik özelliklerini iyileştirmek, muko yapışkan özellikler kazandırmak, korneada kalma süresini uzatmak ve terapötik etkinliklerini artırmak için değiştirilebilmektedir. Ayrıca, NLT ve KLN'ler başka formülasyonlar ile kombine kullanıma da olanak sağlamaktadır. Bu kombinasyonlarda oküler yüzey hastalıklarının tedavisinde umut vadeci olmuştur. Ancak yürütülen çalışmalardan elde edilen tüm umut verici sonuçlara rağmen, araştırmadaki ilerleme henüz klinik uygulamaya aktarılmamıştır. Bu konuyla ilgili problemlerin birincisi saklama sırasında yeterli kolloidal stabilitenin sağlanamaması, bir diğeri de tekrarlanabilir lipit nanopartikül serilerinin üretilmesinin zorluğudur. Bu bağlamda, ön formülasyon aşamasında QbD yaklaşımının değerlendirilmesi uygulanabilir bir strateji olabilir. QbD ile birlikte üretim sırasında oluşacak problemlerin tespiti ile öngörülebilir kalite özelliklerine sahip nihai ürün eldesi ve bu nihai ürünün ticarileşmesi sağlanabilir.

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Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

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DERLEME MAKALE / REVIEW ARTICLE

ALZHEIMER HASTALIĞI, RİSK FAKTÖRLERİ VE TEDAVİ

ALZHEIMER'S DISEASE, RISK FACTORS AND THERAPY

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ÖZ

Amaç: *Alzheimer Hastalığı hem ülkemizde hem de dünya genelinde, yaş ortalamasının da artması ile birlikte görülme sıklığı her geçen gün artan ilerleyici ve zorlu bir hastalıktır. Hastalığa yakalanma nedenleri ve hastalığın patolojisi hala tam olarak aydınlatılamamış, hastalığa yakalanmayı önleyen bir yol bulunamamış ve hasta olduktan sonra da kullanıldığı takdirde hastayı tamamen iyileştirdiği kanıtlanmış bir molekül keşfedilememiştir. Konvansiyonel ilaçlar ile tedavi halen daha klinikte en çok başvurulan ve sadece semptomatik yarar sağlayan tedavi yöntemidir. Günümüzde innovatif ilaç çalışmaları Alzheimer Hastalığına ışık olabilmek için devam etmektedir.*
Sonuç ve Tartışma: *Hastalığın patofizyolojisi tam olarak anlaşılamadan tedavi edilmesi mümkün olmamakla birlikte gelişen ilaç teknolojisi ile umut vaat eden yeni moleküller klinikte kullanıma sunulmuştur. Etkili ve güvenli bulunmalarının devamı halinde ilaç pazarında yerini sağlamlaştırarak hastalara umut olacaklardır.*
Anahtar Kelimeler: *Alzheimer Hastalığı, amiloid beta, tau*

ABSTRACT

Objective: *Alzheimer's Disease is a progressive and challenging disease whose incidence is increasing day by day with the increase in the average age both in our country and around the world. The causes of the disease and the pathology of the disease are still not fully clarified, a way to prevent the disease has not been found, and a molecule that has been proven to completely cure the patient if used after the disease has not been discovered. Treatment with conventional drugs is still the most commonly used treatment method in clinics and provides only symptomatic benefit. Today, innovative drug studies continue to shed light on Alzheimer's Disease.*
Result and Discussion: *Although it is not possible to treat the disease without fully understanding its pathophysiology, promising new molecules have been put into use in the clinic with developing drug technology. If they continue to be found to be effective and safe and strengthen their place in the pharmaceutical market they will be a hope for patients.*
Keywords: *Alzheimer's Disease, amyloid beta, tau*

GİRİŞ

Alzheimer Hastalığı (AH) 1906 yılında Alois Alzheimer tarafından keşfedilmiş, çoğu zaman yaşa bağlı olarak bilişsel ve işlevsel gerileme ile kendini gösteren ve nihayetinde ölümle sonuçlanan

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nörodejeneratif bir hastalıktır. Alois Alzheimer'ın 1901 yılında Auguste Deter isimli hasta ile tanışması ve hastanın bilişsel bozukluk, oryantasyon bozukluğu, delüzyonlar ve başka davranışsal değişiklikler göstermesi; 1906 yılında hastanın ölümüyle birlikte postmortem olarak beyinde histolojik yöntemlerle Alois Alzheimer'ın çalışma yapması ve difüz beyin atrofisi ve kortikal hücre kümelerinde belirli değişiklikler gözlemlemesi ile kendi adı verilen AH ilk defa 1907'de tanımlanmıştır. Yaygın klinik semptomların bazıları planlama, öğrenme, problem çözme, hesaplama, hafıza, konuşma ve karar vermede anormalliklerdir. AH, gelişmiş ülkelerde geriyatrik popülasyondaki (65 yaş üzeri) mortalite nedenleri arasında beşinci sırada yer almaktadır ve geriyatrik popülasyonun yaklaşık %13'ünün AH ile mücadele ettiği bilinmektedir. Dünya genelinde 44 milyonun üzerinde insanın AH veya diğer bağlantılı demans ile yaşadığı belirtilmektedir [1]. AH en yaygın nörodejeneratif demanstır ve dünya çapında milyonlarca hastayı etkilediği gibi hasta yakınlarının da yaşam kalitesini önemli ölçüde değiştirmektedir. Bu kadar büyük çapta etki gösteren hastalığın iki majör patolojik prosesi bilinmektedir: Senil plaklar ve nörofibriler yumaklar [2]. AH'nin etiyolojisi hala tam olarak bilinmediğinden bu hastalığa neden olabilecek tüm risk faktörlerinin araştırılması gerekliliği devam etmektedir [3]. Bu derlemede AH oluşumunda yer alan risk faktörlerinden, hastalığın patofizyolojisinden ve kısaca tedavi yöntemlerinden bahsedilmiştir.

Epidemiyoloji

ABD'de yapılan bir çalışmaya göre her 68 saniyede, yeni bir AH tanısı konmaktadır. Çoğu 65 yaşın üzerinde olan 5,4 milyon Amerikalı hali hazırda AH tanısı almıştır [4]. Nüfus sayımı verileri, sadece ABD'de 2050 yılında, yarısından fazlasının 85 yaşın üzerinde olduğu 13.8 milyon insanın, AH demansı teşhisi alacağı yönünde tahminler ortaya koymaktadır. Günümüzde, dünya üzerinde 36,5 milyon insan demanstan etkilenmekte ve bu vakaların büyük çoğunluğunu AH oluşturmaktadır. Geriyatrik popülasyonda her yıl, tahmini 5-7 milyon yeni AH vakasının oluştuğu değerlendirilmektedir [5]. Demans oranının popülasyonun yaşlanması ile artmaya devam edeceğine olan yaygın inanışın aksine, çok yeni bir çalışma demans prevalansının aslında 2000-2012 yılları arasında azaldığını rapor etmektedir. Bu çalışmada, Langa ve arkadaşları, 10 yıllık bir sürede demans prevalansının %24 oranında azaldığı yönünde veriler sunmuştur. Demans prevalansındaki bu azalma kohortlar arasındaki eğitim seviyesinin yaklaşık bir yıllık artışına bağlanmaktadır [6]. AH insidansının, 85 yaşına kadar yaşla birlikte katlanarak arttığı [7]; platoya ulaşıldıktan sonra ise azaldığı veya platonun devam edip düşüşün gözlenmediği yönünde farklı sonuçların gösterildiği çalışmalar mevcuttur. AH'nin gelecekte oluşturacağı sağlık ekonomisindeki yük ve toplum sağlığı üzerindeki olumsuz etkileri düşünüldüğünde küresel ölçekte daha fazla çalışmaya ihtiyaç vardır [5].

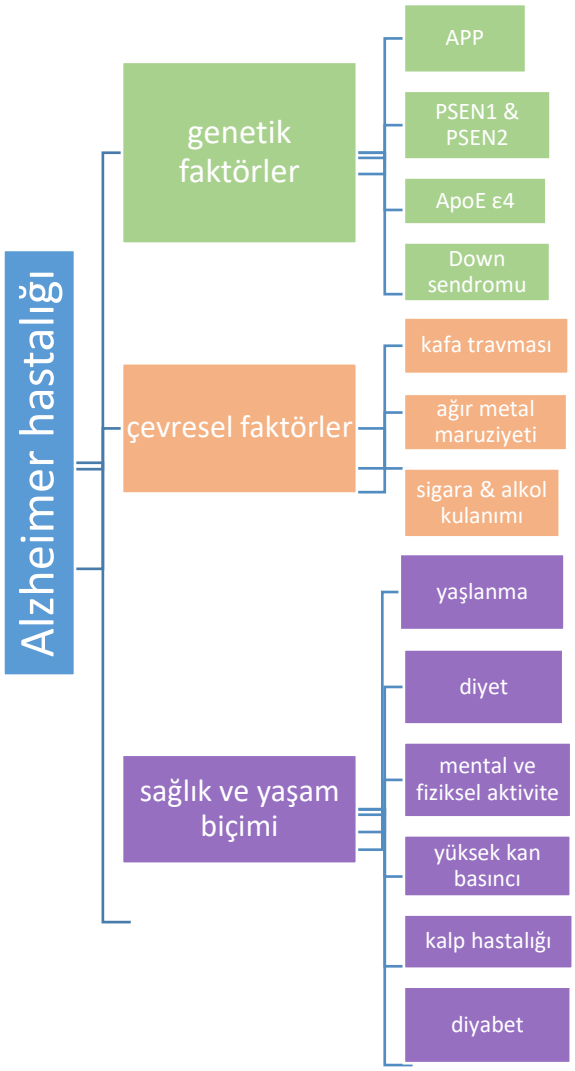
Ekonomik Yük

Yapılan bir çalışmaya göre 2006 senesinde dünya üzerindeki toplam Alzheimer hastası sayısı 26.6 milyon olarak belirlenmiştir. Bu sayının 2050 yılında dört katına çıkması beklenmektedir [7]. Yapılan başka bir çalışma ile Alzheimer hastalarının neredeyse %43'ünün evde bakım ve kurumsal bakım hizmetine (yüksek seviyede ilgi gerektiren) gereksinim duyduğu yönünde tahminler yürütülmüştür. Uzun dönemli kurumsal bakım hizmetinin gelişmiş ülkelerde önemli bir gider kaynağı olacağı düşünülmektedir. Demans hastalarının aile bireyleri tarafından evde bakımı ise bu hasta tipi için gelişmiş ülkelerde genellikle tek seçenektir [8]. O nedenle Alzheimer ve demans hastalarının yeterli derecede bakımı için önemli ölçüde maddi kaynaklara ihtiyaç vardır [7].

Risk Faktörleri

AH dünya çapında yaygın ve altta yatan pek çok nedene bağlı patofizyolojik bir hastalıktır. Temel anlamda hastalığa neden olan belli başlı üç kategori vardır: Genetik faktörler (APP, PSEN1, PSEN2, Down Sendromu, Apolipoprotein E4), çevresel faktörler (kafa travması, sigara ve alkol kullanımı, hava kirliliği, ağır metal maruziyeti gibi), sağlık ve yaşam biçimi (yaşlanma, diyet, mental ve fiziksel aktivite, yüksek kan basıncı, kalp hastalığı, diyabet gibi kardiyovasküler ve metabolik hastalıklar). Bunlardan genetik faktörler bireylerin değiştiremeyeceği durumlar olduğundan hastalığa engel olmak mümkün değildir fakat çevresel faktörlere olan maruziyeti değiştirmek veya daha sağlıklı bir yaşam biçimi tercih

etmek modern insanın bir parça da olsa elindedir. Maalesef Alzheimer yine de bütünüyle önlenebilir bir hastalık değildir [9].



Şekil 1. AH'de risk faktörleri

Değiştirilemez Risk Faktörleri

Cinsiyet

Yapılmış olan geniş çaplı epidemiyolojik çalışmalarda cinsiyetin AH'de tek başına bir risk faktörü olmadığı görülmüştür [10]. Ancak kadınların ortalama yaşam sürelerinin erkeklere göre daha uzun olması ve yaşla birlikte riskte doğrusal artış görülmesi AH'den etkilenenlerin üçte ikisini kadınların oluşturmasına neden olmuştur [11]. Çeşitli çalışmalar AH'li kadınların erkeklere kıyasla daha kötü zihinsel bozulma yaşadıklarını göstermiştir. Ek olarak, genetik düzeyde, Apolipoprotein E4 (ApoE ε4) aleli gibi bazı genlerin varyasyonlarının, erkeklerle karşılaştırıldığında kadınlarda AH riskini önemli ölçüde artırdığı vurgulanmıştır [12,13].

Yaş

Yaş, AH için en güçlü risk faktörüdür. 65 yaşından sonra her beş senede bir AH riski ikiye katlanır, 85 yaşından sonra ise AH görülme ihtimali %50'dir. Aynı zamanda AH, hastalığın başladığı yaşa göre kategorize edilebilir. Erken başlangıçlı AH 65 yaşın altındakileri, geç başlangıçlı AH ise 65

yaşın üzerindekileri etkilemektedir. Erken ve geç başlangıçlı AH, semptomların başlangıç yaşının yanı sıra klinik, nöropsikolojik, nöropatolojik açıdan ve nörogörüntüleme açısından da farklı seyreder [14].

Erken Başlangıçlı Alzheimer Hastalığı Genleri

Erken başlangıçlı vakalarda etkilerin daha güçlü olması gerçeğinin yanısıra, erken başlangıçlı ve geç başlangıçlı da dahil olmak üzere, tüm AH formlarında genetik faktörler önemli rol oynar. Erken başlangıçlı AH, AH'ye bağlı demansın 65 yaşından önce başlaması olarak tanımlanır. Erken başlangıçlı AH ile ilişkili olan otozomal dominant mutasyonlar amiloid prekürsör protein (APP), presenilin 1 (PSEN1) ve presenilin 2 (PSEN2)'dir [15]. Bu genlerden APP kromozom 21'de, PSEN1 kromozom 14'te, PSEN2 ise kromozom 1'de lokalizedir [16]. Erken başlangıçlı Alzheimer hastalığı (EOAD), AH vakalarının % 5-10' unu oluşturur fakat bu vakaların da sadece %10-15'inde AH ile ilişkili olduğu bilinen PSEN1, PSEN2 ve APP genlerinde mutasyon görülmüştür [17]. Geriye kalan kısım ise açıklanamamaktadır [18]. Söz konusu bu üç gen de amiloid proteinin oluşumuna, birikimine ve agregasyonuna neden olur. Ailesel AH'nin en yaygın nedeni PSEN1'dir. Ek olarak EOAD'li bireylerde hastalığın ilerlemesi genellikle daha agresiftir ve hayatta kalma süresi göreceli daha kısadır [15]. EOAD vakalarındaki patolojik hasarın da (nöritik plaklar ve nörofibriler yumaklar) geç başlangıçlı Alzheimer hastalığı (LOAD) hasarına göre daha büyük olduğu gösterilmiştir [19].

Geç Başlangıçlı Alzheimer Hastalığı Genleri

Geç başlangıçlı AH genetik olarak Apolipoprotein E4 (ApoE ε4) mutasyonları ile ilişkilidir. Apolipoprotein E (ApoE) kandaki lipoproteinlerde bulunan belli başlı apolipoproteinlerden biridir ve kromozom 17 üzerinde lokalize olur. ApoE lipidlerin transportunda ve metabolizmasında görev alır. Nöronlar arası transportta, hücre membranı ve sinapsların dayanıklılığında önemli bir role sahiptir. Ayrıca, oluşan hasar sonrası onarımda da görev alır [20]. ApoE ε4 proteininin oligomerik amiloid beta ile çapraz karıştığı ve Aβ'nın sinaptik lokalizasyonunu artırdığı; bunun da sinaptik kayba neden olduğu belirtilmektedir [21,22]. Corder ve arkadaşları ile Strittmatter ve arkadaşları ApoE'nin beyinden ayrılıp kan damarlarına doğru gidecek olan Aβ'yı taşıma işinde görevli olduğunu belirterek ApoE alellerinin AH'de risk faktörü olarak önemini ilk olarak vurgulayanlardır [16]. ApoE'nin tanımlanmış 3 adet aleli bulunmaktadır (ε2, ε3 ve ε4). Aleller, proteinin yapı ve fonksiyonunu etkileyen iki izoform-spesifik amino asitle birbirinden ayrılır. Bu üç alelden ε4, ε2'nin aksine [23,24] AH riskini artırır ve AH'nin başlangıç yaşını düşürür. Bir ε4 aleli AH'nin ömür boyu görülme riskini 2 ila 4 kat artırırken homozigot aleller 8 ila 12 kat artırır [25,26]. ApoE ε4 aleli kolin asetiltransferazın hipokampus ve temporal loblardaki düşük aktivitesi ile ilişkilendirilmektedir; bu da teorik olarak beyinde daha az rezidüel kolinerjik fonksiyona neden olur [4,27]. ApoE ε4'ün hipolipide olduğu ve kolesterol efluksunda ApoE ε3'ten daha etkisiz olduğu gösterilmiştir. Bu da ApoE ε4'ün alzheimerdaki patolojik etkisinin lipid metabolizması ile ilgili olduğunu düşündürmektedir [28]. Bunun aksine, ApoE ε2 taşıyıcılarının, ApoE ε3 ve ApoE ε4 taşıyıcılarına göre 'korunan' bir durumda olduğu ve bu nedenle ApoE ε4 proteini 'toksik' gibi görünürken, ApoE ε2'nin AH'ye karşı 'koruyucu' olduğu yönünde görüşler bulunmaktadır [29,30].

Down Sendromu

AH'nin temel iki patolojisinden biri olarak kabul edilen Aβ'nın kaynağı olan APP'nin geni 21. kromozom üzerinde lokalizedir. Bu durum 21 trizomisi (Down senromu) olan bireylerdeki erken başlangıçlı AH'nin yüksek insidansını açıklayıcı niteliktedir. APP geninin 3 kopyasına sahip olan Down sendromlu hastalar erken yaşta, bilişsel gerilemeye neden olan, AH-benzeri Aβ ve tau nöropatolojisi geliştirebilir [31-33].

Değiştirilebilir Risk Faktörleri

Yaşam Biçimi

AH'de patofizyolojik değişiklikler bilişsel bozukluklar gözlenmeden çok daha önce başlamaktadır. Bu nedenle erken teşhis ve birincil önlem hastalığın önüne geçilmesinde en çok dikkat edilmesi gereken hususlardan biridir [34]. Çeşitli araştırmalar, sedanter yaşam tarzının ve sağlıksız beslenmenin (yüksek yağlı diyet, yüksek karbonhidrat ağırlıklı beslenme) amiloidojenik yolağı

hızlandırabileceğini ortaya koymaktadır. Aksine sağlıklı yaşam tercihinin (Akdeniz diyeti, omega-3 yağ asidi takviyesi, kalori kısıtlaması gibi) AH semptomlarının azalmasına katkıda bulunduğu bildirilmiştir. Yapılan çalışmalar fiziksel aktivitenin beyin sağlığını geliştirdiğini ve beyin vaskülarizasyonunu, plastisiteyi, nörojenezi artırarak AH'yi azalttığını göstermiştir. Aynı zamanda yüksek eğitim seviyesinin ve entelektüel aktivitenin AH'nin progresyonunu ve hafıza kaybını azalttığı, beyin kapasitesini ve bilişsel fonksiyonları artırdığı ortaya koyulmuştur [35]. Fratiglioni L. ve arkadaşlarının 2004 yılında yaptığı sistematik bir incelemede, psikososyal faktörlerin ve hayat boyu aktif yaşam tarzının AH'yi de içine alan demansı azalttığı bulunmuştur [36].

Düşük Eğitim Seviyesi

Bilişsel birikim düzeyinin, eğitimsel veya mesleki becerinin artmasının klinik semptomların başlangıcını geciktirebileceği ve böylece fonksiyonel bağımlılığın süresinin kısalabileceği belirtilmektedir [37].

Kafa Travması

Travmatik beyin hasarı, AH için olası bir risk faktörü olarak kapsamlı bir şekilde araştırılmıştır. Vaka kontrol çalışmalarının meta-analizi, geçmişteki kafa travması öyküsü ile AH gelişme riski arasında bağlantı olduğunu destekler niteliktedir [38]. Buna karşılık, bazı boylamsal çalışmalar AH riskinin kafa travması ile ilişkili olmadığını veya yalnızca ciddi kafa travması ile ilişkili olduğunu iddia etmektedir [7]. Kafa travmasının AH üzerindeki etkisi hakkında yorum yapabilmek için daha fazla çalışmaya ihtiyaç vardır.

Kardiyovasküler ve Kronik Hastalıklar

Aterosklerotik karotid hastalığı, miyokard infarktüsü, atriyal fibrilasyon, hipertansiyon, hiperinsülinemi, hiperkolesterolemi gibi hastalıklar da AH ile ilişkili görülmekle birlikte hiçbirinin ilgili mekanizması açıkça ortaya konulamamaktadır. Damar sertliği olarak tabir edilen patolojinin, serbest radikal oluşumunun artmasının, artmış kolesterol seviyesinin artmış amiloid seviyesi ile ilişkili olduğunun düşünülmesinin, artmış pulsatil basıncın beynin mikrovasküler sistemine hasar verdiği bilgisinin daha pek çok çalışma yapılarak pekiştirilmesi ve kanıtlanması gerekmektedir [39].

Sigara

Sigara içmek ve AH'yi de kapsayan demans arasındaki bağlantı günümüzde de belirsizdir. Geçmiş dönemdeki çalışmalar nikotinin kısa süreli bilişsel performansı artırdığı [40] ve amiloid oluşumunu [41] engellediği yönündedir. Bu bulgular sigaranın demansa karşı koruyucu etkisi olduğunu ve nikotinin bilişsel olarak güçlendirici olabileceğini düşündürmüştür. Daha yakın zamanlarda bu bulgular sorgulanmış ve sigaranın kardiyovasküler hastalıklar üzerindeki bilinen negatif etkisinin vasküler demans üzerinde de risk faktörü olduğu üzerinde iddialar oluşmuştur [42]. Demansın artan prevalansı ile birlikte ilişkili hastalık, morbidite ve güçsüzlüğün yükünün de artmış olması nedeniyle [40], bilişsel gerileme ve demans için değiştirilebilir olan risk faktörlerinin, açıkça tanımlanmasına acil olarak ihtiyaç duyulmaktadır [43]. Çeşitli boylamsal çalışmalar sigara içmeyi demans ve AH için bir risk faktörü olarak tanımlamıştır [43]. Farklı iki grup tarafından yapılan çalışmalarda (Rotterdam Study ve Honolulu-Asia Aging Study) sigara içicilerdeki demans riskinin içmeyenlere göre daha yüksek olduğu bulunmuştur. Ayrıca, Honolulu-Asia Aging Study grubu tarafından yapılan çalışma sonucunda, bir yılda tüketilen sigara paketi sayısı ile beyindeki amiloid yük "doz-yanıt" şeklinde ilişkili bulunmuştur [44]. Sigara içmenin ateroskleroza katkıda bulunduğu ve serebral küçük damar hastalığı ile ilişkili olduğu pek çok çalışma ile ortaya konmuştur. Tüm bunlara ek olarak tütünün, doğrudan nöronal hasara neden olabilecek çok sayıda nörotoksin içerdiği yapılan çalışmalarda gösterilmiştir [39]. Sonuç olarak, sigara içmek ve demans arasındaki ilişkinin altında yatan mekanizmaların kesinleştirilebilmesi için daha fazla araştırma yapılması gerekmektedir.

Alkol

Alkol tüketiminin bilişsel fonksiyonlar üzerindeki etkisi yapılan epidemiyolojik çalışmaların sonuçlarında hala tartışmalıdır. Yapılan bir çalışmada iki hafta içinde 5 veya daha fazla alkol

tüketiminin, iki haftada 1-4 kez tüketimine kıyasla AH riskini %47 oranında artırdığı iddia edilmiştir [45]. Tüketilen alkollü içeceğin türüne göre farklılıklar görülmüştür. Randomize bir çalışmada, biradaki olgunlaşmış şerbetçiotu acı asit desteğinden sonra bilişsel durumun iyileştiği doğrulanmıştır [46]. Özellikle alkolün şarap formunda tüketiminin demans riskini güçlü bir şekilde azalttığı [47,48]; şaraptaki polifenolik ve antioksidan içeriklerin nörodejenerasyona karşı güçlü koruyucu etkileri olduğu görülmüştür [49]. Yüksek miktarda alkol tüketicilerinde, ekstraselüler soğukla-indüklenen RNA-bağlanma proteininin (e CIRP) alkolle indüklenmiş AH progresyonuna neden olan tau fosforilasyonuna aracılık edebileceği bildirilmiştir [50]. Orta yaştaki ağır alkol tüketicilerinin, özellikle ApoE ε4 aleli taşıyıcısı olanların, ileriki yaşlarında demans ve AH geliştirme riskinin diğer bireylere göre üç kat fazla olduğu bulunmuştur [51]. Alkol bağımlılığının alkol demansına neden olduğu iyi bilinmekle birlikte bu konuda daha fazla araştırmaya ihtiyaç duyulmaktadır çünkü tüketilen alkolün türünün ve miktarının AH'de değişken bir risk faktörü olduğu yapılan çalışmalar ile de doğrulanmıştır.

Depresyon

Birçok çalışma depresyon ve geç başlangıçlı demans ve AH arasında bir birliktelik olduğunu raporlamıştır fakat depresyonun demans ve AH için preklinik bir semptom veya risk faktörü olup olmadığı tartışmaya açıktır [52]. Yapılan çalışmalarda Aβ oligomerlerinin farklı tiplerde sinaptik defektlere, nörotransmitterlerin alımında/salımında değişikliklere, hücre iskeleti anormalliklerine, hücresel reseptörlerin lokalizasyonunda değişikliklere ve sinaptik plastisitenin bozulmasına (örneğin uzun süreli potansiyel artışının inhibisyonu (LTP) ve uzun süreli depresyonun artışı (LTD)) etki ettiği; bunun da hafıza bozukluklarına neden olabileceği düşünülmüştür. Fakat hiçbiri AH hastalarında henüz kanıtlanamamıştır [53-55].

İmmünolojik ve İnflamatuar Faktörler

Demansın başlı başına bir hastalık olarak düşünülmemesi gerektiği; beyni etkileyen bazı hasarlar ve bozukluklar nedeniyle oluşan bir dizi semptomu açıklamak için kullanılan bir terim olduğu iddia edilmektedir. Bu semptomların pek çok hastalık nedeniyle olabileceği, beynin etkilenen bölümüne bağlı olduğu belirtilmekte; çeşitli bilişsel, davranışsal, duygusal, motor ve psikiyatrik bozukluklar olarak ortaya çıktığı bilinmektedir. Demansın, beyinde protein birikiminden kaynaklanan nörodejeneratif hastalıklar olarak bilinen çeşitli rahatsızlıklar nedeniyle de oluşabileceği yapılan araştırmalarla gösterilmiştir (Alzheimer, Lewy cisimcikleri, Huntington, Parkinson gibi) [56]. Muhtemelen Aβ birikimi tarafından tetiklenen immün aktivasyon ve inflamasyon, AH'nin patogenezinde dikkate değer bir rol oynar [57]. Aβ birikiminin temel anlamda, Aβ üretimi ve temizlenmesi arasındaki dengesizliğin sonucu olduğu; bu dengesizliğin beyinde kronik iltihaplanma durumuna yol açtığı gösterilmiştir. Amiloid birikimi, santral sinir sisteminin (SSS) temel doğal bağışıklık hücreleri olan mikrogliaların aktivasyonuna neden olur ve sitokinler ve kemokinler gibi bir dizi proinflamatuar mediyatörün üretimini indükler [58]. Mevcut kanıtlar, beyindeki inflamasyonun AH'nin patolojik bir özelliği olduğunu vurgulamaktadır. İnflamatuar reaksiyona proinflamatuar sitokinlerin aracılık ettiği ve ilk olarak, aktifleştirilmiş mikroglia ve astrositler, stresli nöronlar ve Aβ plakları arasında kronik ve kendi kendine devam eden bir inflamatuar etkileşimin meydana geldiği varsayılmıştır [59]. Aktifleşmiş hücreler sadece pro-inflamatuar sitokinler değil ayrıca kemokinler, monosit kemo-çekici proteinler, makrofaj inflamatuar proteinleri, prostaglandinler, lökotrienler, tromboksanlar, pıhtılaşma faktörleri, reaktif oksijen türleri ve diğer radikaller, nitrik oksit, tamamlayıcı faktörler, proteazlar, proteaz inhibitörleri ve pentraksinler de üretir [60,61]. Bu bağlamda, Aβ plakları ve yumakları, glia hücrelerinde proinflamatuar sitokinlerin ekspresyonunu indükleyerek kronik bir inflamatuar reaksiyonu uyarır. Aktive edilmiş mikroglia ve astrositlerden sırayla salınan inflamatuar mediatörler $A\beta_{42}$ peptid üretimini indüklemek için APP üretimini ve APP'nin amiloidojenik işlenmesini artırır [62]. Bu nedenle artmış olan amiloid yük, immun yanıt kaskatı ile daha da artmış olur.

Enfeksiyonlar

SSS, moleküllerin beyne girip çıkmasını kontrol eden mikrovasküler endotel hücreleri, astrositler ve perisitlerden oluşan kan beyin bariyeri (KBB) sistemi tarafından yüksek oranda korunur. Bununla birlikte, virüsler, bakteriler, mantarlar ve protozoalar gibi geniş bir patojen yelpazesi KBB'yi aşıp

SSS'ye erişim sağlayabilir ve birçok ciddi hastalığa neden olabilir. Enfeksiyonlar ve AH etiyolojisi arasındaki ilişki uzun yıllardır tartışma konusu olmuştur. Son yıllarda, yapılan pek çok çalışma farklı mikrobiyal infeksiyonlar, bilişsel gerileme ve AH arasındaki ilişkiyi doğrular niteliktedir. Yapılan çalışmalarda AH ile ilgili olabileceği belirlenen sistemik bakteri ve virüs enfeksiyonlarına neden olabilecek patojenlerden bazılarının insan herpes virüsleri, spiroketler, Klamidya pneumonia veya Borrelia burgdorferi vb. olabileceği; bu bakteri/virüslerin inflamatuar durumu ve AH gelişimine yatkınlığı artırabileceği söylenmektedir [63]. Bunların yanında toplumlarda çok sık rastlanan *Helicobacter pylori*'nin AH patogenezinde önemli bir yer tuttuğu yönündeki kanıtlar gün geçtikçe artmaktadır. Birçok çalışma *Helicobacter pylori'*nin nörodejeneratif, respiratuar ve diğer hastalıklar ile ilişkili olabileceği yönünde sonuçlar ortaya koymaktadır. Alzheimer hastalarının serumunda ve beyin omurilik sıvısında (BOS) *Helicobacter pylori* spesifik IgG antikor seviyelerinin ölçüldüğü bir çalışmada, bu antikorların önemli ölçüde arttığı bulunmuştur [64]. Bunların yanında fungal enfeksiyonların, prionların ve hepatit B enfeksiyonunun da AH ile ilişkili olduğu yönünde veriler mevcuttur [65]. Bütün bu çalışmalar nihayetinde AH ile ilişkilendirilmiş spesifik bir patojen bulunmamaktadır fakat pek çok enfeksiyöz ajanın AH ile ilgili olabileceği yönünde bulgular mevcuttur.

Sistemik Hastalıklar (Medikal Faktörler)

Birçok risk faktörü AH gelişimi ile ilişkilendirilmiştir. AH'li birçok yaşlı birey kardiyovasküler hastalıklar, obezite, diyabet ve diğerleri gibi tıbbi durumlara sahiptir. Bu koşulların tümü, yapılmış çalışmalarda hem birbirleri ile hem de artmış AH riski ile ilişkili bulunmuştur [66]. Örneğin nöral doku kaybına bağlı olarak artmış demans riski ile ilişkili olan inmenin, dejeneratif etkiyi, amiloid ve tau patolojisini artırdığı gösterilmiştir. Hafıza ve bilişsel işlevlerde azalmaya yol açtığı bilinen atriyal fibrilasyon ayrıca embolilere neden olur. Kardiyovasküler hastalıklar değiştirilebilir risk faktörlerindendir ve AH ile aralarındaki ilişkiye odaklanılarak AH'yi önleme ve geciktirme yolu elde edilebilir [39,66].

Obezite

Günümüzde obezite, birden fazla nedenden dolayı küresel popülasyonda artmaktadır. Bunlar: Yaşam tarzı, stres, beslenme, genetik geçmiş ve egzersiz eksikliği vb. [67-69]. Obezite, bilişsel eksiklikler, bozulmuş uzun vadeli potansiyel ve sinaptik plastisite ve daha küçük bir beyin hacmi ile ilişkilendirilerek AH ve diğer demansların gelişme riskini artırmaktadır [70]. Ayrıca obezite, yağ dokusunda homeostatik sistemlerin düzensizliğine yol açan düşük dereceli bir kronik inflamasyon durumuna neden olur ve bu da nörodejenerasyonla ilgili olanlar da dahil olmak üzere çeşitli hastalıkların gelişmesine yol açar [67-69].

Diyabet

Diyabet kendi başına AH ile doğrudan ilişkili değildir çünkü plaklar ve yumakların diyabetik hastaların beyninde arttığı yönünde bir bilgi bulunmamaktadır. Bununla birlikte çokça çalışma diyabetin AH için bir risk faktörü olduğunu göstermektedir. Diyabetin serebrovasküler hastalıklar için bir risk faktörü olduğu ve serebrovasküler hastalıkların AH'de amiloid birikimine ve nöronal ölüme yol açan patolojik süreçleri artırdığı bilinmektedir [71]. Tip 2 diyabet ve AH arasında ortak bazı patogenetik faktörler bulunmaktadır: Kronik inflamasyon, oksidatif stres, mitokondriyal disfonksiyon, adiponektin eksikliği, plazma kolinesteraz aktivitesinin farklı ekspresyonu ve vasküler hasar bu iki hastalığın birlikte aynı hastada mevcut oluşunu açıklamak için olası bir yol gösterir [72]. Giderek artan bir şekilde bilişsel disfonksiyonun diyebetin önemli bir komorbiditesi olduğu kabul edilmektedir [73]. Tip 2 diyabet, hücresel hassasiyeti artırarak yaşa bağımlı geç başlangıçlı AH'nin oluşma riskini artırabilecek olan bilişsel bozukluk ve metabolik faktörlerle ilişkilidir. Ayrıca Tip 2 diyabet beynin nörovasküler ünitesinin, nörogliaların ve nöronların hassasiyetini, bozulmuş serebral kan akışına neden olan yaşlanma (yaşla ilgili kronik hastalıklar) ve metabolik hastalıklar (hiperinsülinemi, hiperglisemi, oksidatif stres vb) sonucunda artırır [74]. Her iki hastalığın da oldukça heterojen olduğu gerçeği göz önünde bulundurulduğunda, ikisi arasındaki bağlantının, çözülmesi zor ve farklı moleküler, selüler ve sistemik faktörlerin birleşimi olduğu muhtemeldir. Spesifik mekanizmalar veya tedaviler önerilirken bu içiçe geçmiş durumun dikkate alınması gerekmektedir [75].

Metaller

Bazı esansiyel (demir, bakır, çinko, manganez, selenyum) [76] ve esansiyel olmayan (kurşun, aluminyum, kadmiyum) metallerin çok çeşitli biyolojik mekanizmalarda görev almalarından dolayı nörodejeneratif hastalıklarda ve AH'de oldukça etkili oldukları yapılan araştırmalarda ortaya konmuştur [77] ve bu konudaki çalışmalar devam etmektedir.

AH ve Biyobelirteçlerin Tanımı

2011 yılında, National Institute on Aging and Alzheimer's Association (NIA-AA) AH'nin preklinik, hafif bilişsel bozukluk ve demans evreleri ile ilgili ayrı ayrı diyagnostik tavsiyeler oluşturmuştur. 2011 kılavuzunun yayınlanmasından bu yana AH'deki bilişsel gerilemenin uzun bir süre boyunca sürekli olarak meydana geldiğini [78] ve biyobelirteç ölçülebilirliğinin progresyonunun semptomlardan önce başlayan ve devam eden bir süreç olduğunu [79, 80] gösteren veriler birikmeye devam etmiştir. Bu nedenle hastalık artık klinik olarak tanımlanmış birbirinden farklı üç aşama olarak değil de tek ve uzun bir "süreç" olarak kabul edilmektedir. Bu kavram kabul edilmiş ancak 2011 NIA-AA kılavuzunda resmileştirilmemiştir. 2011 tavsiyelerindeki ortak temalardan biri de görüntüleme ve BOS biyobelirteçlerinin kullanılmasıdır. Semptomatik bireylerde, AH'ye ait patolojik değişikliklerin kişinin bilişsel bozukluklarına katkıda bulunduğuna dair kanıt sunabilmek için biyobelirteçler kullanılır. Preklinik AH durumunda ise biyobelirteçler, hastalığın yapısını tanımlamak için kullanılmaktadır. 2011 önerilerinde amiloid, preklinik biyobelirteç hiyerarşisinin en tepesinde yer almıştır. Bunun aksine bütün AH biyobelirteçleri, nörodejenerasyonu yansıtanlar da dahil olmak üzere, hafif bilişsel bozukluk ve demans kılavuzlarında eşit zemine oturtulmuştur. Çeşitli görüntüleme ve BOS biyobelirteçleri, beyin yaşlanması ve AH araştırmalarında yaygın olarak kullanılmaktadır ve genelleştirilebilir bir araştırma çerçevesi için organize bir yaklaşım gereklidir. Komite, yakın tarihli bir durum makalesindeki tavsiyeleri izleyerek AH ve beyin yaşlanma araştırmalarında kullanılan biyobelirteçler için tarafsız, tanımlayıcı bir sınıflandırma şeması ile bu sorunu ele almıştır. Şema (AT(N) olarak nitelendirilmiş olan), her birinin ölçtüğü patolojik sürecin doğasına bağlı olarak üç genel biyobelirteçler grubu tanır [78]. Jack Jr., C.R ve arkadaşları tarafından AH'deki patolojik değişikliklerin klinik ölçümleri terminolojide "ATN sınıflandırma sistemi" olarak harmanlanmıştır. Her üç belirteç de ayrı ayrı "negatif" veya "pozitif" olacak şekilde bir değer taşır. "A" Aβ biyobelirteci anlamına gelir, yüksek ligand retansiyonlu pozitif amiloid Pozitron emisyon tomografisi (PET) taraması veya BOS'ta düşük $A\beta_{42}$ seviyesi gibi; "T" tau biyobelirteci anlamına gelir, yüksek ligand retansiyonlu pozitif tau PET taraması veya BOS'ta yüksek forsforile tau (p-tau) gibi; "N" nonspesifik nörodejenerasyon veya nöronal hasar biyobelirteci olarak tanımlanır, [18 F]-fluorodeoksiglukoz – PET hipometabolizması, manyetik rezonans görüntülemede (MRI) AH'ye özgü bölgelerde yapısal atrofi veya artmış BOS total tau (t-tau) [78,81,82].

Tablo 1. A/T/N Sınıflandırması [78]

A	T	N	Biyobelirteç kategorilerinin temsil ettiği durum	
-	-	-	Normal/Sağlıklı	
+	-	-	Alzheimer patolojik değişikliği	AH süreci
+	+	-	AH	
+	+	+		
+	-	+	Alzheimer ve eşlik eden şüpheli alzheimer dışı patolojik değişiklik	
-	+	-	AH dışı patolojik değişiklik	
-	-	+		
-	+	+		

*A: Agrege Aβ veya ilişkili patolojik durum, BOS $A\beta_{42}$ veya $A\beta_{42}/A\beta_{40}$ oranı, Amiloid PET, T: Agrege tau (nörofibriler yumaklar) veya ilişkili patolojik durum, BOS'ta fosforile tau, Tau PET, N: Nörodejenerasyon veya nöronal hasar, Anatomik MRI, FDG PET, BOS'ta total tau, AH: Alzheimer hastalığı

2011 NIA-AA önerilerindeki sınırlama, biyogöstergelerin sadece iki kategoride gruplandırılmış olmasıdır; amiloid ve tau ilişkili nörodejenerasyon. Taupati ve nörodejenerasyon aynı biyobelirteç kategorisine yerleştirilmiştir. Sadece AH'li bireylerde, nörodejenerasyonun patolojik tau ile yakından ilişkili olduğunu varsaymak makul görülmektedir [83].

Hastalığın Mekanizması

Alzheimer patolojisinin başlıca özellikleri ekstraselüler amiloid plaklar ve intraselüler nörofibriler yumaklardır. İlaveten nörofil iplikler, distrofik nöritler, ilişkili astrogliozisler ve mikroglial aktivasyon görülür; serebral amiloid anjiyopati sıklıkla eşlik eder [84]. Bu patolojik proseslerin sonuçları, makroskopik atrofiye yol açan sinaptik ve nöronal kayıplı nörodejenerasyonu içerir [85]. AH'nin patofizyolojisi hala bazı tartışmalara konu olsa bile kabul görmüş başlıca iki major teori bulunmaktadır. Bunlar amiloid kaskat hipotezi ve tau hipotezidir. Bu iki hipotez dışında AH oluşumunda etkili olduğu varsayılan mekanizmalara sinaptik disfonksiyon ve nörotransmitter dengesizliği, nöroinflamasyon, enfeksiyon hastalıkları, bağırsak mikrobiyomunun bozulması, genetik mutasyonlar ve oksidatif stres örnek verilebilir [86].

Kolinerjik Hipotez

AH için ilk atılımın 1970'li yıllarda, Alzheimer hastalarının beyinlerinde kolin asetiltransferaz enziminin eksikliğinin aracılık ettiği kolinerjik açığın kanıtlanması olduğu bilinmektedir. Bu durum, asetilkolinin hafıza ve öğrenme üzerindeki rolünün anlaşılması ile birlikte, AH'nin kolinerjik hipotezine zemin oluşturmuş ve kolinerjik aktivitenin artırılmasına yönelik terapötik girişimleri teşvik etmiştir. Kolinesteraz inhibitörleri, sinaptik boşlukta asetil kolini parçalayan kolinesteraz enzimini inhibe ederek kolinerjik iletimi güçlendirirler. Kolinerjik tükenme nörodejeneratif kaskatın geç bir öğesidir [87].

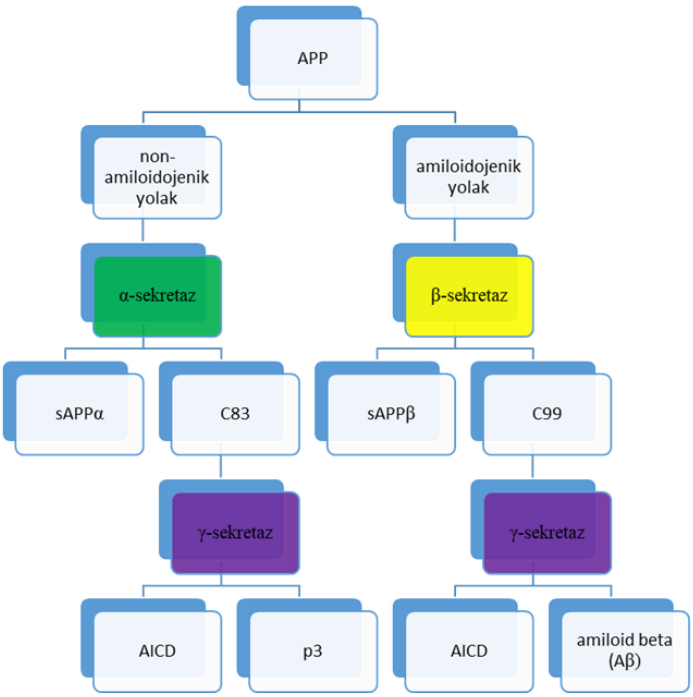
Eksitotoksisite

Eksitotoksisite bir nörotransmitter olan glutamata aşırı maruz kalma veya onun reseptörü olan N-metil-D-aspartat (NMDA)'ın aşırı stimülasyonu olarak tanımlanmaktadır. AH'deki progresif nöron kaybında önemli bir rol oynar [88]. Kolinerjik nöron kaybının bu süreçten etkilendiği ve bunun da hücre içine aşırı miktarda kalsiyum girişi ile sonuçlandığı düşünülmektedir [87].

Amiloid Kaskat Hipotezi

APP'nin amiloidojenik prosesi sonunda 37-43 aminoasitli heterojen Aβ karışımı oluşur [89,90]. Monomerik $A\beta_{1-42}$'nin, "fizyolojik nöroprotektör" özellikte bir nöropeptid olduğu; pikomolar konsantrasyonlarda sinaptik işlevi, sinir devrelerini, organel trafiğini, nörogenezi, nöroinflamasyonu ve bilişsel süreçleri düzenlediği bilinmektedir [91]. $A\beta_{1-42}$ düşük konsantrasyonda mikrobiyal enfeksiyonları baskılar ve kan-beyin bariyerindeki sızıntıları kapatabilir (damar tıkacı) [90,92]. Fizyolojik konsantrasyonlarda Aβ'nın, anjiyogenezi ve vaskülarizasyonu sürdürdüğü, KBB'yi koruduğu, beyin hasarından sonra iyileşmeyi desteklediği ve bir tümör baskılayıcı olarak görev yaptığı çeşitli çalışmalarla ortaya konulmuştur [92]. APP'nin yıkılımındaki sürecin patolojik yolağa kayması ise Aβ plaklarının oluşumunun ilk aşamasını oluşturur. Aβ, APP'nin farklı bölünme bölgelerinden, farklı proteazlarla bölünmesiyle oluşan ve biyolojik fonksiyonları birbirinden farklı olan peptitlerden biridir [93]. AH patofizyolojisinde genel hipotez, beta-amiloidin akümülasyonunu prosesin merkezine oturtur ve bu "amiloid kaskat hipotezi" olarak anılmaktadır [16]. İnsan amiloid prekürsör proteini ilk olarak 1987 yılında çeşitli laboratuvarlar tarafından tanımlanmıştır [93]. APP, çeşitli hücre tipleri tarafından üretilen tip 1 transmembran proteindir; kromozomu 21q21'de lokalizedir ve üç alandan oluşan tip 1 transmembran proteini kodlar: N-terminal uzun ekstraselüler bölge, kısa endotelyal segment ve sitoplazmada bulunan kısa C-terminal segment [94]. APP geninin 19 ekzon içerdiği ve ekzonların (1-13, 13a ve 14-18) alternatif şekillerde birleşmesi ile oluşan birçok izoformu olduğu bilinmektedir. Baskın olan transkriptler APP695, APP751 ve APP770'dir. APP posttranslasyonel proteolitik prosesle, α -, β -, ve γ - sekretaz aracılığı ile, yıkıma uğrar. β -, ve γ -sekretaz amiloidojenik özellikte APP komponenti üretirken α-sekretaz çözünebilir amiloid protein üretir [93]. SSS'de ise APP iki ayrı yolakla art arda bölünmeye uğrar. Bu iki yolakta da ikinci aşama γ-sekretaz aracılığı ile yıkımı içerir. Bu yolaklardan ilki olan non-amiloidojenik yolakta APP önce α-sekretaz ile yıkıma uğrar. sAPPα olarak

isimlendirilen "salgılanmış" ekstraselüler ürün ve C83 isimli, 83 amino asitli membran bağımlı C-terminal fragman oluşur. C83'ün de γ-sekretaz ile yıkımı sonucu P3 (nonamiloidojenik) adı verilen başka bir "salgılanmış" fragman üretilmiş olur ve membran bağımlı APP intraselüler domain (AICD)'den ayrılır. Amiloidojenik yolakta ise APP önce β-sekretaz (veya BACE-1; β-site APP cleavage enzyme 1) aracılığı ile ekstraselüler ürün olan sAPPβ ve membran bağımlı 99 aminoasitli C-terminal fragmanı olan C99'u üretir. C99'un γ-sekretaz ile yıkımından sonra ise ekstraselüler Aβ ve yine aynı membran bağımlı AICD oluşur [16].



Şekil 2. APP'nin yıkılımı

Sekretaz enzimleri APP'yi böler ve bu prosesin normal seyrinde ilerlemeyişi, özellikle gama ve beta sekretazlardaki mutasyonlar, Aβ'nın anormal üretimine neden olur [95]. Çözünebilir olan P3 peptidi kümelenmeye meyilli değildir. Beta-amiloid proteini ise bunun tam tersi kümelenmeye meyillidir [96-98]. AH'ye neden olan en yaygın genetik mutasyonlar AH'de anahtar patojenik faktör olduğu bilinen Aβ prosesi ile ilgilidir [15]: AH'de görülen senil plakların ana bileşeni, APP'nin uygun olmayan proteolitik yıkımı sonucu artan beta-amiloid proteinidir. Aβ iki farklı formda bulunur: $A\beta_{40}$, patolojik akümülasyona neden olmayan protein ve $A\beta_{42}$ amiloidojenik protein, amiloid plaklardaki ana bileşendir [99]. Beta-amiloidin 40 veya 42 amino asitli olması γ-sekretazın protein zincirini ayıracağı bölgenin varyasyonuna göre değişir [100,101]. $A\beta_{42}$ nörotoksik olan formdur çünkü hidrofobik özellikler gösterir ve AH'nin karakteristik bulgusu olan senil plakların oluşumundan sorumludur. Bu senil plaklar da nörodejenerasyon, nöronal kayıp ve atrofi (özellikle beynin parietal ve temporal loblarında) ile karakterizedir [99]. $A\beta_{42}$ kümeleşerek AMPA (α-amino-3-hidroksi-5-metil-4-izoksazolpropiyonik asit) reseptörlerine (glutamat nörotransmitterinin bağlandığı) ve Ca^{2+} kanallarına yapışır, böylelikle Ca^{2+} influksu ve intraselüler Ca^{2+} seviyeleri artar [102]. Bu da hücre ölümüyle sonuçlanan nöronal hücrelerin apoptozisini indükler [103]. Ayrıca bu kümeleşmeler, nöronal hücre ölümüne neden olan lokal inflamatuar cevabı başlatır [104]. Agregasyon prosesi sürecinde Aβ, Fe^{2+} ve Cu^{2+} ile de yoğunluğu artan bir şekilde hidrojen peroksit üretir. Aβ ile birlikte olan Cu^{2+} iyonları elektrokimyasal olarak aktif ve reaktif oksijen türleri üretme kabiliyetindedir [105]. Bu reaktif oksijen türlerinin nöronal hücre membranlarındaki lipidlerin peroksidasyonuna dolayısıyla da glukoz transporterlarının ve iyon kanalları ATPaz'larının disfonksiyonuna neden olduğu bilinmektedir. Aβ'nın neden olduğu oksidatif stresin hücresel iyon homeostazı ve metabolizması üzerinde oluşturduğu bu

rahatsızlığın nöronları apoptozise duyarlı hale getirdiği yapılan çalışmalarda ortaya konmuştur [103,106]. Çok çeşitli Aβ monomer tipi vardır: Amiloid plak oluşturmak için birikebilen geniş ve çözünemeyen amiloid fibriller ve beynin dışına yayılabilen çözünebilen oligomerler gibi. Aβ nörotoksisite ve nöronal fonksiyonda majör rol oynar, bu nedenle hipokampus, amigdala ve serebral kortekste plakların daha yoğun akümülasyonu bilişsel bozulmanın yanı sıra astrositlerin ve mikroglialarının stimülasyonuna, aksonlarda ve dendritlerde hasara ve sinapslarda kayıplara neden olur [66,107]. Aβ proteininin ekstraselüler birikintilerinin konumlandığı alanlar, hafıza ve bilişsel fonksiyonun kontrolünde önemli bir yere sahip olan alanlardadır. APP genindeki birçok mutasyonun EOAD'ye öncülük ettiği bulunmuştur. Mutasyonların çoğu APP geninin Aβ kodlayan bölgesinde lokalize olmuş kümelerdir. Buna ek olarak 21 trizomileri ve Down Sendromu gibi 3 adet Kromozom 21'e sahip olan bireylerde AH nöropatolojisi gelişmiş fakat APP geni içermeyen kısmi 21 trizomili bireylerde AH nöropatolojisi gelişmemiştir [108]. Endoplazmik retikulumda lokalize olan PSEN1 ve PSEN2, γ-sekretaz protein kompleksinin kofaktörleridir; Aβ'nın nörotoksik formunun üretiminden ve ailevi Alzheimer hastalığına sahip bireylerde hastalığın başlangıcından, farklı mekanizma ve katkılarla sorumlulardır [109]. Presenilinler 8-9 transmembran bölgesi olan oldukça homolog proteinlerdir. Sinir hücrelerinde, homeostazı sağlayan membran reseptörleri ve kalsiyum kanalları gibi davranırlar ve yüksek düzeyde ekspresyonu çoğu kez hipokampus ve Purkinje hücrelerinde görülür [110]. PSEN1 geninde EOAD'nin takriben %80'ine neden olan 185 dominant mutasyon tanımlanmıştır [111]. PSEN2 geninde ise daha az sayıda mutasyon tanımlanmıştır ve yaklaşık %5 oranında EOAD nedenidir [108]. Amiloidin etkileri multifaktoriyeldir fakat amiloid hipotezine göre, artmış oksidatif stres, mitokondriyal disfonksiyon, sinaptik kesinti ve amiloid bağlantılı tau hiperfosforilasyonunun AH'ye neden olduğu düşünülmektedir. Ancak son zamanlarda birden fazla amiloid spesifik tedavinin başarısızlığı, amiloidin ortadan kaldırılmasının AH sürekliliğinde daha erken evrede başlatılması gerektiğini düşündürmekte, patogenez amiloid birikiminden bağımsız olarak görülebilmekte, ve/veya amiloidin, AH'de nörodejenerasyona neden olan ve geri dönüşü olmayan olaylar zincirinin sonu için sinyal verebileceği belirtilmektedir [4]. Aβ plaklarının birikimi nöronların hücre iskeletinde yapısal protein olan taunun hiperfosforilasyonunu başlatır, sonrasında tau proteininin yanlış katlanmasına ve nörofibriler yumaklar (Neurofibrillary tangles, NFT) adı verilen agregatların oluşmasına neden olur. Bu da nöronlar arasında iletişim bozukluğu ve ardından da nöronun ölümü ile sonuçlanır [112,113]. Oluşan bu patolojik durum AH için farklı bir hipotez oluşturur.

Tau Hipotezi

AH'de ikinci en yaygın agregat beynin çeşitli bölgelerinde bulunan NFT'lerdir. Hiperfosforile tau proteininin çoğunlukla çift sarmal filamentlerinden oluşur [114,115]. Tau proteinini kodlayan gen (MAPT (microtubule-associated protein tau) geni) 17. kromozomda (17q21) lokalizedir ve 16 ekzondan oluşur [93,116]. Tau proteini nöronlarda eksprese edilen, normalde hücre iskeletinde mikrotübüllerin stabilizasyonunda görev yapan bir proteindir [117]. Hiperfosforilasyonun, taunun, sinir hücresi gövdelerinin içindeki bu NFT kitlelerinde birikmesine neden olduğu; bu yumakların daha sonra hücresel proteinlerle anormal bir şekilde etkileşime girerek normal işlevlerini yerine getirmelerini engellediği yapılan çalışmalarla gösterilmiştir [118]. Hiperfosforile tau proteini formunun hücre içinde kümeleşmesinin mikrotübül fonksiyonun, aksonal iletimin bozulmasına neden olduğu ya da nöron dejenerasyonu ile sonuçlanan sinir hücresi iskeletinin yıkılmasına yol açtığı belirtilmiştir [27,119]. Sırasıyla taunun fosforilasyonu ve defosforilasyonuna neden olan kinazlar ve fosfatazların faaliyetinin serbest tau proteini ve mikrotübül ilişkili protein arasındaki dengenin düzenlenmesini etkilemekte olduğu; protein fosforilasyon ve defosforilasyonu arasındaki dengesizliğin bu proteinin mikrotübüllere bağlanmasının bozulmasına ve eşleştirilmiş helikal filamentler (paired helical filaments (PHF)) ve NFT oluşumuna yol açtığı gösterilmiştir. Fosforile tau-proteininin yapısının Aβ, oksidatif stres, nöroinflamasyon ve kinazları ve fosfatazları etkileyen enzimlerden etkilenebileceği ihtimali düşünülmektedir [120,121]. Muhtemelen en önemli etkiye sahip olan üç enzimin glikojen sentaz kinaz 3 (GSK3), siklin bağımlı kinaz 5 (Cyclin-dependent kinases 5 (CDK5)) ve mikrotübül affinite-düzenleyici kinaz (MARK) olduğu iddia edilmektedir [122]. Birçok çalışma CDK5'in tau fosforilasyonu ve NFT progresyonu içinde olduğunu göstermiştir [123,124]. Ayrıca, ilginç bir şekilde CDK5'in, AH'nin başlangıcına dahil olan GSK3'ün regülatörü olarak davrandığı yapılan çalışmalarda

gösterilmiştir [125]. Bu hiperfosforile tau yumaklarının normal fonksiyonların yürütülmesine engel olacak şekilde ve düzensiz bir biçimde hücresel proteinlerle etkileştikleri bildirilmiştir. Hiperfosforilasyon, Aβ'nın aşağı akış yönündeki mekanizmaları ile meydana gelir, yani yapılan araştırmalarda Aβ'nın akümülasyonunun fosforilasyon prosesini başlattığı ileri sürülmüştür [118]. Ek olarak, toksik taunun Aβ üretimini geri bildirim döngü mekanizması aracılığı ile artırdığına yönelik kanıtlar mevcuttur [126]. Tau aynı zamanda nöronal disfonksiyon üretmek için amiloid ile sinerjik olarak hareket edebilir [4]. Tau'nun hiperfosforilasyonunun, amiloid proteininin toksik bir işlev kazanımına yol açtığı da tau hipotezinde ileri sürülen diğer bir görüştür [119]. Tau seviyelerinin azaldığı hayvan modellerinde, tau proteininin yok olmasının, Aβ ile indüklenmiş eksitotoksisite veya farelerdeki APP veya PRES1'in fazla ekspresyonundaki eksitotoksinler karşısında nöronlar üzerinde koruyucu etkisi olduğu bulunmuştur. Tau patolojisi AH'deki demansın yanı sıra frontotemporal demans, argirofilik tahıl hastalığı, kortikobazal dejenerasyon, progresif supranükleer palsi ve çeşitli diğer hastalıkların nedeni olarak görülmektedir. Beyinde anormal tau proteininin akümülasyonunun oluştuğu bu hastalıklar tauopatiler olarak adlandırılır [127]. İnsana ait fizyoloji ve patolojide taunun görevleri kısaca aksonal transportta görev almak, sinapslarda yer almak, nöronların çekirdeğinde fonksiyon göstermek olarak özetlenebilir. Taunun post-translasyonel modifikasyonlarının hiperfosforilasyon, asetilasyon, karboksi terminali kesilmesi ve *O*-GlcNAsilasyon (*O*-glikozilasyon türü) ve N-glikozilasyon olduğu bilinmektedir. *O*-GlcNAsilasyon dışındaki tüm değişimler taunun yanlış katlanmasına ve nöronal hasara neden olmasına yol açmaktadır. Bu nedenle bu modifikasyonlardan (*O*-GlcNAsilasyon hariç) biri ya da kombine olarak birkaçı, tau agregasyonunun engellenmesi ve proteinin normal fonksiyonunun yeniden sağlanması için tedavide hedef haline getirilebilir [128]. Ubikinasyon da bir modifikasyon türüdür. Tau ubikinasyonunun tau patolojisinde (taunun dendritlerdeki yanlış yerleşimi veya tau agregasyonunun teşfik edilmesi gibi) yer aldığı [129] fikrinin aksine tau ubikinasyonunun nörotoksik olduğunu gösteren doğrudan kanıt bulunmamaktadır. Ubikinasyonun birincil fonksiyonu degradasyon iken taunun ubikinasyonunun nontoksik olduğu belirtilmektedir [128].

Amiloid Beta ve Tau İlişkisi

Tau proteini pek çok enzim tarafından fosforile edilir. Bu enzimlere A-kinaz, C-kinaz, siklin bağımlı kinaz-5 (CDK-5), CaM kinaz II, glikojen sentaz kinaz-3β (GSK-3β) ve mitojen aktif protein kinaz (MAPKs) örnek verilebilir [130]. Patolojik şartlar altında bu kinazların tau proteininin hiperfosforilasyonunu artırdığı, bunun da taunun mikrotübüllerden disosiye olmasına ve NFT'lerin oluşmasına neden olduğu yapılan çalışmalarla gösterilmiştir [125,131]. Agrege Aβ, CDK-5 [132] ve GSK-3β'nın [133] aktivasyonuna aracılık ederek tau'nun hiperfosforilasyonunu hızlandırır. Bu da nörofibriler dejenerasyona neden olan NFT'lerin oluşumunu artırır [132]. Taunun fosforilasyonu üzerindeki itici etkisine ek olarak, Aβ, tau oligomerizasyonu ve agregasyonu ile de etkileşime girer. NFT oluşumundan önce oluşan taunun orta seviye formu olan tau oligomerlerinin toksik olduğu bilinmektedir. Oluşan bu oligomerlerin nöronal hasarı artırdığı ve bu hasarın nörodejenerasyona neden olduğu ortaya konmuştur [134,135]. Bunların yanı sıra Aβ, taunun kaspaz-3 aracılığı ile C-terminalinden bölünmesini tetikleyerek N-terminalli kaspaz bölünme ürünü oluşmasına neden olur. C-terminali kesilince 20 aminoasit kaybederek oluşan kısa tau, uzun tauya göre filamentler arasına çok daha hızlı bir şekilde girer [136]. Bu mekanizma ile Aβ tau oligomerlerini artırmış olur, oluşan bu oligomerler taunun en nörotoksik formudur; bu kanalla Aβ tau nörodejenerasyonuna katkı sağlamış olur.

Dolayısıyla, Aβ ile indüklenmiş nörotoksisitenin sadece Aβ'nın aşırı birikmesi ile ilgili değil ayrıca 17-kDa nörotoksik tau fragmanı gibi nörotoksik tau fragmanlarının üretilmesine yol açan proteaz aktivasyonu yoluyla da ilgili olduğu yapılan çalışmalar sonucu bildirilmektedir. Bu olay akışında Aβ tetikleyiciye, tau ise kurşuna benzetilmektedir [137,138]. Tüm bu sonuçlar, Aβ'nın toksisitesinin tauya bağlı olduğu fikrini daha da desteklemektedir. Özetle, tau proteini Aβ ile indüklenmiş mekanizmada anahtar rol oynayabilir; taunun tükendiği nöronlarda insan tau proteininin rekombinant ekspresyonu nöronların Aβ toksisitesine duyarlılığını geri kazandırır. Bunların yanı sıra Aβ ve taunun, mitokondriye hasar vermek için birlikte hareket ettiği [137,139], nöronal olmayan hücrelerde mikroglia [140,141] ve astrositler [142] üzerindeki ortak etkileri; Aβ plaklarının, nöritik plak tau agregasyonunu ve yayılmasını kolaylaştırdığı belirtilmektedir [136].

AH’nin Tedavisi

Onaylanmış Tedavi Yöntemleri

Dünya çapında 47 milyon insanı etkileyen demansın yıkıcı bir formu olan AH’nin, hastaların tam anlamıyla iyileşmesini sağlayan tedavisi henüz yoktur [143]. Mevcut tedavi yöntemleri ancak semptomları hafifletmeye yöneliktir. Onaylı moleküllerden hiçbiri ilerlemiş olan hastalığı geriye döndürebilecek etki mekanizmasına sahip değildir [87]. Günümüzde AH tedavisinde uzun süredir kullanılan onaylı iki ayrı farmakolojik grup bulunmaktadır. Bu gruplardan biri donepezil, rivastigmin ve galantamin içeren hafif, orta ve ciddi seviyedeki AH’de ve Parkinson hastalığı demansında kullanılan kolinesteraz inhibitörleri [144]; diğeri ise hem non-kompetitif N-metil-D-aspartat reseptör antagonisti hem de dopamin agonisti olan ve dikkat ve uyanıklık halinde zorluk çeken orta ve ciddi seviyedeki Alzheimer hastalarında kullanımı onaylı olan memantindir [145].

Kolinesteraz İnhibitörleri

AH tedavisinde ilk ilerleme, 1970'lerde AH'li hastaların beyinlerinde, kolin asetiltransferaz enzimindeki eksikliklerin aracılık ettiği kolinerjik bir eksikliğin gösterilmesiyle başlamıştır [146]. Kolinesteraz inhibitörleri ile nörotransmitter artışı tedavisi hafif-orta seviyedeki AH'li hastalar için klinik olarak kanıtlanmış bir yaklaşımdır. Kolinesteraz inhibitörleri, sinaptik boşluktaki asetilkolinesterazı inhibe ederek kolinerjik sinaptik iletimi artırırlar. Böylelikle presinaptik nöronlardan salınan asetilkolinin hidrolizi azalmış olur. Bu grup ilaçlar küçük ama ölçülebilir düzeyde anlamlı klinik fayda sağlar [147].

Takrin

Food and Drug Administration (FDA) tarafından onaylanan ilk jenerasyon kolinesteraz inhibitörüdür [148]. Muskarinik nöronlarda asetilkolini artırarak etki eder fakat hepatotoksik yan etkileri nedeniyle uzun yıllardır klinikte kullanılmamaktadır [147,149].

Donepezil

İkinci jenerasyon asetilkolinesteraz inhibitörüdür ve AH tedavisi için önde gelen ilaç olarak kabul edilir. Asetilkolinesteraz enzimine geri dönüşümlü olarak bağlanır ve asetilkolinin hidrolizini engeller. Bu da sinapslarda asetilkolin konsantrasyonunu yükseltir. Donepezil iyi tolere edilir, gastrointestinal sistem ve sinir sistemiyle ilgili hafif ve geçici kolinerjik yan etkiler görülebilir. AH’nin ilerlemesini engellemez, biliş ve davranışlar ile ilgili semptomlar üzerinde iyileştirici etkisi vardır [66,150].

Rivastigmin

Rivastigmin, asetilkolinesteraz ve butirilkolinesterazın psödo geri dönüşsüz inhibitörüdür. Asetilkolinesterazın aktif iki bölgesine (anyonik ve esterik bölgesine) bağlanarak etki gösterir. Bu da asetilkolin metabolizmasını engeller. Rivastigmin asetilkoline kıyasla asetilkolinesterazdan daha zor ayrılır, bu nedenle de psödo irreversibl olarak adlandırılır. AChE ve BuChE tarafından sinapsta metabolizmaya uğrar. İlaç olarak hafif ila orta seviyedeki AH vakalarında kullanılır. Bilişsel fonksiyonları ve günlük yaşam aktivitelerini geliştirdiği bilinmektedir. İlacın oral olarak uygulanması bulantı, kusma, dispepsi, asteni, anoreksiya ve kilo kaybı gibi pek çok yan etki ile ilişkilendirilmiştir. Çok sayıda vakada bu yan etkiler ilacı kullanmayı kesmenin temel nedeni olmuştur. Rivastigminin kontrollü ve kesintisiz bir şekilde hastaya verilebilmesi için transdermal flasterler ile uygulama çok elverişlidir. Bu sayede, hem hasta tarafından tolere edilebilirliği hem de hastaya bakım sağlayan kişilerin memnuniyeti artar. Aynı zamanda flasterler sayesinde oral formlara göre verilen doz azaltılabilmektedir, bu da yan etkilerin de azalması ile sonuçlanır. Çoğu AH’li bireyin hafıza kaybı ve yutma sorunları yaşaması düzenli aralıklarla oral ilaçlar kullanabilmelerine uyuncu azaltmaktadır. Bu nedenle de transdermal flasterlerin kullanılması AH’li bireylere ilaç uygulanmasının en uygun yöntemidir [66].

Galantamin

Galantamin hafif ila orta seviyedeki AH vakalarında standart birinci basamak tedavi olarak tercih edilir. Çift yönlü etki mekanizmasına sahiptir. Asetilkolinesterazın yarışmalı inhibitörüdür, aynı

zamanda nikotinik asetilkolin reseptörlerine bağlanarak aktivasyon sağlar. Galantamin iyi etkililik ve tolere edilebilmesi ile diğer asetilkolinesteraz inhibitörleri gibi davranışsal semptomları, günlük yaşam aktivitelerini ve bilişsel performansı geliştirir. Farklı uygulama yolları konusunda çalışmalar yapılmış olmasına rağmen ruhsatlı olan ilaçlar sadece oral yolla kullanıma uygundur [151,152].

Genel olarak takrin dışındaki kolinesteraz inhibitörleri iyi tolere edilir. Donepezil ve galantamin günde bir kere, rivastigmin ise günde iki kere verilmek üzere reçete edilir. Rivastigmin ve galantaminin doz titrasyon aralığı, donepezilden daha geniştir [153]. Sadece rivastigminin transdermal flaster formu vardır, günde bir kere uygulanır [154]. Amerika ve Avrupa kılavuzları asetilkolinesteraz inhibitörlerini hafif ila orta seviyedeki AD'de birinci basamak farmakoterapi olarak sınıflandırır. Bununla birlikte asetilkolinesteraz inhibitörleri hafif ila orta dereceli AH'deki bilişsel eksiklikler üzerinde yalnızca orta düzeyde etkinlik gösterirken, fonksiyonel kapasite üzerinde etkileri anlamlı değildir [155]. Bu üç ajanın etkililikleri benzerdir. Bu nedenle tedavide yapılacak seçim hastanın bireysel toleransına, hekimin deneyimine ve maliyete göre belirlenmektedir [156].

N-metil-D-aspartat Reseptör Antagonistleri

N-metil D-aspartat (NMDA) reseptörleri, hipokampus ve korteksteki (biliş, öğrenme ve hafızayla ilgili alanlar) piramidal hücrelerde bol miktarda bulunur. Öğrenme ve hafızada yer alan mekanizma, NMDA reseptörü aracılığıyla nörotransmiter glutamatın aracılık ettiği uzun vadeli güçlenmeyi gerektirir. Bununla birlikte artmış glutamat seviyeleri de normal fizyoloji koşullarında istenmeyen bir durumdur ve nöronların eksitotoksisitesi ile ilişkilidir [157,158]. NMDA reseptörlerine aşırı miktarda glutamat bağlanmasının, sinaptik fonksiyon kaybına ve nöronal kayba neden olan Ca^{2+} akışına yol açtığı bilinmektedir ve bunun da AH'deki nörodejenerasyonda yer aldığı düşünülmektedir [159].

Memantin

Memantin, AH vakalarında nörotoksisitede rol oynayan glutaminerjik sistemin aşırı aktivasyonunu önleyen, glutamat reseptörü alt tipi olan NMDA reseptörlerinin düşük afiniteli rekabetçi olmayan bir antagonistidir [160]. AH'de meydana gelen nöronal hasarı azalttığı; ayrıca Aβ'nın indüklediği Ca^{2+} dengesizliğini düzenlediği yapılan çalışmalarla gösterilmiştir [161]. Bu nedenle, memantin AH için semptomatik tedavi sağlarken bir yandan da nöroprotektif bir rol oynar [112]. Memantin orta ila ciddi seviyedeki AH'nin semptomatik tedavisinde kullanılır [162]. Güvenlidir ve iyi tolere edilir [160]. Glutamatın öğrenme ve hafıza üzerindeki fizyolojik etkilerine izin verirken, nöronun glutamat kaynaklı eksitotoksisitesini azalttığı varsayılmaktadır. Yapılan klinik çalışmalarda memantinin, plaseboyla karşılaştırıldığında, orta-ciddi seviyedeki AH 'de biliş, günlük yaşam aktiviteleri ve davranışlar üzerinde küçük ama klinik olarak anlamlı bir yararlı etkiye yol açtığı gösterilmiştir. Hastaların ruh halinde bozulma, ajitasyon, sinirlilik veya sanrılar yaşama olasılığının düştüğü bildirilmiştir [157,158]. Memantin hafif ila orta seviyedeki AH'de fonksiyonel iyileşme olmaksızın bilişsel semptomlar üzerinde çok sınırlı bir etki göstermektedir [163]; günlük yaşam aktiviteleri, davranış ve klinik izlenim açısından herhangi bir fayda sağlamaz [157,158].

Önerilen başlangıç dozu günde oral yolla alınan 5 mg'dır. Dozlama 5 mg'lık artışlarla günde en fazla 20 mg'a kadar çıkartılır [157]. Günde bir kez uygulanan uzatılmış salımlı formülasyonda ise günlük maksimum doz 28 mg'dır [16]. Aynı zamanda memantin ve donepezilin kombinasyonu, orta ila ciddi seviyedeki AH vakalarında beşinci tedavi seçeneği olarak "sabit doz kombinasyonu" statüsünde 2014 yılında onaylanmıştır [164,165]. Hem Klinik Uygulama Kılavuzları hem de Bilimsel Dernekler otoritesi, memantin ve kolinesteraz kombinasyonunun ciddi seviyede AH'li hastalarda önemli ölçüde etkili olduğunu bulmuşlardır. Bunun nedeni, iki ilacın semptomatik tedavide birbirinin eksikliklerini telafi edebildiğinin ve beş ilaçla monoterapiden daha etkili olduğunun gösterilmiş olmasıdır [166]. 28 mg memantin hidroklorür (uzatılmış salım) + 10 mg donepezil hidroklorür içeren (oral kullanım, kapsül) kombinasyon ilk kez 2014 yılında FDA'den onay almıştır [167]. Bu ilaç tedavileri (asetilkolinesteraz inhibitörleri ve NMDA reseptör antagonisti), hastalığın ilerlemesini geciktirmek, bilişsel işlevleri geçici de olsa stabilize etmek veya iyileştirmek, davranış bozukluklarını kontrol altına almak amacıyla kullanılır; hastalığı tümüyle iyileştirmez fakat yine de Alzheimer hastaları ve bakıcıları için bireysel bağımsızlığı artırır, yaşam kalitelerinin iyileşmesine yardımcı olur. Bununla birlikte, etkinliği en iyi ihtimalle sadece kısmi ve geçici olan bu tedaviler, AH 'nin nedeninden ziyade sadece sonuçlarını etkiler.

Bu ilaç tedavilerinin, nörodejenerasyon süreci gerçekleşmeden önce erken asemptomatik aşamada daha faydalı olabileceği açıktır [168].

Günümüzde onaylı olan bu dört etkin madde ve beş farklı tedavi yöntemi sadece semptomatik fayda sağladığından, hastalığı iyileştirecek ve/veya hastalığın oluşmasını engelleyecek moleküllere ihtiyaç devam etmektedir.

Gelecek Vaat Eden Yeni Tedavi Yaklaşımları

Hastalık Modifiye Edici Tedaviler

Hastalık modifiye edici tedaviler (Disease-Modifying Treatment (DMT)) pek çok patofizyolojik mekanizma üzerinde çalışarak AH'nin progresyonunu değiştirmeyi hedefler. Bu durum, AH tedavisinde hastalığı etkilemeden ve değiştirmeden bilişsel fonksiyonları geliştiren, depresyon ve delüzyon gibi semptomları azaltan semptomatik tedavilerin aksine bir yaklaşımdır. Hastalık modifiye edici tedaviler (İmmünoterapiler ya da küçük moleküller) AH'yi engellemek veya ilerlemesini azaltmak için tasarlanmaktadır. Pek çok DMT geliştirilmiştir. Bunların önemli bir kısmı klinik çalışma aşamasına bile geçemeden *in vitro/in vivo* çalışmalar esnasında, önemli bir kısmı ise klinik çalışmaların farklı fazlarında etkililik/güvenlilik sorunları ve göstermiş oldukları yan etkiler nedeniyle elenmiştir. DMT'lerin başarısızlığına neden olan faktörlerin bazıları, tedaviye çok geç başlanması, yanlış ana hedefe yönelik tedavi uygulanması, uygun olmayan ilaç dozlarının kullanılması ve AH patofizyolojisinin yanlış anlaşılmasıdır [66]. Tasarlanan tedavilerin etkililiğinin başarı oranının düşük olmasının diğer nedenlerinden biri de beyin hedefli ilaçların kan beyin bariyerini aşma ve santral sinir sistemine geçmesindeki zorluktur [169]. AH'de pek çok ilaç geliştirme denemesi, kan beyin bariyerindeki permeabilite sorunları nedeniyle başarısız olmuştur. Kan beyin bariyeri engeli nedeniyle daha yüksek dozlarda etkin madde gerekmiş fakat bu sefer de istenmeyen etkilerin oluşma ihtimali artmıştır [170, 171]. Kan beyin bariyeri santral sinir sistemine ilaç ulaştırmada çok büyük bir engeldir; bu engeli aşmak için araştırma-geliştirme çalışmalarında pek çok strateji geliştirilmektedir [172]. İlaç etkililiğinin, yaşa bağlı olarak nöronal membranlarda ve membran reseptörlerinde gelişen modifikasyonlar nedeniyle azalıyor olabileceği de göz önünde bulundurulmalı, pre-klinik çalışmalarda bu ihtimal mutlaka değerlendirilmelidir [173]. Tedavilerdeki başarısızlığın bir diğer nedeninin ise tedavilerin AH'nin geç dönemlerinde uygulanması olduğu söylenebilir [174]. Uygulanmaya çalışılan tedavilerde AH seviyesi ne kadar ilerideyse, başarı oranının da o ölçüde düştüğü belirtilmektedir. Tüm bu bulgular erken teşhisin önemini ve AH'nin erken teşhisi için daha çok biyobelirtecin keşfedilmesi ihtiyacını ortaya koymaktadır [175].

Farmakolojik girişimlerde potansiyel ajanların keşfi zordur. AH multifaktöriyel bir hastalıktır ve pek çok patojenik mekanizma içerir: Yanlış katlanmış protein agregasyonu, nöroinflamatuvar süreç, nörodejenerasyon ve insülin düzensizliği gibi. Günümüzde AH patolojisinde yer aldığı bilinen temel unsurlar gözetildiğinde özgün ilaç geliştirme stratejilerindeki yaklaşımlar şu şekilde özetlenebilir: Anti-amiloid tedavisi (sekretaz inhibitörleri, Aβ agregasyon inhibitörleri, Aβ immünoterapisi) anti-tau tedavisi (fosfataz modifiye ediciler, kinaz inhibitörleri, tau agregasyon ingibitörleri, mikrotübül stabilize ediciler, tau immünoterapisi), anti-nöroinflamatuar tedavisi (mikroglia modülatörleri, astrosit modülatörleri, insülin direnci yönetimi, mikrobiyom tedavisi), nöroprotektif ajanlar (antiepileptik ilaçlar, NMDAR modifikasyonu, omega-3 çoklu doymamış yağ asidi takviyeleri) ve beyin stimülasyonu (derin-beyin stimülasyonu, vagus siniri stimülasyonu, transkraniyal manyetik stimülasyon) [176].

Geliştirilen pek çok molekül ve yapılan pek çok *in vivo/in vitro*/klinik çalışmalar sonunda Aβ-hedefleyici bir ilaç olan "aducanumab" 2021 yılında FDA tarafından AH'nin tedavisinde kullanılmak üzere 2003 yılından sonra onaylanan ilk ilaç olmuştur. "Aducanumab" bir insan immünglobulin G1 monoklonal antikorudur (IgG1 mAB). Seçici olarak agrege Aβ fibrillerine bağlandığı; doza ve zamana bağlı bir şekilde Alzheimer hastalarının beyinlerindeki Aβ plaklarını azalttığı yapılan klinik çalışmalarla gösterilmiştir [177]. Ancak ruhsat sahibi firma, 20 Nisan 2022 tarihinde aducanumabın pazarlama izni başvurusunu European Medicines Agency (EMA)'den geri çektiğini açıklamıştır [178-180]. Başvurunun geri çekilmesinin nedeni ise EMA'nın bilimsel komitesinin (The Committee for Medicinal Products for Human Use (CHMP)), şu ana kadar sağlanan verilerin aducanumabın pazarlama iznine ilişkin olumlu bir görüşü desteklemek için yeterli olmayacağını belirtmesidir [181]. Aducanumabın

FDA tarafından onayı devam etmektedir [182]. Aducanumabın onaylanmasından kısa bir süre sonra "lecanemab" da FDA tarafından onaylanmıştır [183]. Lecanemab, nöronlar için monomerlerden veya çözünmeyen fibrillerden daha toksik olduğu gösterilen çözünür Aβ protofibrillerine yüksek afinite ile bağlanan insanlaştırılmış bir monoklonal antikordur. AH'nin erken safhalarında olan bireylerde lecanemabın, beyin amiloid düzeylerini azalttığı; 18 aylık sürede, biliş ve fonksiyonlara ilişkin klinik ölçümlerde plaseboya kıyasla orta derecede daha az düşüşle ilişkilendirildiği, ancak advers etkilerin de gözlemlendiği yapılan klinik çalışmalar sonucunda bildirilmiştir [184]. 01.06.2023'te FDA tarafından AH tedavisi endikasyonu ile onay almıştır [185,186]. Yapılan bir çalışmada 2022 yılında FDA tarafından tüm terapötik kategoriler arasından 37 yeni tedavinin (22 yeni kimyasal ve 15 biyolojik ürün olmak üzere) onaylandığı; bunlardan 9 tanesinin SSS'de etkili olduğu bildirilmiştir. 2022 yılında AH tedavisi için FDA tarafından kabul edilen herhangi bir etkin madde olmamıştır. 2021'de aducanumab, 2023'te lecanemab AH tedavisi endikasyonu ile onay almıştır. Her iki etkin madde de immünoterapötik monoklonal antikor kategorisinde yer alır. 2003'ten bu yana AH tedavisi için sadece iki ilacın onaylandığı göz önünde bulundurulduğunda AH'nin hala en zorlu hastalıklardan biri olduğu söylenebilir [187].

SONUÇ VE TARTIŞMA

Geçmişten günümüze yapılan çalışmaların sonucunda AH patolojisi halen tam olarak anlaşılamamıştır. Hastalığın seyri nedeniyle semptomların geç fark edilmesi; erken teşhis için sağlıklı olan bireylerde yapılması gereken tanı yöntemlerine ait dünyada kabul görmüş bir protokol olmayışı, nörodejenerasyona neden olan bu hastalığın uzun yıllardır ancak semptomatik olarak iyileştirilmesine olanak sağlamaktadır. Hastalığın tamamen iyileşesini sağlayan bir yöntem bulunamamıştır. AH tedavisinde kullanılmak üzere tasarlanan pek çok etkin madde olmuş fakat 2003 yılından bugüne kadar ancak iki tanesi sağlık otoriteleri tarafından kabul görmüştür. Kabul edilen bu iki yeni etkin maddenin de ancak hastalığın erken evrelerinde kullanıldığı durumda fayda sağlayacağı yapılan çalışmalarla gösterilmiştir. Hedefleme yaklaşımları ile geliştirilen ilaçların sayısının gün geçtikçe artması umut edilmektedir.

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Yayım Koşulları

1. Ankara Üniversitesi Eczacılık Fakültesi Dergisi (Ankara Ecz. Fak. Derg. – J. Fac. Pharm. Ankara), açık erişimli, hakemli bir dergi olup yılda üç kez (Ocak-Mayıs-Eylül) yayımlanır.

2. Dergiye Eczacılığın her alanında daha önce hiç bir yerde yayınlanmamış, Türkçe veya İngilizce olarak hazırlanmış makaleler kabul edilir. Deneylerde, insan için "the Declaration of Helsinki" ve hayvan için "European Community Guidlines"'a bağlı kalınmalıdır. Etik Kurul Onayının zorunlu olduğu çalışmalarda, etik kurul onayı alınan kurumun adı ve etik kurul onay numarası, gereç ve yöntem bölümünde ve Etik Kurul Onay bölümünde belirtilmeli ve ilgili belge makale gönderim sırasında yüklenmelidir.

3. Yayın Komisyonuna gelen makaleler en az 2 danışmana gönderilir. Ankara Üniversitesi Eczacılık Fakültesi Dergisi'nin makale değerlendirme süreci çift taraflı kör hakemlik ilkesiyle yürütülür.

4. Makaleler yayına kabul ediliş sırasına göre yayınlanır.

5. Danışmanlar tarafından önerilen düzeltmelerin yapılması için yazar/ yazarlara geri gönderilen makaleler, düzeltilip yayınlanmak üzere 3 ay içinde tekrar yayın kuruluna gönderilmezse, yeni başvuru olarak işlem görür. Makale yayımlanmadan önce yazarların yayımcıya makalenin "Copyright Transfer Form'unu doldurarak telif hakkını göndermesi gerekmektedir.

6. Yayımlarda intihal olup olmadığı kontrol edilmelidir. Ankara Üniversitesi Eczacılık Fakültesi Dergisi'ne yayımlanmak üzere gönderilen makaleler intihal tarama programları (iThenticate) ile taranmalı ve çevrim içi makale gönderim sırasında makalelerin intihal içermediğine dair rapor yüklenmelidir.

7. Ankara Üniversitesi Eczacılık Fakültesi Dergisi'nin makale yayın ücreti (APC) veya abonelik ücreti yoktur.

8. Ankara Üniversitesi Eczacılık Fakültesi Dergisi'ne aşağıdaki makale türleri kabul edilir:

    a) **Özgün makaleler**: Türkçe veya İngilizce hazırlanmış, şekiller ve tablolar dahil tamamı en çok 25 A4 kağıdı sayfası olan, orjinal araştırmaların bulgu ve sonuçlarını açıklayan makalelerdir. Araştırma makalelerinin yenilikçi ve bilime katkı sağlayan çalışmalar olması beklenir. Makaleler, yazım kurallarında belirtilen ana başlıkları taşımalı ve Windows uyumlu bir program kullanılarak hazırlanmalıdır.
    b) **Derleme makaleler:** Türkçe veya İngilizce hazırlanmış, şekil ve tablolar dahil tamamı en çok 30 A4 kağıdı sayfası olan, yeterli sayıda bilimsel makale taranarak, o güne kadarki gelişmeleri özetleyerek ortaya koyan ve sonuçlarını yorumlayarak değerlendiren makalelerdir. Makaleler, yazım kurallarında belirtilen ana başlıkları taşımalı ve Windows uyumlu bir program kullanılarak hazırlanmalıdır.
    c) **Kısa bildiriler:** Devam etmekte olan bir çalışmanın bulgularını zaman kaybetmeden duyurmak için Türkçe veya İngilizce yazılan en çok 5 A4 kağıdı sayfası olan makalelerdir. Makaleler, yazım kurallarında belirtilen ana başlıkları taşımalı ve Windows uyumlu bir program kullanılarak hazırlanmalıdır.

Yazım Kuralları

1. Metinler, A4 normunda (21 x 29,7 cm) yazılmış olmalıdır.
2. Metinler A4 normundaki sayfanın sağ ve sol tarafından 2,5 cm., üst ve alt kenarlarından 3 cm. boşluk bırakılarak 1 satır aralıkla yazılmalıdır. Yayımı kabul edilen makaleler doğrudan "Microsoft Word" dosyası halinde çevrim içi olarak sisteme yüklenecektir (online submission). Ana metin yazı karakteri "**Times New Roman**" ve **11 punto** olmalıdır.
3. Sayfa numaraları makalede **belirtilmemelidir**.
4. Paragraf başları **1 cm içeriden** başlamalıdır. Paragraflar arası ilave boşluk bırakılmamalıdır.
5. Başlık sayfasında yayın adı, yazar/yazarların adları, ORCID noları ve yazışma yapılacak yazarın açık adresi, telefon ve e-mail adresi belirtilmeli ve ortalı yazılmalıdır. İlk sayfada başlıktan önce yukarıdan 3 satır aralığı bırakılmalıdır. Başlık ile Öz/Abstract arası 1 satır aralıkla yazılmalıdır. Sorumlu yazarın soyadının üstüne (*) işareti konularak belirtilmelidir. Bu kişinin Adı Soyadı, açık adresi, telefon numarası ve e-mail adresi başlık sayfasının en altında belirtilmelidir.
6. **Yazar Adı** (ilk harfi büyük diğerleri **küçük harf**) ve **SOYADI** (tamamı **büyük harf**) **koyu** olarak başlığın altına bir satır aralık verildikten sonra altına unvan belirtmeden yazılmalıdır. Birden çok yazar varsa virgülle ayrılıp bir boşluk bırakılarak yazılmalıdır. Yazarların soyadları üzerine konulacak rakamlarla hemen isimlerin altındaki satıra kurum adları ve posta adresleri (Örneğin: Ankara Üniversitesi Eczacılık Fakültesi, Farmasötik Kimya Anabilim Dalı, 06560, Ankara, Türkiye) açıkça yazılmalıdır.
    - **Tüm yazarlar için ORCID numarası** mutlaka beyan edilmelidir. Yazarların ORCID ID'leri ilgili logoya köprü oluşturularak URL linklerinin eklenmesiyle gerçekleştirilmelidir.
7. Uluslararası kısaltmalar kullanılabilir. Metin içinde mililitre için ml; dakika için dak. olarak belirtilen şekliyle yazılmalıdır.
8. Birimler metrik sistemi kullanılarak ifade edilmelidir.
9. Bütün tablo ve şekiller metin içindeki yerlerine yazım alanından taşmadan yerleştirilmiş olmalıdır.
10. Tablolar üstlerine, şekiller (formül, grafik, şema, spektrum, kromatogram, fotoğraf vb.) de altlarına arabik rakamlarla (**Şekil 1**., **Tablo 2**.,) numaralandırılmalı ve metin içinde yer verilmelidir. "Tablo", "Şekil" sözcükleri ile bunlara ait numaralar koyu yazılmalı ve 11 punto olmalıdır. Şekil/Resim (**JPEG formatında**) makale içinde yerleşmiş ve **resimler 300 dpi veya daha yüksek çözünürlükte** olmalıdır. Üzerinde oynanmış (parlaklık, kontrast, gama ayarı vb.) şekillerde şekil altı metninde yapılan ayarlar belirtilmelidir. **Yazarlar, önceki makalelerinden alıntılanmış olsalar bile, diğer kaynaklardan herhangi bir görüntüyü çoğaltmak için ilgili yayıncılardan yazılı izin almalıdır**.
11. **Tablo** başlıkları Tabloların üstüne ve iki yana yaslı ve bunların genişliğini aşmayacak şekilde 11 punto ve bir satır aralıkta yazılmalıdır. Tabloya ait açıklama varsa tablonun altına 9 punto ile yazılmalıdır. Tablo içindeki metin 8-11 punto arasında yazılabilir. **Şekil** başlıkları ise şekillerin altına birer satır aralıkla ortalı ve 11 punto yazılmalıdır. Şekil başlığı ve şekil arasında 6 nk aralık olmalıdır. Tablo ve Şekiller metin içine yerleştirilirken metin ile aralarında 18 nk aralık olmalıdır.

    Örnek tablolar için bakınız.
    - Tüm satır ve sütun çizgileri yer almalı.
    - Tablo tasarımı tüm makalede tek tip ve düz olmalı, herhangi bir renklendirme/gölgelendirme kullanılmamalıdır.
    - Tablo içinde yer alan başlıklar **bold/koyu** renkte yazılmalıdır. Tablo başlığı ve tablo arasında 6 nk aralık olmalıdır.

Tablo 1. Türlere ait morfolojik özellikler

Bitki kısmı*	***C. nummularia***	***C. integerrimus***
Yaprak	Genişçe eliptik-orbikular, 0.9-2.5-(4) x 0.5-2.5-(3-5) cm	Orbikulardan ovata kadar farklı şekillerde, 1.2-(4-5) x 0.9-3 cm
Tohum	3.5-4 x 1-2 mm, koyu kahverengi	3-4 x 1.5-2 mm, açık kahverengi

*Açıklama: 9 punto, 1 aralık olmalı.

Tablo 2. Hastaların özellikleri

Demografik bilgiler	**A grubu***	**B grubu**	**C grubu**
Erkek cinsiyet	10 (%30)	20 (%60)	10 (% 30)
Sigara kullanımı	20 (%60)	10 (%30)	20 (%60)

*Açıklama: 9 punto yazılmalıdır.

Örnek şekil;

Şekil 1. *C. nummularia*'nın genel görünüşü (Yazı karakteri "Times New Roman" ve 11 punto, "1" aralık, ortalı)

12. Makalelerin bölümleri **BAŞLIK** (Türkçe ve İngilizce), **ÖZ**, **ABSTRACT**, **GİRİŞ, GEREÇ VE YÖNTEM, SONUÇ VE TARTIŞMA, TEŞEKKÜR** (varsa eklenmeli), **YAZAR KATKILARI**, **ÇIKAR ÇATIŞMASI**, **ETİK KURUL ONAYI** (varsa eklenmeli) ve **KAYNAKLAR** sırasına uygun olarak hazırlanmalıdır. Bu bölümleri ifade eden başlıklar (Makalenin ilk başlığı hariç) **12 punto ile koyu olarak büyük harflerle ve sayfanın solundan başlanarak** yazılmalıdır. **GİRİŞ**'ten önce ve sonra sırasıyla 18 nk ve 6 nk aralık bırakılmalıdır. Diğer ana başlıklardan önce ve sonra sırasıyla 12 nk ve 6 nk aralık olmalıdır. Bölüm başlıkları ile metin arasında belirtilenin dışında ayrıca aralık **bırakılmamalıdır.**

- **BAŞLIK:** Türkçe ve İngilizce olarak büyük harf ve **ilk başlık** (Türkçe makalelerde Türkçe başlık, İngilizce makalelerde İngilizce başlık ilk başlıktır) **14 punto, koyu** ve ikinci başlık 12 punto, *italik* olarak yazılmalıdır. Başlık metine uygun, kısa, çalışmayı tanıtıcı ve açık ifadeli olmalıdır.

- **ÖZ** ve **ABSTRACT:** Türkçe (**ÖZ**) ve İngilizce (**ABSTRACT**) olarak makalelerin başında **200**'er kelimeyi geçmeyecek şekilde 10 punto ile *italik* olarak yazılmalıdır. Yabancı dilde yazılmış makalelerde önce **ABSTRACT** daha sonra mutlaka Türkçe olarak **ÖZ** bulunmalıdır. **ÖZ** ve **ABSTRACT** başlıkları 12 punto ve koyu yazılıp kendi içlerinde alt başlıklar (aşağıda görüldüğü gibi) halinde makalenin özeti sunulmalıdır. Her bir alt başlık 10 punto, koyu, normal yazılmalıdır. Alt başlıkların içeriğindeki metinler *italik* yazılmalıdır. **ÖZ ve ABSTRACT metni blok halinde sağdan ve soldan 1 cm boşluk bırakılarak yazılmalıdır.**

Özgün makalelerde;

ÖZ için kullanılacak alt başlıklar:
Amaç: *Metin italik yazılmalıdır.*
Gereç ve Yöntem: *Metin italik yazılmalıdır.*
Sonuç ve Tartışma: *Metin italik yazılmalıdır.*
Anahtar Kelimeler: *Metin italik yazılmalıdır, alfabetik sıralama gözetilmelidir*

ABSTRACT için kullanılacak alt başlıklar:
Objective: *Metin italik yazılmalıdır.*
Material and Method: *Metin italik yazılmalıdır.*
Result and Discussion: *Metin italik yazılmalıdır.*
Keywords: *Metin italik yazılmalıdır, alfabetik sıralama gözetilmelidir*

Derleme makalelerde;

ÖZ için kullanılacak alt başlıklar:
Amaç: *Metin italik yazılmalıdır.*
Sonuç ve Tartışma: *Metin italik yazılmalıdır.*
Anahtar Kelimeler: *Metin italik yazılmalıdır, alfabetik sıralama gözetilmelidir*

ABSTRACT için kullanılacak alt başlıklar:
Objective: *Metin italik yazılmalıdır.*
Result and Discussion: *Metin italik yazılmalıdır.*
Keywords: *Metin italik yazılmalıdır, alfabetik sıralama gözetilmelidir*

- **Anahtar Kelimeler (Keywords):** En az 3 sözcükten oluşmalı, ilgili dilde alfabetik, *italik* olarak, yalnızca ilk anahtar sözcüğün ilk harfi büyük olacak şekilde (büyük harf kullanılarak yapılan kısaltmalar hariç) aralara virgül konularak yazılmalı son anahtar sözcükten sonra ise bir imla işareti **kullanılmamalıdır.**

- **METİN:** Orijinal Türkçe makalede metin kısmı **GİRİŞ, GEREÇ VE YÖNTEM, SONUÇ VE TARTIŞMA** olmak üzere 3 ana başlıktan oluşmalıdır. Bu ana başlıkların tamamı 12 punto, **büyük harflerle** ve koyu olacak şekilde yazılmalıdır. Derleme makalelerde ise **GİRİŞ** ile **SONUÇ VE TARTIŞMA ana başlıkları olmalı,** diğer başlıklar yazarın belirleyeceği şekilde **her kelimenin ilk harfi büyük diğerleri küçük ve koyu** olacak şekilde yazılmalıdır. Alt başlıklar 11 punto, 1satır aralık, **bold/koyu** yazılmalı ve sola dayalı olmalıdır Alt başlıklarda numaralandırma sistemi **kullanılmamalıdır**. Alt başlıklardan önce ve sonra 6 nk aralık olmalıdır.

- **GİRİŞ:** Araştırmanın amacı ve konuyla ilgili çalışmaların yer aldığı bölüm olmalıdır.

- **GEREÇ VE YÖNTEM**: Kullanılan gereç belirtilerek, uygulanan yöntem hakkında gerekli bilgiler açıkça ifade edilmelidir. **Bileşiklerin karakterizasyonu** ayrı bir paragraf ile gösterilmeli ve yeni bileşiklerin saflıkları ve yapı aydınlatılmaları sağlanmalıdır. Eğer çalışmada hayvan ya da insan örnekleri/gönüllüler kullanılıyorsa, araştırıcılar tüm işlemlerin ilgili kanun ve kurumsal kılavuzlara uygun şekilde gerçekleştirildiğine ve uygun idari kurul tarafından bu işlemlerin onaylandığına ve Etik Kurul onayı alındığına dair ifadenin çalışma içinde yer almasını sağlamalıdırlar. Etik Kurul onayının zorunlu olduğu çalışmalarda, etik kurul onayı alınan kurumun adı ve etik kurul onay numarası, gereç ve yöntem kısmında belirtilmelidir. Ayrıca, kullanılan protokol ve prosedürlerin etik olarak gözden geçirildiği ve onaylandığı, makalenin gereç ve yöntem bölümüne eklenmelidir. Detaylı bilgi için lütfen http://journal.pharmacy.ankara.edu.tr/en/ethical-principles-and-publication-policy/ web sayfasını ziyaret ediniz.

- **SONUÇ VE TARTIŞMA:** Bulguların verilerek değerlendirildiği bölümdür.
  - Dileyen yazar, RESULT AND DISCUSSION bölümünün son paragrafı olarak "Conclusion" başlığı oluşturabilir. Ancak 11 punto Times New Roman karakterinde İlk harfi büyük diğer harfleri küçük olmalıdır.

- **TEŞEKKÜR:** Varsa araştırmayı destekleyen kuruluşa ve katkısı olan kişilere Yazarların Katkısından önce yer alan bu bölümde kısaca teşekkür edilebilir.

- **YAZAR KATKILARI:** Makalede yer alan yazarların katkısı yazarlar tarafından imzalanan Telif Hakkı Devir Sözleşmesi (*Copyright Transfer Agreement*) uyarınca, çıkar çatışması bildiriminden hemen önce, makalede yer alan isim sırası gözetilerek yazılmalıdır. Lütfen bu bildirim için açık ad ve soyad yerine aşağıdaki örnekte olduğu gibi yazarların baş harflerini kullanınız. Yazar katkısı belirtilmeyecek alanlar için "-" işareti konulmalıdır.

  Örnek:

  YAZAR KATKILARI

  Kavram: İ.Y., M.M.H., C.H., K.B.; Tasarım: İ.Y., C.H., I.Ö.G., Ö.Ü.; Denetim: C.H., I.Ö.G., M.M.H., K.B.; Kaynaklar: Ö.Ü., Z.K., K.B., M.M.H., A.K., İ.A., G.A.G., B.G., B.K.; Malzemeler: I.Ö.G., B.E., G.A.G., B.K., D.Ç.P.; Veri Toplama ve/veya İşleme: A.K., Ö.Ü., M.K., A.S., D.Ç.P., T.C.Ş.T.; Analiz ve/veya Yorumlama: Ö.Ü., B.G., T.C.Ş.T., E.K.S.; Literatür Taraması: B.K., D.Ç.P, B.G., B.E.; Makalenin Yazılması: A.K., İ.A., T.C.Ş.T.; Kritik İnceleme: İ.Y., B.G., Ö.Ü., İ.A.; Diğer: -

- **ÇIKAR ÇATIŞMASI BEYANI**

  Çıkar çatışması varsa ne şekilde olduğu açıkça beyan edilmelidir. Eğer yok ise "Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler." ifadesini kullanmalıdırlar.

- **ETİK KURUL ONAYI**

  Çalışmanın sonunda kaynaklardan önce etik kurul onayı alınmışsa hangi kurumdan ve ne zaman alındığı onay numarası ile mutlaka belirtilmeli ve Etik Kurul Onayını makale gönderim sırasında yüklemelidir. Etik kurul onayına gerek olmayan çalışmalarda aşağıdaki cümle yazılmalıdır.

  "Yazarlar bu çalışma için etik kurul onayının zorunlu olmadığını beyan etmektedir."

- **KAYNAKLAR:** Kaynak yazım stili Amerikan Psikoloji Derneği'ne (APA) göredir. Yazı karakteri "Times New Roman" ve 10 punto, "1" aralık, iki yana yaslı. Metinde, geçiş sırasına göre köşeli parantez içinde, örneğin: [1,6,9], [5-7] gibi numaralandırılmalı ve metin sonunda bu numaralara göre sıralanmalıdır. Alt başlıkların yanına kaynak belirtilmemelidir. Tablo içinde kaynak bildirilmesi gerekiyorsa metin içinde verildiği gibi belirtilmelidir.

  - **Makale için:** Yazarın soyadı, adının baş harfleri (Birden fazla adı olan yazarın her bir isminin baş harfinden sonra nokta konmalı ve arada boşluk bırakılmamalıdır. Birden fazla yazarların arasında virgül yer almalıdır. **Son yazar ile bir önceki yazar arasında "ve" kelimesi veya "&" sembolü <u>kullanılmamalıdır</u>**.), makalenin tam başlığı, derginin adı, cilt no, varsa sayı no (parantez içinde), başlangıç ve bitiş sayfa numarası (veya makale numarası), yıl yazar isimlerinden sonra (parantez içinde) yazılmalıdır. **Birden fazla yazar varsa hepsi yazılmalıdır.** Makalenin adı yazılırken ilk kelimenin ilk harfi büyük diğer kelimelerin ilk harfi küçük yazılmalıdır. Kaynaklarda verilen **dergi adları kısaltma yapılmadan açık olarak yazılmalıdır.**

Her bir referansın sonuna [CrossRef] **ekleyerek aşağıdaki formatta DOI numarasını köprü olarak giriniz. Lütfen https://www.crossref.org/'da yer almayan makaleleri** [CrossRef] şeklinde **belirtmeyiniz.**
https://doi.org/10.1016/0006-2952(89)90403-6

Örnekler:

1. Martinez, M.J.A., Del Olmo, L.M.B., Benito, P.B. (2005). Antiviral activities of polysaccharides from natural sources. Studies in Natural Products Chemistry, 30, 393-418. [CrossRef]
2. Bahiense, J.B., Marques, F.M., Figueira, M.M., Vargasa, T.S., Kondratyuk, T.P., Endringer, D.C., Scherer, R., Fronzaa, M. (2017). Potential anti-inflammatory, antioxidant and antimicrobial activities of *Sambucus australis*. Pharmaceutical Biology, 55(1), 991-997. [CrossRef]

- **Elektronik Makale için:**

Örnek:
Perneger, T.V., Giner, F. (1998). Randomized trial of heroin maintenance programme for adults who fail in convential drug treatments. British Medical Journal, 317, from http://www.bmj.com/cgi/content/full/317/7150/ Erişim tarihi: 14.03.2021

- **Web sitesi için:**

Örnek:
Clinical Pharmacology Web site. (2001). Erişim adresi http://cpip.gsm.com/ Erişim tarihi: 14.03.2021.

- **Kitap için:** Yazarın soyadı, adının baş harfleri, kitabın adı, cilt no (varsa), kitabevi, yayınlandığı şehir, sayfa no, basıldığı yıl (parantez içinde) yazılmalıdır.

Örnek:
Franke, R. (1984). Theoretical Drug Design Methods, Elsevier, Amsterdam, p.130.

- **Kitap bölümü için:** Yazarın soyadı, adının baş harfleri, bölümün başlığı, editör/editörlerin soyadı, adının baş harfleri, (Ed./Eds.) ibaresi, kitabın adı, varsa cilt no, kitabevi, yayınlandığı şehir, sayfa no, basıldığı yıl (parantez içinde) yazılmalıdır.

Örnek:
Weinberg, E.D. (1979). Antifungal Agents. In: M.E. Wolff and S.E. Smith (Eds.), Burger's Medicinal Chemistry, (pp. 531-537). New York: John Wiley and Sons.

- **Tez için:** Yazarın soyadı, adının baş harfleri, yıl yazar isimlerinden sonra (parantez içinde) yazılıp nokta işareti konmalıdır. Ne tür tez olduğu belirtildikten sonra tezin başlığı, nerde yapıldığı yazılmalıdır.

Örnek:
Ahmed, J. (2008). PhD Thesis. Pharmaceutical Botany investigations on *Prangos* Lindl. (Umbelliferae) growing in Konya province. Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, Ankara, Turkey.

- **Patent için:** Yazarın soyadı, adının baş harfleri, yıl yazar isimlerinden sonra (parantez içinde) yazılıp nokta işareti konmalıdır. Patent başlığı ve patent numarası yazılmalıdır.

Örnek:
Mahoney, S., Molz, L., Narayan, S., Saiah, E. (2018). Heteroaryl RHEB Inhibitors and Uses Thereof. WO 2018/191146 A1.

ETİK İLKELER VE YAYIN POLİTİKASI

Ankara Üniversitesi Eczacılık Fakültesi Dergisi, açık erişimli, hakemli bir dergi olup Türkçe veya İngilizce olarak farmasötik bilimler alanındaki önemli gelişmeleri içeren orijinal araştırmalar, derlemeler ve kısa bildiriler için bir yayım ortamıdır. Ankara Üniversitesi Eczacılık Fakültesi Dergisi'nin makale yayın ücreti (APC) veya abonelik ücreti yoktur.

Yayın kurulu olarak dergi kapsamında önemli katkı sağlayan kaliteli yeni çalışmaların yayınlanması amaçlanmaktadır. Bu amaca ulaşmak için gönderilen makaleler, dergide yayınlanmak için bilimsel ve biçimsel gerekli kriterleri karşıladıklarından emin olmak adına baş editör ve/veya editör yardımcıları tarafından ilk değerlendirmeye tabi tutulur. Yalnızca bu ön değerlendirme sürecini geçen çalışmalar, daha ileri değerlendirme için diğer aşamalara devam ettirilir.

Ön Değerlendirme

- Çalışmanın bilimsel kalitesi ve yeniliği dergide yayınlanmak için yeterli olmalıdır.
- Dergiye gönderilen çalışmalar derginin amaç ve kapsamına uygun olmalıdır.
- Metin İngilizce veya Türkçe olarak dilbilgisi kurallarına uygun ve bilimsel olarak iyi yazılmış olmalıdır.
- Dergiye gönderilen çalışmaların benzerlik oranı %20'i geçmemelidir.
- Çalışmalar derginin yazım kurallarına ve şablonuna uygun olacak şekilde düzenlenmelidir.
- Telif hakkı devir formu, etik kurul onay belgesi, yazar katkı formu mutlaka yüklenmeli ve imzalı olmalıdır.
- Çalışmalar elektronik online başvuru sistemi aracılığı ile dergiye gönderilmiş olmalıdır.

Bu yeterlikleri taşımayan çalışmaların ileri değerlendirme süreci başlatılamaz.

Dergi yayınlanma sürecinde dergi editörleri, hakemler ve yazarlara bazı sorumluluklar düşmektedir. Bu sorumluluklar aşağıdaki şekilde açıklanmıştır.

1. Editörün Görevleri ve Etik Sorumlulukları

Editör, dergiye gönderilen makalelerden hangilerinin yayınlanması gerektiğine bağımsız olarak tek başına karar verebileceği gibi editör kurulunun üyelerine veya hakemlere de danışabilir. Derginin etik ilkeleri ve yayın politikası çerçevesinde, çalışmaların ön değerlendirme, hakem değerlendirmesi ve yayınlanma aşamalarının tarafsız, denetlenebilir, adil, çıkar ilişkisinden bağımsız ve gizlilik ilkelerine uygun şekilde yürütülmesinden sorumludur. Yayın politikası ve etik ilkeleri açısından ihlal yoksa derginin amacına ve kapsamına uygun çalışmaları, ön değerlendirme aşamasına almalıdır.

Baş editörün, editör yardımcılarının, alan editörlerinin ve editoryal danışma kurulunun görevleri ve tanımları aşağıdaki gibidir:

Baş Editör: Dergi içeriğinin yayınlanması konusunda tam yetkiye sahip kişidir. Editör yardımcıları, alan editörleri ve editöryal danışma kurulu ile birlikte çalışır.
Editör Yardımcıları: Dergi ilgili sorulara cevap vermek, dergi hakem ve kuruluna önerilerde bulunmak, makale yayın sürecinde baş editöre yardımcı olan kişilerdir.
Alan Editörleri: Çift kör hakem atamalarının gerçekleşmesi ve dergi ile ilgili sorulara cevap vermek konusunda yazarlara yardımcı olan kişilerdir.
Editoryal Danışma Kurulu: Editoryal Danışma Kurulu, Ankara Üniversitesi Eczacılık Fakültesi Dergisinin, amacına uygun ve kaliteli yayın üretilmesine ilişkin konularda Baş Editör ve Editör Yardımcılarına kılavuzluk eder.

1.1. Yayın Politikası

- Baş editör, dergiye gönderilen makalelerden hangilerinin yayımlanması gerektiği kararından tek başına sorumludur. Editörün kararı, derginin editör kurulunun prensipleri doğrultusunda olabileceği gibi, onur kırıcı yayım yapmak, telif hakkı ihlali ve intihal gibi konularla ilgili olarak yürürlükte olan yasal gereklilikler ile sınırlandırılmıştır.
- Baş editör, makale yayımlanmadan önce yazarların yayımcıya makalenin “Copyright Transfer Form” unu, doldurarak telif hakkını gönderdiğinden emin olmaktadır.
- Baş editör, yazarların makale yayımlanmadan önce "Conflict of Interest Form”unu ve “Author Contribution Form” unu doldurduğundan emin olmaktadır.
- Baş Editör, dergiye gönderilen makalelerin biçimsel olarak incelenmesi için editör yardımcılarını görevlendirmektedir. Ankara Üniversitesi Eczacılık Fakültesi Dergisinin kurallarını sağlamayan makaleler kesinlikle değerlendirmeye alınmadan reddedilmektedir.

1.2. Yayın Değerlendirmesi

- Baş editör, yayın değerlendirme sürecinin adil, tarafsız ve zamanına uygun şekilde gerçekleşmesini sağlamaktan sorumludur.
- Editör, tüm makaleleri genel olarak dışardan ve bağımsız en az iki hakem ile değerlendirilmesini sağlamaktadır. Gerek olması durumunda editör üçüncü bir hakemden ek görüş istemektedir.
- Editör, hakem seçimini makale kapsamına uygun olan uzmanları değerlendirerek yapar.
- Editör, olası çıkar çatışmaları için yapılan açıklamaları, hakemler tarafından yapılan “self-citation” önerilerini ve herhangi bir taraflılık olasılığını değerlendirmek ve karar vermek için dikkatli bir şekilde yayın sürecini gözden geçirmektedir.
- Baş editör/editörler, hakem değerlendirmesi veya değerlendirme/yayım sürecinin herhangi bir noktasında bir benzerlik tespit yazılımı (iThenticate) tarafından taratılmasını yazardan istemektedir veya kendisi yapmaktadır. Bu anlamda ifadelerin veya cümlelerin yazarın/yazarların kendileri olsa dahi metin daha önce yayınlanmış verilerle kabul edilemez bir benzerliğe sahip olmamalıdır.
- Baş editör, bir makaledeki hataları yayımlanmadan önce tespit ederse düzeltmektedir. Eğer daha sonra tespit ederse bu durumda düzeltmeleri yayımlamak zorundadır. Tüm düzeltme veya geri çekme bildirimlerini dergide belirgin bir şekilde yayımlamalıdır. Ayrıca içindekiler sayfasında listelemelidir.
- Ankara Üniversitesi Eczacılık Fakültesi Dergisinin editörleri, Yayın Etiği Komitesi (Committee on Publication Ethics (COPE)) tarafından yayınlanan "COPE Code of Conduct and Best Practice Guidelines for Journal Editors" ve "COPE Best Practice Guidelines for Journal Editors" kılavuzlarına uyarak çalışmalarını sürdürür.

1.3. Adil Değerlendirme

- Baş editör/editörler, makaleleri yazarların ırk, cinsiyet, cinsel eğilim, inanç, etnik köken, vatandaşlık ya da politik görüşlerine bakmaksızın bilimsel içeriklerine göre değerlendirmektedir. Derginin editoryal prensipleri şeffaf ve tümüyle dürüst değerlendirmeyi desteklemektedir.
- Editör, hakemlerin ve yazarların kendilerinden bekleneni tam olarak anladıklarından emin olmalıdır.
- Editör, dergi ile ilgili tüm iletişimini derginin elektronik başvuru sisteminden yapar ve kararlarında itirazlar olması halinde şeffaf ve hakkaniyetli bir yol izler.

1.4. Gizlilik İlkesi

- Baş editör/editör, dergiye yapılan başvurudaki tüm materyallerin ve hakemlerle yapılan tüm iletişimin gizliliğini (ilgili yazar ve hakemlerle aksi onaylanmadığı sürece) korumakla yükümlüdür.

- Baş editör/editör, hakemlerin isimlerinin açıklanmasını kabul etmediği sürece, hakemlerin kimliklerini ve haklarını korumakla sorumludur.
- Başvurusu tamamlanmış bir makaleye ait basılmamış materyaller, yazarın yazılı onayı alınmadan editörün kendi çalışmaları/araştırmaları için kullanılmamalıdır.
- Baş editör/editör, makale değerlendirme sürecinde edinilen tüm bilgileri veya fikirleri gizli tutmalı ve kişisel amaçlar için kullanmamalıdır.

2. Hakemlerin Görevleri ve Etik Sorumlulukları

Ankara Üniversitesi Eczacılık Fakültesi Dergisi'nin makale değerlendirme süreci çift taraflı kör hakemlik ilkesiyle yürütülmektedir. Dolayısıyla hakemler yazar/yazarlarla iletişim kuramazlar, değerlendirmeler dergipark yönetim sistemi üzerinden paylaşılır. Değerlendirme sürecinde tam metinlere ilişkin değerlendirme formları hakem yorumları editör aracılığı ile sorumlu yazara iletilir. Hakemler, değerlendirme süreci boyunca tarafsızlık, gizlilik, nesnellik, bilimsel yönden inceleme ilkelerine uygun hareket etmelidir. İlgili alanda uzman ve yetkinliğe sahip olmalıdır. Değerlendirmesine sunulan çalışmaya ilişkin raporunu belirtilen zaman aralığı içinde bitirmelidir. Zamanında sunulamayacak raporlar için gecikmeden editör ile iletişime geçilmelidir. Etik ilkeleri, telif hakkı ihlali, olası çıkar çatışması ve intihal yapıldığının fark edilmesi durumlarında editör kurulunu bilgilendirmelidir.

Ankara Üniversitesi Eczacılık Fakültesi Dergisi için makaleleri değerlendiren hakemlerin aşağıda belirtilen görevlere ve etik sorumluluklara uyması beklenmektedir.

2.1. Editöryal Kararlara Katkı

- Hakemler, yazarların sundukları çalışmaları yapıcı ve uygun şekilde değerlendirmelidirler.
- Hakemler, makalede yer alan araştırmayı değerlendirmeye yetkin olmadığını düşünüyorsa veya yeterli sürede tamamlayamayacaksa editöre durumu bildirmelidirler.
- Hakemler, yazarlara yönelik sert ve kişisel eleştirilerde bulunmamalıdırlar.
- Hakemler, makale değerlendirmesi için davet aldığında eğer kendilerini makalede çalışılan konu hakkında yetersiz hissederlerse makaleyi değerlendirmeyi reddetmelidirler.
- Hakemler, makale değerlendirmesini verilen süre içinde yapmalıdırlar.
- Hakemler, sadece çalışmanın içeriğine ilişkin değerlendirmeyi objektif olarak yapmalıdırlar.

2.2. Gizlilik

- Hakemler, değerlendirmeyi tarafsızlık ve gizlilik içerisinde yapmalıdırlar.
- Hakemler, makale hakkındaki değerlendirmelerini ya da bilgilerini üçüncü kişilerle paylaşmamalıdırlar.
- Hakemler, makale değerlendirme sürecinde edinilen bilgileri, fikirleri ve basılmamış materyal veya çalışmaları gizli tutmalı ve kişisel amaçlar için kullanmamalıdırlar.
- Hakemler, makalenin bir kopyasını elinde bulundurmamalı veya çoğaltmamalıdırlar.

2.3. Etik Sorunları Fark Etme

- Hakemler, makalede yer alan etik sorunları fark etmeli ve editörün dikkatine sunmalıdırlar.
- Hakemler, makalenin daha önce başka bir yerde basıldığını veya basılmış önceki bir makale ile önemli ölçüde benzerlik ya da örtüşme tespit ederse editöre bildirmelidirler. Daha önce yayımlanmış olan herhangi bir gözlem ve/veya argüman, ilgili referans ile birlikte verilmelidir.

2.4. Tarafsızlık ve Rekabet Standartları

- Hakemler, tarafsız olarak değerlendirmelerini yapmalı ve önyargıdan uzak şekilde değerlendirmelidirler. Yazarın kişi olarak eleştirilmesi uygun değildir. Hakemler, görüşlerini destekleyici argümanlarla ifade etmelidirler.

- Hakemler, makale değerlendirmeyi kabul etmeden önce olası çıkar çatışmasını kontrol etmelidirler. Eğer çıkar çatışmasıyla karşı karşıya olduğunu düşünüyorsa makaleyi incelemeyi reddetmeli ve editörü bilgilendirmelidirler.
- Hakemler, yazar tarafından hakemin (ya da hakemle çalışan kişilerin) çalışmalarının kaynak olarak alındığını ileri sürerse, gerçek bilimsel gerekçeler sunmalılar, bu durumun hakemin kaynak gösterilme sayısını ya da çalışmalarının görünürlüğünü artırmaya yönelik bir girişim olmamasına özen göstermelidirler.
- Hakemler, değerlendirmelerini yaparken bilimsel gerçeklikten uzaklaşmamalı ve gerekirse kaynak gösterme yoluna başvurmalıdırlar.

3. Yazarların Görevleri ve Etik Sorumlulukları

Ankara Üniversitesi Eczacılık Fakültesi Dergisi'ne gönderilen makaleler, daha önce herhangi bir yayın organında yayımlanmamış olmalıdır veya yayımlanmak üzere aynı zaman diliminde başka bir yayın organına gönderilmiş olmamalıdır. Çalışmalarda yararlanılan araştırmaların ve yayınların, alıntılarının veya atıflarının bilimsel araştırma ilkelerine uygun olarak eksiksiz yapılması ve kaynakların belirtilmesi zorunludur. Çalışmada yer alan yazar sayısı birden fazla ise, yazarların çalışmaya bilimsel ve akademik olarak somut ve yeterli düzeyde katkı sağlaması beklenir. Çalışmaya ait tüm finansal destek kaynakları açıklamalıdır. Olası çıkar çatışması durumlarını yayın kuruluna bildirmelidir.

Ankara Üniversitesi Eczacılık Fakültesi Dergisi'ne makale gönderen yazar/yazarların aşağıda belirtilen görevlere ve etik sorumluluklara uymalıdır.

3.1. Bildirim Standartları

- Yazar(lar)ın gönderdiği makale (araştırma, derleme veya kısa bildiri) özgün olmalıdır.
- Yazar(lar), çalışmanın önemine ilişkin tarafsız bir tartışma ile gerçekleştirilen araştırmayı net bir şekilde sunmalıdır.
- Yazar(lar), makalede verileri açık bir şekilde sunmalıdır.
- Yazar(lar)ın başka çalışmalardan faydalanması halinde tam ve doğru bir şekilde alıntı yapmalıdır.
- Makale, diğer araştırmacıların çalışmayı tekrar edebilmesine olanak verecek şekilde yeterli detay ve kaynak içermelidir.
- Yazar(lar), etik dışı davranarak yanıltıcı ya da net olmayan ifadeleri makalelerinde kullanmamalıdır.
- Yazar(lar), dergi kurallarına uymadıkları ve belirtilen sürede aksiyon almadıkları sürece makalelerinin dergi tarafından yayımlanmayacağını bilerek hareket etmelidir.

3.2. Veri Ulaşımı ve Saklama

- Yazarlardan editöryal değerlendirme için makalelerini destekleyici araştırma verisi istenebilir.
- Yazarlar, değerlendirme sürecinde makalelerine ilişkin ham verilerin veya makalelerini destekleyecek verilerin talep edilmesi durumunda belirtilen verileri yayın kuruluna sunmaya hazır bulunmalıdırlar.

3.3. Orijinallik, İntihal ve Kaynakların Belirtilmesi

- İntihal, yazarın başka bir makaleyi kendi çalışması olarak göstermesi, kaynak göstermeden başka birine ait çalışmanın belli bölümlerinin kopyalanması ya da başka sözcüklerle anlatılması veya başkaları tarafından yapılan çalışmanın sonuçlarının alınarak sunulması şeklinde olabilir. İntihalin her biçimi etik olmayan davranıştır ve kesinlikle kabul edilmemektedir. Yazarlar intihalden uzak durmalıdır. İntihal tanımı için buraya bakınız.
- Yazarlar çalışmalarının tümüyle orijinal olduğunu garanti etmelidirler. Yazarlar, başkalarının fikirlerini veya metinlerini kullanıyorlarsa mutlaka uygun şekilde kaynak ya da alıntı

göstermeliler ve gerekliyse izin almalıdırlar.

- Yazarlar kendilerine ait olan çalışmayı etkileyen ve çalışmaya ait uygun içeriğin oluşturulmasında katkısı olan tüm yayınları veya eserleri kaynak olarak göstermelidirler. Özel olarak (görüşme, yazışma ya da üçüncü taraflar ile tartışma) ile elde edilen bilgiler kullanılmamalı ya da kullanılacaksa izin alınarak bildirilmelidir.
- Yazarlar, Ankara Üniversitesi Eczacılık Fakültesi Dergisi'ne yayımlanmak üzere gönderdikleri makalelerini intihal tarama programları (iThenticate) ile taramalı ve dergipark sisteminde çevrim içi makale gönderim sırasında makalelerinin intihal içermediğine dair raporu yüklemek zorundadırlar.

3.4. Çoklu, Gereksiz ve Tekrar Yayınlama

- Aynı makale ile birden fazla dergiye başvuruda bulunmak etik olmayan bir davranıştır ve asla kabul edilmemektedir. Genel olarak, yazar daha önce basılmış bir yayını, özet formunda ya da yayınlanmış bir ders, akademik tez ya da elektronik ön baskının bir parçası olması dışında, değerlendirme için başka bir dergiye göndermemelidir.
- Yazarlar başvuru sırasında makaleyi başka bir dergiye daha aynı anda göndermediklerini garanti etmelidirler.
- Yazarlar, gönderilen yazının değerlendirme aşamasında olmadığını veya başka bir yerde yayımlanmak üzere kabul edilmediğini ve eğer kabul edilirse, aynı biçimde, başka bir dilde, elektronik ortam da dahil olmak üzere, yazarın yazılı izni olmaksızın başka bir yerde yayımlanmayacağını garanti etmelidir.

3.5. Yazar Katkıları

- Yazar katkıları, çalışmanın konseptine, tasarımına, gerçekleştirilmesine ya da yorumlanmasına önemli katkı sağlayan kişiler ile sınırlandırılmalıdır.
- Yazarlar, çalışmaya katkı veren yazarların listesini dikkatli bir şekilde hazırlamalıdır. Bazı durumlar eşyazar (co-author) olmayı bazı durumlar ise çalışmanın "Teşekkür" (Acknowledgement) bölümünde yer almasını hak edebilir.
- Sorumlu yazar, tüm eşyazarların çalışmada uygun şekilde yer aldığına, tüm eşyazarların çalışmayı görüp onayladıklarına ve yayınlanmak üzere başvuru yapılmasına dair verdikleri onaya ilişkin sorumluluğu üstlenmelidir.
- Sorumlu yazar, makaledeki tüm yazarların yazar sıralaması, çalışmanın kesinliği ve bütünlüğü gibi konularda fikir birliğinin sağlanmasından sorumludur ve orijinal başvuru sırasında kesin bir yazar listesi sunmalıdır.
- Çalışmanın başvurusu tamamlandıktan sonra, sadece istisna durumlarda, editör yazar listesinde ekleme, silme ya da yeniden düzenleme yapabilir. Tüm yazarlar bu şekilde yapılacak ekleme, silme ve yeniden düzenleme konusunda fikir birliği içinde olmalıdırlar. Tüm yazarlar çalışmanın ortak sorumluluğunu aldıklarını kabul ederler. Her yazar, uygun şekilde araştırılan ve karara bağlanan çalışmanın kesinliği ve bütünlüğü ile ilişkili sorulardan sorumludur.
- Sorumlu yazar, editör ile iletişime geçen kişi olarak Ankara Üniversitesi Eczacılık Fakültesi Dergisi'ne makale ile birlikte "Yazar Katkı Formu"nun da doldurulup gönderilmesinden sorumludur.

3.6. Çıkar Çatışması Beyanı

- Yazarlar, çalışmalarını uygunsuz bir şekilde etkileyebilecek olarak gördükleri diğer kişi veya organizasyonlarla çıkar çatışması oluşturabilecek her türlü durum ve ilişkileri beyan etmelidirler.
- Sorumlu yazar, editör ile iletişime geçen kişi olarak Ankara Üniversitesi Eczacılık Fakültesi Dergisi'ne makale ile birlikte "Çıkar Çatışması Beyanı Formu"nun da doldurulup gönderilmesinden sorumludur.

- Yazarlar çıkar çatışmalarının olduğu durumları mutlaka açıklamalıdırlar.

3.7. Temel Hataların Bildirimi

- Yazarlar, yayımlanmış, erken görünüm veya değerlendirme sürecinde olan bir çalışmasında önemli bir hata ya da eksiklik fark ettiğinde, acil olarak dergi baş editörüne/yayınevine veya ilgili editöre bildirmek ve editör tarafından gerekli görülmesi durumunda makaleyi geri çekmek veya düzeltmek için editörle işbirliği yapmak ile yükümlüdür.
- Editör/yayınevi yayımlanmış olan makalenin bir hata içerdiğini üçüncü bir taraftan öğrenirse, editör ile işbirliği yapmak ve gerektiğinde destekleyici kanıt sağlamak yazarın yükümlülüğüdür.

3.8. Olası Riskler ve İnsan veya Hayvan Konuları

- Yazarlar, kullanımları sırasında olağan dışı risk yaratan kimyasallar, işlemler ya da malzemeler ile çalışmışlarsa açıkça belirtmelidirler.
- Eğer çalışmada hayvan ya da insan örnekleri/gönüllüler kullanılıyorsa, araştırmacılar tüm işlemlerin ilgili kanun ve kurumsal kılavuzlara uygun şekilde gerçekleştirildiğine ve uygun idari kurul tarafından bu işlemlerin onaylandığına ve Etik Kurul Onayı alındığına dair ifadenin makale içinde yer alması sağlamalıdırlar.
- Yazarlar, Etik Kurul Onayının zorunlu olduğu çalışmalarda, etik kurul onayı alınan kurumun adı ve etik kurul onay numarasını, gereç ve yöntem kısmında ve Etik Kurul Onay bölümünde belirtmelidirler. Ayrıca, kullanılan protokol ve prosedürlerin etik olarak gözden geçirildiğini ve onaylandığını, makalenin gereç ve yöntem bölümüne eklemelidirler.
- Etik kurul raporu alınması gerektiği halde, etik kurul raporu olmayan çalışmalar reddedilecektir.
- İnsanlar veya insandan elde edilen örnekler üzerinde yapılan klinik araştırmalarda bilgilendirilmiş onam formu mutlaka alınmış olmalıdır ve gereç ve yöntem kısmında belirtilmelidir. İnsan gönüllüleri ile yapılan araştırmalar için araştırma protokolüne uygun olarak hazırlanmış yazılı bilgilendirilmiş gönüllü onam formu alınmalıdır.
- Yazarlar, çalışmalarında, hayvan ya da insan örnekleri/gönüllüler kullanmışsa gerekli etik kurul izinlerini aldığından emin olmalıdır. Etik kurul izin ifadesini makalede mutlaka belirtmelidir.
- Bu anlamda yazarlar aşağıda sıralanmış olan kılavuzlara uyarak çalışmalarını gerçekleştirmiş olmalıdırlar:
  İnsanlar üzerinde gerçekleştirilen tüm araştırmalar Helsinki Bildirgesi ilkelerine göre yapılmalıdır (World Medical Association (WMA) Helsinki Declaration for Medical Research in Human Subject). İnsan gönüllülerinden bilgilendirilmiş onam formu alınmış olmalıdır. Tüm hayvan çalışmaları ARRIVE kılavuzuna uygun olmalı (Animal Research: Reporting of In Vivo Experiments (ARRIVE) Guidelines) ve "Bilimsel Amaçlı Kullanılan Hayvanların Korunmasına İlişkin Konsey Direktifi"ne (EU Directive 2010/63/EU for animal experiments), "Birleşik Krallık Hayvan Yasası"na (The U.K. Animals (Scientific Procedures) Act 1986) ve/veya "U.S. İnsan Bakımı ve Laboratuvar Hayvanlarının Kullanımına İlişkin Halk Sağlığı Hizmeti Politikası" rehberine (U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals) uygun şekilde yürütülmelidir. Bitkiler ile ilgili tüm deneysel araştırmalar, uluslararası yönergelere uygun olmalıdır.

4. Ücret Politikası

- Hiçbir ad altında yazar veya kurumundan ücret alınmaz.
- Dergi ile işleme ve yayınlama ücretsizdir. Gönderilen veya kabul edilen makaleler için makale işleme ücreti veya gönderim ücreti yoktur.

Publication Terms

1. The Journal of Faculty of Pharmacy of Ankara University (J. Fac. Pharm. Ankara) is an open-access, peer reviewed journal and is published three times (January-May-September) a year.

2. The Journal of Faculty of Pharmacy of Ankara University publishes articles in every field of Pharmaceutical Sciences. The manuscript to the journal should not be published previously as a whole or in part and not be submitted elsewhere. Manuscript should be written in Turkish or in English. The experiments used have to be adhered to the Declaration of Helsinki for humans and European Community Guidlines for animals. In studies where Ethics Committee Approval is mandatory, the name of the institution from which ethics committee approval was obtained and the ethics committee approval number should be stated in the material and method section and the Ethics Committee Approval section, and the relevant document should be uploaded during article submission.

3. All manuscripts will be submitted to a review process by the editors and by qualified at least 2 outside reviewers. The article evaluation process of Journal of Faculty of Pharmacy of Ankara University is carried out on the principle of double-blind refereeing.

4. Manuscripts are published in order of final acceptance after review and revision.

5. If a manuscript returned to the authors for revision is not received back to the editor within 3 months it will be treated as a new article. When the article is published, authors must send the copyright of the article to the Publisher by filling out the "Copyright Transfer Form".

6. Manuscript will be controlled using plagiarism checker. Articles sent to Journal of Faculty of Pharmacy of Ankara University for publication must be scanned with plagiarism scanning programs (iThenticate) and a report stating that the articles do not contain plagiarism must be uploaded during online article submission.

7. Journal of Faculty of Pharmacy of Ankara University does not have an article publication fee (APC) or subscription fee.

8. The following types of articles are accepted in the Journal Faculty of Pharmacy of Ankara University:

    a) **Original articles**: Articles written in English or Turkish in scientific format presenting original research. Articles should be printed on A4 size papers not exceeding 25 pages (including tables and figures). Research articles are expected to be innovative and contributing to science. Articles must have the main headings specified in the writing rules and must be prepared using a Windows compatible program.
    b) **Review articles:** An updated comprehensive review of scientific works on a particular subject. Articles written in English or Turkish should be printed on A4 size papers not exceeding 30 pages (including tables and figures). Articles must have the main headings specified in the writing rules and must be prepared using a Windows compatible program.
    c) **Short communications:** Rapid announcement of the results of a continuing research written in English or Turkish, no longer than 5, A4 size pages. Articles must have the main headings specified in the writing rules and must be prepared using a Windows compatible program.

Preparation of Manuscript

1. Texts must be written in A4 norm (21 x 29.7 cm).
2. Texts should be written with 1 line spacing, with 2.5 cm margins on the left and right sides of the A4 norm page, 3 cm margins each from the top and bottom edges (3 line spacing from the top on the first page). Articles accepted for publication will be directly uploaded to the system as a "Microsoft Word" file (online submission). The main text font should be **"Times New Roman"** and **11 pt**.
3. Page numbers **should not be specified** in the article.
4. Paragraph headings must **begin 1 cm inside**. Additional spaces should not be left between paragraphs.
5. On the title page, the title of the manuscript the name/s, the full address/es and ORCID no of the author/s, and the full address, telephone number, e-mail address of the corresponding author should be written and all should be centered in the text. It should be indicated by placing (*) above the surname of the corresponding author. Name, surname, full address, telephone number and e-mail address of this person should be specified at the bottom of the title page.
6. **Author's Name** (**first letter capital, others lowercase**) and **SURNAME** (**all capital letters**) should be written in bold, three lines spaced under the title, and without a title underneath. If there is more than one author, they should be written by separating them with a comma and leaving a space. The numbers to be placed on the surnames of the authors and the institution names and postal addresses (For example: Ankara University Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06560, Ankara, Turkey) should be clearly written on the line just below the names.
   - **ORCID ID number must be declared for all authors**. ORCID IDs of the authors should be created by creating a hyperlink to the relevant logo and adding URL links.
7. International abbreviations may be used. ml for milliliter in the text; min. for minutes It should be written as specified.
8. Units should be expressed using the metric system.
9. All tables and figures should be placed in their places in the text without exceeding the writing area.
10. Tables should be numbered on the top, figures (formula, graph, chart, spectrum, chromatogram, photograph, etc.) should be numbered below with Arabic numbers (**Figure 1., Table 2.)** and should be included in the text. The words "Table", "Figure" and their numbers should be written in bold and in 11 pt. Figure/Picture (**in JPEG format**) must be placed in the article and pictures must be at least **300 dpi or in higher resolution**. Authors must obtain written permission to reproduce any images from other sources.
11. **Table** titles should be written in 11 font size justified on the top of the tables and not exceeding their width. If there is an explanation for the table, it should be written in 9 font size at the bottom of the table. The text in the table can be written between 8-11 points. **Figure titles** should be written at the bottom of the figures with a line spacing, centered and 11 pt. There must be **6 nk** space between the figure and figure title. There should be **18 nk** space between the text and title of figure and/or table.

    See for below examples for tables:
    - All row and column lines should be included.
    - Table design should be uniform and straight throughout the article, no coloring / shading should be used.
    - Headings in the table should be written in **bold**. There must be **6 nk** space between the table and table title.

Table 1. Morphological characteristics of the species

Plant part*	***C. nummularia***	***C. integerrimus***
Leaf	Broadly elliptical-orbicular, 0.9-2.5-(4) x 0.5-2.5-(3-5) cm	From orbicular to ovate, 1.2-(4-5) x 0.9-3 cm,
Seed	3.5-4 x 1-2 mm, dark brown	3-4 x 1.5-2 mm, light brown

* Explanation should be 9 font size, 1 range.

Table 2. Patient demographics

Demographics	**Group A***	**Group B**	**Group C**
Male gender	10 (%30)	20 (%60)	10 (% 30)
Cigarette consumption	20 (%60)	10 (%30)	20 (%60)

* Explanation should be 9 font size, 1 range.

Example for figure:

Figure 1. General view of *C. Nummularia* (The font size must be 11 pt with 1 line spacing and "Times New Roman" font, and must be centered in the text)

12. The sections of the articles should be prepared in accordance with the **TITLE** (Turkish and English), **ABSTRACT, INTRODUCTION, MATERIAL AND METHOD, RESULT AND DISCUSSION, ACKNOWLEDGEMENTS** (if available), **AUTHOR CONTRIBUTIONS, CONFLICT OF INTEREST, ETHICS COMMITTEE APPROVAL** (if available) and **REFERENCES**. Titles expressing these sections (except the first title of the article) should be written in **12 pt**, **bold capital letters and starting from the left of the page**. **There should be 18 nk space before and 6 nk space after the INTRODUCTION.** For, there should be 12 nk space before and 6 nk space after the other titles. Between the chapter titles and the text, a separate space **should not be left** other than the specified in this document.

- **TITLE:** Capital letters and **first title** in Turkish and English (Turkish title is the first title in Turkish articles, English title is the first title in English articles), **14 pt, bold** and the second title should be written in 12 pt, *italic*. The title should be appropriate to the text, short, introducing the work and clearly worded.

- **ABSTRACT** and **ÖZ:** It should be written in English (**ABSTRACT**) and Turkish (**ÖZ**) at the beginning of the articles, not exceeding 200 words, 10 pt, *italic* and within a frame. In articles written in a foreign language, first **ABSTRACT** and then **ÖZ** in Turkish. **ABSTRACT** and **ÖZ** titles should be written in 12 pt. And bold and the summary of the article should be presented as subheadings. Each subtitle should be written in 10 pt, bold, normal and 1 cm indented. **ABSTRACT** and **ÖZ** should be written in blocks with 1 cm margins from the right and left.

For original articles;

Subheadings to be used for **ABSTRACT**:

Objective: *Text should be written in italic.*
Material and Method: *Text should be written in italic.*
Result and Discussion: *Text should be written in italic.*
Keywords:

Subheadings to be used for **ÖZ**:

Amaç: *Text should be written in italic.*
Gereç ve Yöntem: *Text should be written in italic.*
Sonuç ve Tartışma: *Text should be written in italic.*
Anahtar Kelimeler: *Text should be written in italic.*

For review articles;

Subheadings to be used for **ABSTRACT**:

Objective: *Text should be written in italic.*
Result and Discussion: *Text should be written in italic.*
Keywords:

Subheadings to be used for **ÖZ**:

Amaç: *Text should be written in italic.*
Sonuç ve Tartışma: *Text should be written in italic.*
Anahtar Kelimeler:

- **Keywords (Anahtar Kelimeler)**: It should consist of a minimum of 3 words, should be written alphabetically, italic in the relevant language, with only the first letter of the first keyword capitalized (except for abbreviations using capital letters) with commas between them and a spelling mark **should not be** used after the last keyword.

- **TEXT:** The text part of the original Turkish article should consist of 3 main headings: **INTRODUCTION, MATERIAL AND METHOD, RESULT AND DISCUSSION**. All of these main headings should be written in 12 pt, **capital letters** and bold. In review articles, there should be the main headings of **INTRODUCTION** and **RESULT AND DISCUSSION**, other titles should be written with the first letter of each word capital, the others in lowercase and bold, as determined by the author. Subheadings should be written in 11 font size, 1.5 line spacing, **bold** and aligned to the left. Numbering system **should not be** used in subheadings.

- **INTRODUCTION:** There should be a section containing the purpose of the research and studies on the subject.

- **MATERIAL AND METHOD:** Required information about the method should be clearly stated by indicating the material used. **Characterization of compounds** should be shown in a separate paragraph and clarification of the purity and structure of the new compounds should be provided. If animal or human samples/volunteers are used in the study, researchers should ensure that a statement stating that all procedures are carried out in accordance with the relevant laws and institutional guidelines and that these procedures have been approved by the appropriate administrative committee and that the approval of the Ethics Committee is included in the study. In studies for which Ethics Committee approval is mandatory, the name of the institution for which the ethics committee approval was obtained and the ethics committee approval number should be specified in the materials and methods section. It should also be included in the materials and methods section of the article that the protocols and procedures used are ethically reviewed and approved. For detailed information, please visit http://journal.pharmacy.ankara.edu.tr/en/ethical-principles-and-publication-policy/ web page.

- **RESULT AND DISCUSSION:** This is the section where findings are given and evaluated.
  - If the author wishes, "Conclusion" can be added as the last paragraph of the RESULT AND DISCUSSION section. The font size must be 11 pt with 1 line spacing and “Times New Roman” font and the first letter must be uppercase and the other letters must be lowercase.

- **ACKNOWLEDGMENTS**: If any, the organization supporting the research and the people who contributed can be acknowledged briefly in this section prior to the Authors' Contribution.

- **AUTHOR CONTRIBUTIONS:** Contribution of the authors in the article should be written just before the conflict of interest notification, in accordance with the *Copyright Transfer Agreement* signed by the authors. Please use the initials of the authors for this notice instead of the full name and surname as in the example below. If there is not any author contribution for the specified sections, “-” should be added. Please see below example for writing author contributions.

  Example:

  AUTHOR CONTRIBUTIONS

  Concept: İ.Y., M.M.H., C.H., K.B.; Design: İ.Y., C.H., I.Ö.G., Ö.Ü.; Control: C.H., I.Ö.G., M.M.H., K.B.; Sources: Ö.Ü., Z.K., K.B., M.M.H., A.K., İ.A., G.A.G., B.G., B.K.; Materials: I.Ö.G., B.E., G.A.G., B.K., D.Ç.P.; Data Collection and/or Processing: A.K., Ö.Ü., M.K., A.S., D.Ç.P., T.C.Ş.T.; Analysis and/or Interpretation: Ö.Ü., B.G., T.C.Ş.T., E.K.S.; Literature Review: B.K., D.Ç.P, B.G., B.E.; Manuscript Writing: A.K., İ.A., T.C.Ş.T.; Critical Review: İ.Y., B.G., Ö.Ü., İ.A.; Other: -

- **CONFLICT OF INTEREST**

  If there is a conflict of interest, it should be clearly declared in what form it is. If not, "The authors declare that there is no real, potential, or perceived conflict of interest for this article." They should use the expression.

- **ETHICS COMMITTEE APPROVAL**

  If the ethics committee approval is obtained before the sources at the end of the study, the approval number must be specified from which institution and when it was obtained. Approval from the ethics committee should be uploaded during the manuscript submission. In studies that do not require ethics committee approval, the following sentence should be written.

  "The authors declare that the ethics committee approval is not required for this study".

- **REFERENCES:** Bibliography style is according to the American Psychological Association (APA). Typeface "Times New Roman" and 10 font size, "1" spacing, justified. In the text, it should be numbered in square brackets according to the order of appearance, such as: [1,6,9], [5-7] and listed according to these numbers at the end of the text. Reference should not be given next to the subtitles. If it is necessary to provide a source in the table, it should be specified as given in the text. References should be written in accordance with the examples below.

  - **For the article:** Author's surname, the initials of the name (There should be a period after the initial letter of each name of the author with more than one name, and there should not be a space in between. **There <u>should not be</u> “and” between the last author and the previous author. The “&” symbol <u>should not be</u> used**. The full title of the article should be written as the name of the journal, volume number, if available, the number (in parentheses), the beginning and ending page number (or article id), the year after the author names (in parentheses). **If there is more than one author, all of them should be written**. While writing the name of the article, the first letter of the first word should be capitalized

and the first letter of the other words should be written in lowercase. Journal names given in references should be written clearly without abbreviation.
Add the [CrossRef] **sign at the end of each reference and enter the DOI number as a hyperlink with the right click in the format below. Please do not add CrossRef hyperlink if the article is not listed at https://www.crossref.org/.**
https://doi.org/10.1016/0006-2952(89)90403-6

Examples:

1. Martinez, M.J.A., Del Olmo, L.M.B., Benito, P.B. (2005). Antiviral activities of polysaccharides from natural sources. Studies in Natural Products Chemistry, 30, 393-418. [CrossRef]
2. Bahiense, J.B., Marques, F.M., Figueira, M.M., Vargasa, T.S., Kondratyuk, T.P., Endringer, D.C., Scherer, R., Fronzaa, M. (2017). Potential anti-inflammatory, antioxidant and antimicrobial activities of *Sambucus australis*. Pharmaceutical Biology, 55(1), 991-997. [CrossRef]

- **Online articles:**

Example:
Perneger, T.V., Giner, F. (1998). Randomized trial of heroin maintenance programme for adults who fail in convential drug treatments. British Medical Journal, 317. Retrieved August 12, 2005, from ttp://www.bmj.com/cgi/content/full/317/7150/

- **Web sites:**

Example:

Clinical Pharmacology Web site. (2001). Retrieved June 16, 2004, from http://cpip.gsm.com/. Accessed date: 14.03.2021.

- **Books:** The surname of the author, the initials of the name, the name of the book, volume number (if any), the bookstore, the city where it was published, the page number, the year it was published (in parentheses) should be written.

Example:

Franke, R. (1984). Theoretical Drug Design Methods, Elsevier, Amsterdam, p.130.

- **Book chapters:** Author's surname, initials of the name, the title of the section, the editor / editors' surname, the initials of the name, the phrase (Ed./Eds.), The title of the book, if any, the book house, the city where it was published, the page number, the year it was published (in parentheses) should be written.

Example:

Weinberg, E.D. (1979). Antifungal Agents. In: M.E. Wolff and S.E. Smith (Eds.), Burger's Medicinal Chemistry, (pp. 531-537). New York: John Wiley and Sons.

- **For the thesis**: The surname of the author, the initials of the name, the year should be written (in parentheses) after the author's names and a full stop. After specifying the type of thesis, the title of the thesis and where it was made should be written.

Example:

Ahmed, J. (2008). PhD Thesis. Pharmaceutical Botany investigations on Prangos Lindl. (Umbelliferae) growing in Konya province. Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, Ankara, Turkey.

- **For patent:** The surname of the author, the initials of the name, the year should be written (in parentheses) after the author's names and a full stop. The title and number of the patent should

be indicated.

Example:

Mahoney, S., Molz, L., Narayan, S., Saiah, E. (2018). Heteroaryl RHEB Inhibitors and Uses Thereof. WO 2018/191146 A1.

ETHICAL PRINCIPLES AND PUBLICATION POLICY

Journal of Faculty of Pharmacy of Ankara University is an open-access, peer-reviewed journal and a publishing medium for original research, reviews and short communications covering important developments in the field of pharmaceutical sciences in Turkish or English. Journal of Faculty of Pharmacy of Ankara University does not have an article publication fee (APC) or subscription fee.

As the editorial board, it is aimed to publish high-quality new studies that make a significant contribution to the scope of the journal. To achieve this goal, articles submitted are subject to initial evaluation by the editor-in-chief and/or assistant editors to ensure that they meet the scientific and formal criteria to be published in the journal. Only studies that pass this preliminary evaluation process are continued to other stages for further evaluation.

Preliminary Assessment

- The scientific quality and novelty of the study must be sufficient to be published in the journal.
- Studies submitted to the journal must comply with the purpose and scope of the journal.
- The text must be written in English or Turkish, grammatically and scientifically well-written.
- The similarity rate of studies submitted to the journal should not exceed 20%.
- Studies should be arranged in accordance with the journal's writing rules and template.
- Copyright transfer form, ethics committee approval document and author contribution form must be uploaded and signed.
- Studies must be sent to the journal via the electronic online application system.

The further evaluation process of studies that do not meet these qualifications cannot be initiated.

Journal editors, reviewers and authors have certain responsibilities during the journal publication process. These responsibilities are explained below.

1. Editor's Duties and Ethical Responsibilities

The editor can independently decide which of the articles sent to the journal should be published, or can also consult with members of the editorial board or reviewers. Within the framework of the journal's ethical principles and publication policy, it is responsible for carrying out the preliminary evaluation, peer review and publication stages of the studies in an impartial, auditable, fair, independent of conflict of interest and in accordance with confidentiality principles. If there is no violation in terms of publication policy and ethical principles, studies that comply with the purpose and scope of the journal should be taken to the preliminary evaluation stage.

The duties and descriptions of the editor-in-chief, associate editors, section editors and editorial advisory board are as follows:

Editor-in-Chief: Editor in chief has full authority over the publication of the journal content. Editor in chief works with Associate Editors, Section Editors and the Editorial Advisory Board.
Associate Editors: Associate Editors are primarily responsible for answering questions about the journal, making suggestions to the journal reviewers and board, and assisting the Editor-in-Chief during the article publication process.
Section Editors: Section Editors assist authors in assigning double-blind referees and answering questions about the journal.
Editorial Advisory Board: The Editorial Advisory Board guides the Editor-in-Chief and Associate Editors on issues related to the production of quality publications that are appropriate for the purpose of Journal of Faculty of Pharmacy of Ankara University.

1.1. Publication Policy

- The editor-in-chief is solely responsible for deciding which articles sent to the journal should be published. The editor's decision may be in line with the principles of the journal's editorial

board or is limited by applicable legal requirements regarding issues such as defamatory publication, copyright infringement and plagiarism.

- The editor-in-chief ensures that the authors fill out the "Copyright Transfer Form" and send the copyright of the article to the publisher before the article is published.
- The editor-in-chief ensures that the authors fill out the "Conflict of Interest Form" and the "Author Contribution Form" before the article is published.
- The editor-in-chief assigns associate editors to formally review the articles sent to the journal. Articles that do not comply with the rules of the Journal of Faculty of Pharmacy of Ankara University are rejected without being evaluated.

1.2. Publication Review

- The editor-in-chief is responsible for ensuring that the publication evaluation process is fair, impartial and timely.
- The editor generally ensures that all articles are evaluated by at least two external and independent reviewers. If necessary, the editor requests additional opinion from a third reviewer.
- The editor selects the reviewers by evaluating experts who are suitable for the scope of the article.
- The editor carefully reviews the publication process to evaluate and decide on disclosures made for possible conflicts of interest, “self-citation” suggestions made by reviewers, and any possibility of bias.
- The editor-in-chief/other editors requests for the article to be scanned by a similarity detection software (iThenticate) at any point during the peer review or evaluation/publication process, or they do it themselves. In this sense, even if the expressions or sentences are the author(s) themselves, the text should not have an unacceptable similarity to previously published data.
- If the editor-in-chief detects errors in an article before it is published, he/she corrects them. If he/she detects it later, then he/she has to publish the corrections. All corrections or retraction notices must be prominently published in the journal. It should also be listed on the contents page.
- Journal of Faculty of Pharmacy of Ankara University’s editors follow the "COPE Code of Conduct and Best Practice Guidelines for Journal Editors" and "COPE Best Practice Guidelines for Journal Editors" guidelines published by the Committee on Publication Ethics (COPE).

1.3. Fair Evaluation

- Editor-in-Chief/other editors evaluates articles according to their scientific content, regardless of the authors' race, gender, sexual orientation, belief, ethnicity, citizenship or political views. The journal’s editorial principles support transparent and completely honest review.
- The editor must ensure that reviewers and authors fully understand what is expected of them.
- The editor makes all his communication regarding the journal through the journal’s electronic application system and follows a transparent and fair manner in case of objections to his decisions.

1.4. Privacy Policy

- The chief editor/other editors is obliged to maintain the confidentiality of all materials in the application to the journal and all communication with the reviewers (unless otherwise approved by the relevant authors and reviewers).
- The chief editor/other editors is responsible for protecting the identities and rights of the reviewers, unless the reviewers agree to their names being disclosed.
- Unpublished materials belonging to a submitted article should not be used for the editor's own studies/research without the written consent of the author.
- The chief editor/other editors must keep all information or ideas obtained during the article evaluation process confidential and should not use them for personal purposes.

2. Duties and Ethical Responsibilities of Referees

The article evaluation process of Journal of Faculty of Pharmacy of Ankara University is carried out on the principle of double-blind review. Therefore, reviewer cannot communicate with the author(s), evaluations are shared through the Dergipark management system. During the evaluation process, evaluation forms and reviewers' comments regarding the manuscripts are forwarded to the corresponding author through the editor. Reviewers must act in accordance with the principles of impartiality, confidentiality, objectivity and scientific review throughout the evaluation process. They must be an expert and competent in the relevant field. They must complete their report on the work submitted for evaluation within the specified time period. For reports that cannot be submitted on time, the editor should be contacted without delay. The editorial board should be informed in cases of copyright and/or ethical infringement, possible conflict of interest and plagiarism.

Reviewers who evaluate the manuscript of the Journal of Faculty of Pharmacy of Ankara University are expected to comply with the stated duties and ethical responsibilities describe below:

2.1. Contribution to Editorial Decisions

- Reviewers must evaluate the work submitted by authors constructively and appropriately.
- If the reviewers think that they are not competent to evaluate the research in the article or cannot complete it in sufficient time, they must notify the editor.
- Reviewers should not make harsh and personal criticisms towards the authors.
- When reviewers receive an invitation to evaluate an article, they should refuse to evaluate the article if they feel inadequate about the subject studied in the article.
- Reviewers must evaluate the article within the given time.
- Reviewers should only objectively evaluate the content of the study.

2.2. Privacy

- Reviewers must make the evaluation impartially and confidentially.
- Reviewers should not share their evaluations or information about the article with third parties.
- Reviewers must keep confidential the information, ideas and unpublished materials or studies obtained during the article evaluation process and must not use them for personal purposes.
- Reviewers should not retain or reproduce a copy of the article.

2.3. Detecting Ethical Issues

- Reviewers should notice the ethical problems in the article and bring them to the attention of the editor.
- If the reviewers detect that the article has been previously published elsewhere or that there is a significant similarity or overlap with a previously published article, they must notify the editor. Any previously published observations and/or arguments should be accompanied by the relevant reference.

2.4. Impartiality and Competition Standards

- Reviewers must make their evaluations impartially and free from bias. It is not appropriate to criticize the author as a person. Reviewers must express their opinions with supporting arguments.
- Reviewers must check for possible conflict of interest before agreeing to evaluate the article. If they feel they face a conflict of interest, they should refuse to review the manuscript and inform the editor.
- If reviewers claim that the reviewers' (or people working with the reviewers) work has been taken as a source by the author, they must provide real scientific justifications and be careful that this is not an attempt to increase the reviewers' number of references or the visibility of their work.

- Reviewers should not stay away from scientific reality when making their evaluations and should resort to citing sources if necessary.

3. Authors' Duties and Ethical Responsibilities

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