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ORIGINAL ARTICLE / ÖZGÜN MAKALE

SECONDARY METABOLITES OF ENDEMIC *CENTAUREA APHRODISEA* BOISS.

ENDEMİK CENTAUREA APHRODISEA BOISS.'İN SEKONDER METABOLİTLERİ

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ABSTRACT

Objective: *This study aimed to isolate secondary metabolites from the aerial parts of endemic Centaurea aphrodisea Boiss. using several chromatographic methods and elucidate the structure of the compounds by using spectroscopic methods.*
Material and Method: *Aerial parts of the endemic C. aphrodisea were collected from Bozdağ (Ödemiş, İzmir) and n-hexane, chloroform and metanol extracts were prepared. The chloroform extract was investigated by using various chromatographic methods, and the structures of the isolated compounds were determined using spectroscopic methods (1D-2D NMR and LC-MS).*
Result and Discussion: *One elemane type sesquiterpene (methyl 8α,6α,15-trihydroxyelema-1,3,11(13)-trien-12-oate) and four flavone derivatives (cirsimaritin, 3'-O-methyl eupatorin, eupatorin and salvigenin) were isolated and identified. In addition, the presence of a phenylpropanoid glycoside (syringin) was determined in a fraction by comparison with a reference compound using TLC technique. These compounds are reported for the first time from C. aphrodisea with this study.*
Keywords: *Centaurea aphrodisea, elemane, flavone, secondary metabolite, sesquiterpene*

ÖZ

Amaç: *Bu çalışmada endemik Centaurea aphrodisea Boiss. bitkisinin toprak üstü kısımlarında bulunan sekonder metabolitlerin, kromatografik yöntemlerle saflaştırılması ve spektroskopik yöntemlerlede yapılarının aydınlatılması amaçlanmıştır.*
Gereç ve Yöntem: *Bu çalışmada, endemik C. aphrodisea'nın toprak üstü kısımları Bozdağ'dan (Ödemiş, İzmir) toplanmış ve n-hekzan, kloroform ve metanol ekstreleri hazırlanmıştır. Kloroform ekstresi çeşitli kromatografik yöntemler kullanılarak incelenmiş ve izole edilen bileşiklerin yapıları spektroskopik yöntemler (1D-2D NMR ve LC-MS) kullanılarak aydınlatılmıştır.*
Sonuç ve Tartışma: *Bir eleman tip seskiterpen (metil 8α,6α,15-trihidroksielema-1,3,11(13)-trien-12-oat) ve dört flavon türevi (sirsimaritin, 3'-O-metil öpatorin, öpatorin ve salvigenin) izole edilerek yapıları aydınlatılmıştır. Ayrıca İTK tekniği ve şahit bileşikler kullanılarak bir fraksiyonda fenilpropanoit glikozitinin (siringin) varlığı saptanmıştır. Bu bileşikler C. aphrodisea'dan tarafımızca ilk kez rapor edilmektedir.*
Anahtar Kelimeler: *Centaurea aphrodisea, elemane, flavon, sekonder metabolit, seskiterpen*

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INTRODUCTION

The genus *Centaurea* L. is the fourth largest genus in the Asteraceae family, and there are approximately 700 species in the world [1]. The majority of the species are distributed in Western Asia and the Mediterranean Region. In the flora of Turkey, the *Centaurea* genus is represented by 213 taxa, 125 of which are endemic [2].

Various species of *Centaurea* are used as herbal remedies for stomach upset, abdominal pain, expectorant and antipyretic in common colds and against inflammatory conditions, such as abscesses in Anatolian traditional medicine [3,4]. Bioactivity studies generally revealed that *Centaurea* species have antiinflammatory, cytotoxic, antioxidant, antimicrobial and antiulserogenic properties [5].

Previous phytochemical and bioactivity studies showed that the pharmacological effect of *Centaurea* species are generally related to sesquiterpene lactones (germacranolide, eudesmanolide, elemanolide and guaianolide type) and flavonoids as their main secondary metabolites [6,7].

In a previous study, methanol (MeOH) extract of *C. ensiformis* was reported to have strong antioxidant activity with 86.2% FRSA (Free radical scavenging activity) and the total phenolic content of the extract was determined as 59.33 ± 1.76 GAE (Gallic acid equivalent) mg/l. 11 phenolic compounds were isolated from the MeOH extract, and strong DPPH radical scavenging activities were observed with protocatechuic acid (IC_{50}:6.47 μM), tacioside (IC_{50}:22.87 μM) and dihydrodehydrodiconiferyl alcohol 4-*O*-β-glucopyranoside (IC_{50}:27.7 μM) [8,9].

In another study, three guaianolide type sesquiterpene derivatives (13-acetyl solstitialin A, solstitialin A and chlorojanerin) were isolated from the chloroform ($CHCl_3$) extract of *C. solstitialis* ssp. *solstitialis*. Chlorojanerin (59.2 mg/kg), and a mixture of 13-acetyl solstitialin A (95%) and solstitialin A (5%) (179 mg/kg) were reported to exhibit antiulcerogenic activities in various ulcer models in rats and mice [10].

Demiröz et al., evaluated the *in-vivo* anti-inflammatory activities of *C. calolepis* $CHCl_3$ extract and cnicin (a germacrenolide type sesquiterpene), against edema induced by *Macrovipera lebetina obtusa* and *Montivipera xanthina* venoms in a rat model. The $CHCl_3$ extract demonstrated strong inhibition on edema at all doses and hours against both venoms. Cnicin (27.31% edema increase at 2.5 mg/kg) was more effective than the extract (36.43% edema increase at 25 mg/kg) against rat paw edema induced by *M. lebetina obtusa* venom [11].

C. aphrodisea Boiss. is one of the endemic species naturally distributed in Western and South-Western parts of Anatolia including Aydın, Denizli and Izmir. It is a perennial plant, with rose-purple florets and tomentose leaves. It differs from the other *Centaurea* species with large appendages with distinct hyaline border ending with a terminal mucro, and light brown central parts [12].

In our previous study, composition of the essential oil of *C. aphrodisea* was investigated, and the major metabolites were detected as spathulenol (8.1%), hexahydrofarnesyl acetone (7.8%), tridecanal (5.4%), and heptacosane (4.5%) [13]. *In vitro* cytotoxic and anti-inflammatory activity of different extracts of the plant were also reported. Among the tested extracts, chloroform extract exhibited cytotoxic activity against SK-MEL (malignant melanoma), KB (oral epidermal carcinoma), BT-549 (breast ductal carcinoma), SK-OV-3 (ovarian carcinoma) cancer cell lines. It was also found that chloroform extract showed anti-inflammatory activity by inhibiting NF-κB (Nuclear factor kappa B) and iNOS (inducible nitric oxide synthase) activation (IC_{50}:21 μg/ml and 23 μg/ml, respectively) [5].

In our continuous search on *Centaurea* species, we aimed to isolate the secondary metabolites of the chloroform extract of endemic *C. aphrodisea* by chromatographic techniques and to elucidate their structures by spectroscopic methods.

MATERIAL AND METHOD

General Experimental Procedures

Column chromatography was carried out on silica gel, RP-C_{18} (Merck), and Sephadex LH-20 (GE Healthcare) using analytical grade purity solvents [*n*-hexane, ethylacetate (EtOAc), chloroform ($CHCl_3$), methanol (MeOH) and acetonitrile (ACN); Merck]. Isolation procedure was monitored by thin layer chromatography (TLC, Silica gel 60 F_{254} plates, Merck), and for detection of the metabolites UV

light (254, 366 nm) and vanillin/H_2SO_4 (V/S) reagent were used. 1D and 2D NMR spectra were recorded on Varian Oxford AS-400 spectrometer, and Thermo-Scientific TSQ Quantum Access Max LC-MS/MS was used for mass analysis.

Plant Material

C. aphrodisea was collected from İzmir-Ödemiş-Bozdağ (1700 m) during the flowering period (June 2010) (38019'06.10"N 28004'53.63"E). Prof. Dr. Serdar Gokhan Senol (Section of Botany, Department of Biology, Faculty of Science, Ege University) confirmed the plant material and a voucher specimen was recorded in the IZEF Herbarium of Ege University (IZEF-5915).

Extraction and Isolation

Aerial parts of *C. aphrodisea* (1.5 kg) were dried and finely crushed. The plant material was extracted sequentially with *n*-hexane, $CHCl_3$ and MeOH (3 x 2l, 24 h each) with ultrasonic water bath. The extracts were filtered and separately concentrated to dryness with an evaporator at 40°C and yielded 14.65 g *n*-hexane, 20.68 g $CHCl_3$ and 73.04 g MeOH extracts.

$CHCl_3$ extract (17.8 g) was chromatographed over RP-C_{18} column (400 g) using H_2O:MeOH mixtures (80:20 to 0:100; 10%, 1l each) and 12 main fractions were obtained according to TLC profiles. Three main fractions (A1: Fr.13-18; A2: Fr. 23-28, and A3: Fr.5-9) were selected for further purification steps.

Fr. A1 (2.2 g) was chromatographed using silica-gel column (30 g) and eluted with EtOAc:MeOH:H_2O (100:5:1, isocratic, 1l) and afforded 38 subfractions. Fr.A1/25-36 (402 mg) was chromatographed using silica-gel (15 g) and $CHCl_3$:MeOH:H_2O (90:10:0.5, isocratic, 500 ml), and subfractions 25-26 were combined to afford compound **1** (73 mg).

Fr.A2 (2.5 g) was fractionated using sephadex LH-20 column (75 g) with MeOH, and Fr.A2/30-56 (256 mg) was chromatographed over silica-gel (15 g) ($CHCl_3$:MeOH; 100:0 and 98:2, 200 ml each). Subfraction 1-10 (184 mg) was chromatographed using silica-gel (20 g) with $CHCl_3$:MeOH (100:0, 99:1 and 98:2, 300 ml each) and afforded 144 subfractions. Subfractions 45-81 were combined to yield compound **4** (64 mg).

Fr.A2/30-56/1-10/116-end (22 mg) was chromatographed using RP-C_{18} (10 g) with an elution system of H_2O:ACN (65:35 and 60:40, 100 ml each). Compound **2** (9 mg) was isolated from subfractions 10-33 (19 mg) using silica-gel (7 g) and $CHCl_3$ (100%, 100 ml) as mobile phase.

Fr.A2/30-56/1-10/26-44 (49 mg) was fractionated using RP-C_{18} (20 g) column and H_2O:ACN mixtures (70:30 to 55:45, 5% decreasing polarity, 200 ml each) and afforded compounds **3** (12 mg) and **5** (4 mg).

Fr. A3 (1.5 g) was chromatographed over sephadex column (60 g) using MeOH. Among the 51 subfractions, Fr.A3/13-19 (600 mg) was chromatographed over silica-gel (50 g) using EtOAc:MeOH mixtures (100:0 to 50:50, 10%, 300 ml each) and the presence of compound **6** was detected in Fr.A3/13-19/9 (51 mg) by TLC comparison with an authenthic sample of syringin, which was previously isolated from *C. polyclada* (Figure 1) [11].

RESULT AND DISCUSSION

Previous phytochemical studies on *Centaurea* species have generally resulted in the isolation of sesquiterpene lactones (germacranolide, eudesmanolide, elemanolide and guaianolide type) and flavonoids as main metabolites.

In this study, $CHCl_3$ extract of *C. aphrodisea* was evaluated with a series of column chromatographic separation steps. Isolation studies yielded one elemane type sesquiterpene; methyl 6α,8α,15-trihydroxyelema-1,3,11(13)-trien-12-oate (**1**) [14,15], and four methoxyflavone derivatives; cirsimaritin (**2**), 3'-methoxy eupatorin (**3**), eupatorin (**4**), and salvigenin (**5**) [16], in accordance with previous studies. The structures of the isolated compounds were determined by comparing their NMR data with those of previously reported metabolites. ^{1}H and ^{13}C NMR data of compounds **2-5** are presented in Tables 1 and 2. Additionally, the presence of a phenylpropanoid glucoside; syringin (**6**)

was detected in a fraction by TLC comparison with an authenthic sample, which was previously isolated from *C. polyclada* (Figure 1) [15]. Structures of the compounds **1-6** are given in Figure 2.

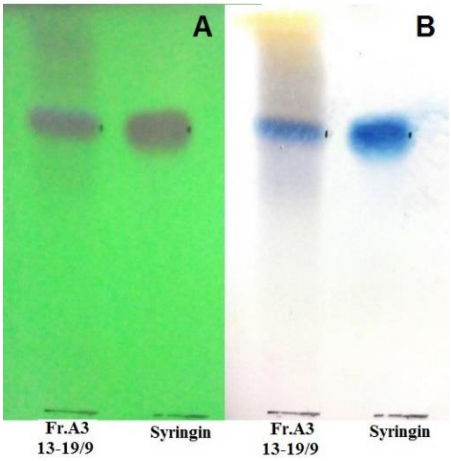

Figure 1. TLC comparison of syringin and Fr.A3/13-19/9. Silica gel 60 F_{254} TLC plate, $CHCl_3$:MeOH:H_2O-70:30:3, A: TLC plate under UV-254 nm, B: TLC plate sprayed with V/S reagent

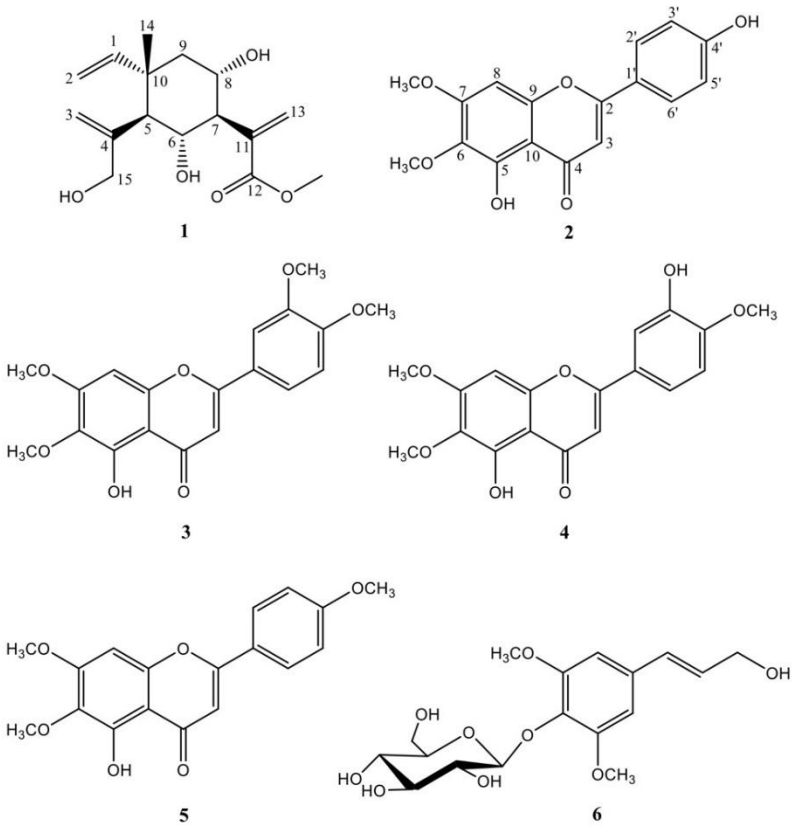

Figure 2. Structures of compounds **1-6**

Methyl 6α, 8α,15-trihydroxyelema-1,3,11(13)-trien-12-oate (**1**): ^{1}H NMR (400 MHz, CD_3OD), δ 5.75 (dd, *J* = 18/10 Hz, H-1), 4.93 (d, *J* = 10.4 Hz, H2a), 4.92 (d, *J* = 18 Hz, H2b), 5.33 (brs, H3a), 4.93 (brs, H3b), 1.85 (d, *J* = 10.8 Hz, H5), 4.12 (t, *J* = 10.4 Hz, H6), 2.31 (t, *J* = 10.4 Hz, H7), 4.10 (td, *J* = 10.8/4.4 Hz, H8), 1.73 (dd, *J* = 12.4/4.4 Hz, H9a), 1.49 (dd, *J* = 12.4/12 Hz, H9b), 6.34 (d, *J* = 1.6Hz,

H13a), 5.74 (d, *J* = 1.6 Hz, H13b), 1.12 (s, 3H, H14), 3.99 (d, *J* = 14.8 Hz, H15a), 3.89 (d, *J* = 14.8 Hz, H15b) and 3.75 (s, 3H, -OCH_3); LC-MS (ESI), *m/z*= 297.13 $[M+H]^+$ and 319.12 $[M+Na]^+$

Cirsimaritin (**2**): ^{1}H NMR and ^{13}C NMR data: see Tables 1 and 2, LC-MS (ESI), *m/z*= 315.02 $[M+H]^+$ and *m/z*= 313.13 $[M-H]^-$

3'-methoxy eupatorin (**3**): ^{1}H NMR and ^{13}C NMR data: Tables 1 and 2, LC-MS (ESI), *m/z*= 359.06 $[M+H]^+$

Eupatorin (**4**): ^{1}H NMR and ^{13}C NMR data: Tables 1 and 2, LC-MS (ESI), *m/z*= 345.08 $[M+H]^+$

Salvigenin (**5**): ^{1}H NMR and ^{13}C NMR data: Tables 1 and 2, LC-MS (ESI), *m/z*= 329.08 $[M+H]^+$

Table 1. ^{1}H NMR data of compounds **2-5** [400 MHz, DMSO-d_6, δ_H (*J* in Hz)]

Position	2	3	4	5*
3	6.81, s	6.80, s	6.71, s	6.50, s
8	6.92, s	6.91, s	6.79, s	6.48, s
2'	7.94, dd (8.8)	7.55, d (1.6)	7.41, d (2.4)	7.76, d (8.8)
3'	6.90, d (8.4)	-	-	6.95, d (8.8)
5'	6.90, d (8.4)	7.11, d (8.8)	7.02, d (8.4)	6.95, d (8.8)
6'	7.94, dd (8.8)	7.67, dd (8.8/1.6)	7.48, dd (8.4/2.4)	7.76, d (8.8)
6-OCH_3	3.73, s	3.72, s	3.71, s	3.89, s
7-OCH_3	3.92, s	3.88, s	3.88, s	3.93, s
3'-OCH_3	-	3.91, s	-	-
4'-OCH_3	-	3.85, s	3.84, s	3.85, s

* in $CDCl_3$

Table 2. ^{13}C NMR data of compounds **2-5** (100 MHz)

Position	2[a]	3[b]	4[a]	5[b]
2	164.2	163.9	164.2	163.9
3	102.3	104.4	103.7	103.9
4	182.1	182.6	182.5	182.5
5	152.0	153.2	152.5	153.1
6	131.8	132.7	132.3	132.6
7	158.5	158.7	159.0	158.7
8	91.5	90.6	91.8	90.5
9	152.6	153.0	153.0	153.0
10	105.0	106.1	105.5	106.0
1'	120.3	123.8	123.3	123.4
2'	128.5	108.9	113.5	127.9
3'	116.1	149.4	147.2	114.4
4'	162.2	152.3	151.6	162.6
5'	116.1	111.2	112.4	114.4
6'	128.5	120.1	119.1	127.9
6-OCH_3	60.0	60.8	60.4	60.8
7-OCH_3	56.4	56.1	56.8	56.2
3'-OCH_3	-	56.1	-	-
4'-OCH_3	-	56.3	56.2	55.5

[a] in DMSO-d_6
[b] in $CDCl_3$

In our previous study, chloroform extract exhibited cytotoxic activity against SK-MEL (IC_{50}=65 µg/ml), KB (IC_{50}=60 µg/ml), BT-549 (IC_{50}=86 µg/ml), SK-OV-3 (IC_{50}=60 µg/ml) cell lines, and non-cancerous LLC-PK1 cells (kidney epithelial cells) (IC_{50}=58 µg/ml) [5]. The elemane derivative, compound **1,** was previously reported from *Centaurea aspera* var. *subinermis* [14], *C. polyclada* [15],

Onopordum acaulon [17], and *O. cynarocephalum* [18], and was reported to exhibit cytotoxic activity against A375 (human melanoma) cell line (IC_{50}: 9.2 µM) [18]. Among the methoxyflavone derivatives, cirsimaritin was reported to have cytotoxic activity against HL-60 (human leukemia), COLO-205 (human colon carcinoma), MCF-7 (human breast adenocarcinoma), and NCI-H520 (lung squamous carcinoma) cell lines (IC_{50}: 61 and 13.1, 59.86, and 23.29 µM respectively) [19-21]. *In vivo* studies have also shown that, salvigenin exhibited antitumor effects in drug-resistant HCC (hepatocellular carcinoma) cell lines by increasing cell sensitivity and decreasing the IC_{50} of 5-fuorouracil, and suppressing MCF-7 tumor growth [22,23].

Best to our knowledge, all compounds are reported for the first time from Turkish endemic *C. aphrodisea*. When the molecules obtained during this phytochemical study and the literature data are evaluated together, it may be thought that the isolated molecules may be responsible for the cytotoxic activity of the plant or may contribute to this activity.

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AUTHOR CONTRIBUTIONS

Concept: F.G., C.K.; Design: F.G., C.K.; Control: S.D., C.K.; Sources: F.G., C.K.; Materials: F.G., S.D., S.B.O., C.K.; Data Collection and/or Processing: F.G., S.D., C.K.; Analysis and/or Interpretation: F.G., S.D., S.B.O., C.K.; Literature Review: F.G., S.D., S.B.O., C.K.; Manuscript Writing: F.G., S.D., S.B.O., C.K.; Critical Review: S.B.O., C.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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ORIGINAL ARTICLE / ÖZGÜN MAKALE

A CONVERSATION ANALYTIC INVESTIGATION ON EXPERTISE DEMONSTRATION IN COMMUNITY PHARMACY INTERACTION

TOPLUM ECZANESİNDEKİ ETKİLEŞİMDE KONUŞMA ÇÖZÜMLEMESİ YÖNTEMİ İLE UZMANLIK BİLGİSİ GÖSTERİMİ

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ABSTRACT

Objective: *In recent years, there have been incresing numbers of the studies based on pharmacist communication skills in community pharmacy. However, different expertise knowledge provision within community pharmacy interaction is an under-researched area. This article aims to investigate how the expertise demonstration is deployed in a community pharmacy interaction.*
Material and Method: *30-hour audio and video recordings were collected in a community pharmacy in Türkiye, and analysed through data-driven and bottom-up research perspective of Conversation Analysis.*
Result and Discussion: *The findings of the study show that the pharmacist displayed his expertise knowledge within three different sequential organizations initiated by the patient, pharmacy staff and pharmacist through a wide range of interactional practices such as providing extended explanations and advices, referring to external authority, and using both professional and trade knowledge. This micro-analytic study in pharmacy interaction makes an important contribution to pharmacy services and education.*
Keywords: *Conversation analysis, display of expertise knowledge, pharmacy interaction*

ÖZ

Amaç: *Son yıllarda, toplum eczacılığında eczacıların iletişim becerilerini temel alan çalışmaların sayısı giderek artmaktadır. Ancak, toplum eczanesndeki etkileşimde farklı uzmanlık bilgisi sağlama uygulamaları yeterince araştırılmamış bir alandır. Bu makale, bir toplum eczanesindeki etkileşimde uzmanlık gösteriminin nasıl kullanıldığını araştırmayı amaçlamaktadır.*
Gereç ve Yöntem: *Türkiye'deki bir eczanede 30 saatlik ses ve video kayıtları toplanmış ve Konuşma Çözümlemesi'nin veri güdümlü ve tabandan yukarı işlemlemeye dayalı araştırma bakış açısı ile analiz edilmiştir.*
Sonuç ve Tartışma: *Çalışmanın bulguları, eczacının uzmanlık bilgisini hasta, eczane personeli ve eczacı tarafından başlatılan üç farklı sıralı organizasyon içinde, genişletilmiş açıklamalar ve tavsiyeler sunma, dış otoriteye atıfta bulunma ve hem mesleki hem de ticari bilgiyi kullanma gibi çok çeşitli etkileşimsel uygulamalar yoluyla sergilediğini göstermektedir. Eczane etkileşimi üzerine yapılan bu mikro-analitik çalışma, eczacılık hizmetleri ve eğitimine önemli bir katkı sağlamaktadır.*
Anahtar Kelimeler: *Eczane etkileşimi, konuşma çözümlemesi, uzmanlık bilgisinin gösterimi*

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INTRODUCTION

Communication in Pharmacy

The communication role, which is one of the nine-star pharmacist concepts, has a very important place in the communication of pharmacists with patients and other healthcare professionals [1]. It is known that using effective communication skills can improve patient care outcomes, achieve the desired patient satisfaction, and improve the results of medication and treatment [2,3]. In this sense, effective, motivating, and targeted communication is one of the most important tools in medicine counselling to reach institutional goals [4].

In the interactive field of counselling, Heritage and Sefi's study of health visitor interactions with new mothers may be the first document to identify and define the context of counseling and counselling practices. In this study, counseling is identified as “describing, recommending, or otherwise informing a health visitor about a preferred course of action” [5]. “Effective counseling” on the zone of the pharmacist involves effective knowledge and know-how relationships, counseling process and content. First of all, the pharmacist needs to have “current knowledge of pharmacotherapy” as well as the capability to “give effective and accurate patient education and counseling” [6]. The purpose of the consultation is to provide patients with the tools to use all medicines more safely and effectively. This can improve the perception of pharmacists as the “first point of contact” for medicines and health recommendations [7]. Pharmacists’ expertise plays a crucial role in the patients’ decision-making and pharmacy interaction [8]. In addition, expertise is not easily observed, but analysts need to learn about the relationship between participants' actions and "responsible expertise" in a given field, or how recipients perceive actions as expertise. In this sense, 'becoming an expert' and ‘demonstrating expert knowledge’ are interactive presentations for task accomplishment in pharmacy interaction [9].

Conversation Analysis in Pharmacy Interaction

Conversation Analysis (CA) is performed as a persuasive system of discipline and inquiry to obtain precise details in social interaction and to analyse what the actors of social interaction do and what they achieve in talk-in-interaction [10]. The existing CA literature on the professionals’ practices shows how they transfer general knowledge in a contextual manner with various discursive functions. For example, physicians can initiate questions and interruptions to align a patient's personal plan with the facility's plan [11] and may strategically use invitations to present perspective to perform advanced diagnostics in a non-confrontational manner [12] while healthcare seekers may raise issues to provide context for giving advice [5]. Over the past decade, there has been increasing number of the studies focusing on how practitioners and patients/clients construct their expertise through dialogue [13-16]. In these studies, the pharmacists are regarded as information providers and experts while the patients are seen as information receivers and laypeople as well as other interactional features including action sequencing, turn-taking and content administration. In addition, the interactants (i.e., pharmacists and patients) continuously position the pharmacist as an expert and the patients as a layperson through different sequential actions in patient consulting conversations, including medicine information, access to medicine names, counseling, asking questions, and showing current directions [6].

Knowledge Construction

Knowledge is a collective category that includes expert knowledge, specialized news, and private information, as well as interactive aspects of understanding, perception, awareness, cognition, evaluation, and experience [17]. While institutional collaboration is inherently asymmetric because professionals have expertise and experience about the problems around which the service is focused, the provision of recommendations is an activity in which these asymmetries are most apparent [18]. Participants display and/or claim knowledge while initiating sequences or orienting to co-participants’ turns, thus co-construct knowledge in a dynamic and context-sensitive way, which is known as epistemics in interactional research [19].

In counselling sessions, there is always an epistemic asymmetry between the participants [20], and they dynamically position themselves and each other on an epistemic gradient according to their state of being less to more knowledgeable or vice versa [19]. Stivers et al. identified three main

dimensions of knowledge asymmetry; 1) epistemic access (ranging from unknowing (K-) to knowing (K+); the degree of certainty with which a speaker displays an unknowing or knowing position varies dependent on the interactional context [19], 2) epistemic advantage, and 3) epistemic responsibility. The first dimension represents the source of knowledge and relates to practices for direct access to it, the second dimension refers to participants' relative right to know, and the third dimension relates to the duty to know. All these aspects play significant roles in accomplishments of the institutional situations. Although differences in participants' knowledge are ubiquitous and inevitable in any human relationship, they are particularly evident in institutional settings. Because participants are involved in institutional cooperation within the framework of their institutional roles, structuring them as 'experts' and 'owners' of particular knowledge areas and assigning them different epistemological positions, i.e., socially-based positions of epistemological authority [21,22].

There are very few studies that have investigated the community pharmacy interaction and how the pharmacist demonstrates her/his expertise in talk-in-interaction [16]. The community pharmacy interaction including information and medicine presentations of pharmacists who do not receive any consultancy fee has not been closely examined in the existing literature. Against this background, the current study aims to show how the pharmacist displays his expertise knowledge using diverse interactional practices within community pharmacy interaction initiated by the patient, pharmacy staff, and pharmacist.

MATERIAL AND METHOD

Data and Research Context

The data of the study come from the face-to-face interaction between a pharmacist, two pharmacy staffs, and patients in a community pharmacy in Ankara, Türkiye. The whole database includes 30-hour recordings collected with two cameras and two audio recording devices. Before the data collection procedure, ethical approval was received from the official ethical board. In order to receive the patients' consents, an explanatory notice about the data collection was hung on the door of the focal pharmacy. Before the patients entered the pharmacy, the researcher also asked for their permission. In total, 300 patients were involved and all of them accepted to participate in the study. Also, the pseudonyms were used to secure the participants' identities while representing the extracts in the results section.

Conversation Analysis

This study adopts Conversation Analysis (CA) as the research methodology to show the micro--analytic details of naturally occurring pharmacy interaction. CA is identified as 'a set of methods and approach that describes, analyses and aims to understand conversation as the basis of people's social life in social sciences' [23]. Through this methodology, researcher(s) record, transcribe, and analyse "naturally occurring" conversations of the type of interaction of interest [24]. At the beginning phase of the data analysis, the recordings of the pharmacy interaction were watched over and over through unmotivated looking [25]. Then, both audio and video recordings were transcribed using Jefferson Transcription System [26] (Table 1) transcription conventions to grasp all the interactional details including verbal utterances and embodied actions. Moreover, line-by-line analysis of interactional sequences was based on the scrutiny of interactional features of CA including turn-taking, preference organization, and repair. Following the CA-based data analysis procedure, we built a collection of expertise knowledge demonstration of the pharmacist as the main phenomenon of this study. We explored that the pharmacist showed his expertise following the patient-, the pharmacy staff- or his own initiated sequences in three different subcollections. Thus, the broader collection consists of 80 extracts including 30 pharmacist-initiated, 7 pharmacy staff-initiated, and 43 patient-initiated sequences through diverse interactional practices providing extended explanations and advice, referring to external authority, and using both professional and trade knowledge within pharmacy interaction. The average length of a consultation was around 4 minutes, but can range from 3 to 10 minutes. In this paper, three representative extracts will be closely examined to demonstrate the displays of pharmacist's expertise knowledge with different interactional practices.

Table 1. Jeffersonian Transcription Conventions

Symbol	Description
(.)	A micropause- a pasue of no significant length.
(0.7)	A timed pause- long enough to indicate a time
[ ]	Square brackets show where speech overlaps.
> <	Arrows showing that the pace of speech has quickened.
< >	Arrows showing that the pace of speech has slowed down.
( )	Unclear section
(( ))	An enrty requiring comment but without a symbol to explain it.
Underlining	Denotes a rise in volüme or emphasis.
↑	Rise in intonation
↓	Drop in intonation
→	Entered by the analyst to Show a sentence of a particular interest. Not usually added by the transcriber.
CAPITALS	Louder or shouted words
(h)	Laughter in the conversation/speech.
=	Will be at the end of one sentence and the start of the next. It indicates that there was no pause between them.
:::	Colons-indicate a stretched sound

RESULT AND DISCUSSION

In this section, three long representative extracts will be represented as (1) patient (Pt)-, (2) pharmacy staff (Ps)-, and (3) pharmacist (P)-initiated sequences. Firstly, Figure 1 (Extract 1-Solkasen) illustrates how the pharmacist displays his expert knowledge including both professional and trade knowledge about the alternative medicines and companies through different interactional practices within patient-initiated pharmacy interaction.

In line 1, the patient (Pt) initiates a Yes/No question [27] about if there is a particular medicine (`solkasen patch- diclofenac sodium`) or not, and the pharmacist provides a dispreferred response thereby sharing a trade knowledge about medicine production as the first attempt of expertise knowledge demonstration [28], and using '`we`' as an institutional pronoun of pharmacy staff [29]. Following Pt's verbal dissatisfaction (`don't do it`), P announces another alternative medicine (`kuromet-ibuprofen`), mitigates his suggestion through the silences (0.6, 1.0) and using 'can' structure from line 6 to 10. Then, Pt explains her health problem, and provides account for her previous request for solkasen patch, and she extends the interaction through clarification questions about solkasen patch (lines 11, 12). This signals that Pt needs more information about the demanded medicine [7] although the pharmacist suggests another alternative medicine. Then, the pharmacist starts his extended explanation turn (e.g., [30] with "`honestly`", and displays his epistemic knowledge based on trade knowledge in pharmacy field with references to different possibilities and particular company names [19] (lines 13-15). This extended display of his expertise knowledge leads to Pt's demonstration of understanding and acceptance. After 0.5 sec of silence, the pharmacist orients to the pain stand to show alternative medicines, and P elaborates various types of medicines with detailed explanation and exemplification. Such detailed explanations based on P's higher epistemic knowledge and authority [10] is followed by Pt's lack of knowledge [31] and experience about different medicines for pain (`i have never used, i never know`). Then, the pharmacist upgrades his expertise demonstrations with references to the Ministry of Health which is an external source of authority [32] and recommends the same alternative medicine (`kuromet`). However, unlike the previous mitigated recommendation in line 8, he highlights "`kuromet`" as the most similar one to the demanded medicine. Thus, P's increasing demonstrations of his expertise knowledge result in the patient's overlapped repetition. After P's detailed knowledge provision about the company producing his suggested medicine, the patient requests

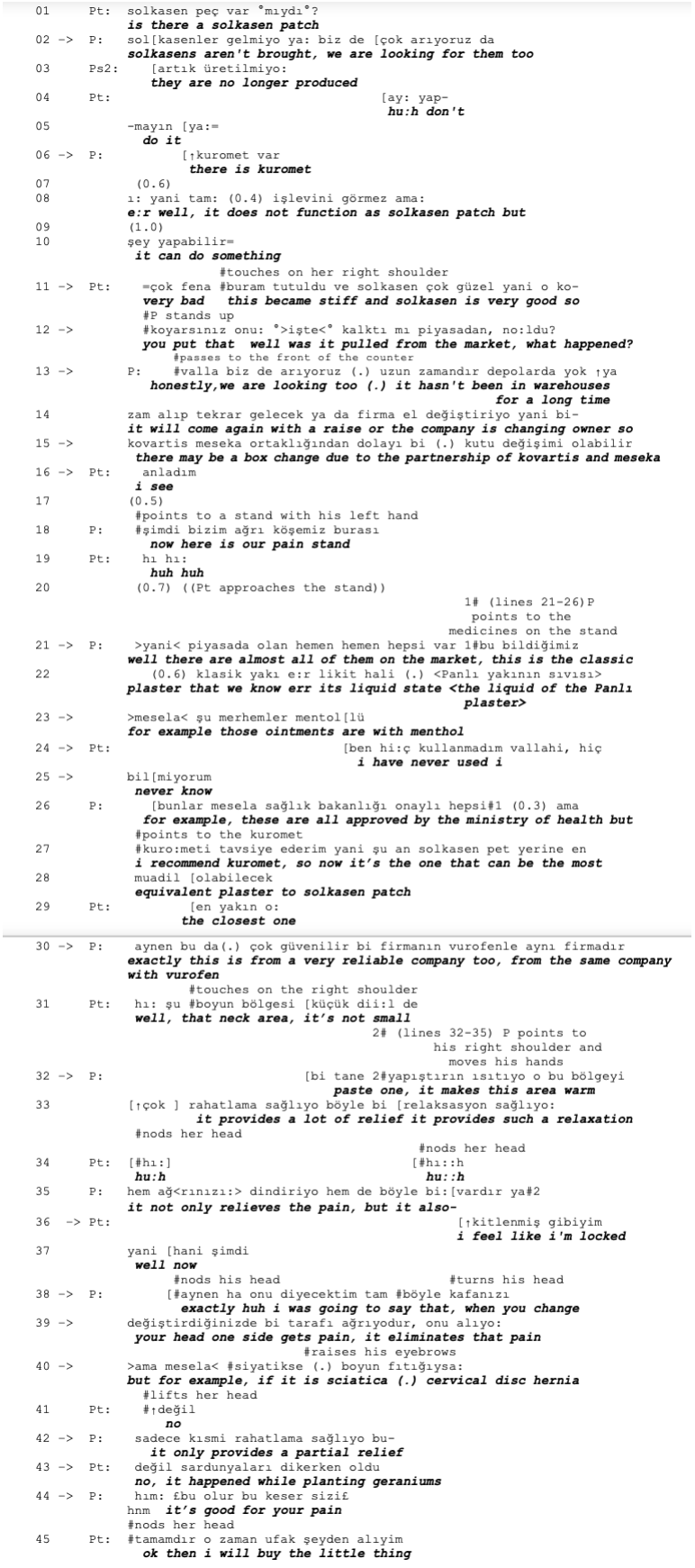

Figure 1. Extract 1-Solkasen

another mitigated clarification thereby stating that the pain area (her neck) isn't small and touching her neck (line 31). From line 32 to 35, P explains how to conduct the medicine and get the solution for her pain while Pt displays her listenership [33]. Also, Pt extends her previous request for clarification with reference to her pain (`i feel like i'm locked`). Following this, the pharmacist not only introduces how the medicine influences on her pain, but also exemplifies her pain using a medical terminology (`sciatica`) and a specific instance for sciatica with a formulated medical expression (`cervical disc hernia`). Therefore, the pharmacist demonstrates his expertise knowledge through medical terminology as well as his information-sharing on the effect of the medicine [34]. Finally, after Pt's medical history sharing (line 43), and P's explicit advice about the medicine (line 44), this extract closes with Pt's confirmation with "`okay`" [35] and request for buying the small package of the suggested medicine (kuromet).

In brief, Extract 1 shows the dynamic knowledge asymmetry of the patient-initiated pharmacy interaction through the pharmacist's displays of his expertise knowledge. In doing so, after the patient requests for detailed information about the production of her demanded medicine (solkasen) and the impact of the suggested medicine (kuromet), the pharmacist shares his professional knowledge (usage of the medicine, impact of the medicine, etc.) and trade knowledge (medicine production, companies) through different practices such as using medical terminology and formulated instance, referring to the external epistemic authority (i.e., Ministry of Health). Thus, it can be clearly seen that various displays of the pharmacist's expertise knowledge enable the patient to persuade for buying the medicine existing in the pharmacy.

Figure 2 (Extract 2-Pastille) represents how the pharmacist responds another patient's question through diversified expertise knowledge demonstration practices within a pharmacy staff-initiated sequence. The following extract comes from moment during which one of the pharmacy staff (Ps1) inputs a patient's prescription data on the system and prepares the patient's medicine while the patient asks a question about the possible damage of the prescribed medicine to her body.

At the beginning of the extract, when the pharmacy staff shows a pastille from the medicine corner, the patient asks a Y/N question about if she can use pastille while breastfeeding (lines 1-3). After Ps1's request for confirmation about whether she is breastfeeding (line 4), Pt's confirmation (`huh huh`) (line 5), and P's announcement that he is leaving the pharmacy (line 6), the pharmacy staff orients to the pharmacist by uttering his name (line 8), and then asks the patient's question about the medicine usage thereby announcing the the name of the pastille to elicit the pharmacist's expert knowledge [36]. In line 10, the pharmacist shows the medicine to the Pt and displays her expertise through his account provision (`sugar free`) [37]. The pharmacy staff also confirms P's explanation with reference to the written explanation on the medicine, which leads the Pt's change of state token (`huh`) [38] and acknowledgement token (`ok`). This signals that the pharmacist's expertise knowledge through account provision and the Ps1's written evidence to this knowledge result in epistemic change on the patient's knowledgeability. After they talk about off-task topic (see omitted part-lines 13-19), Pt initiates another Yes/No question by displaying her epistemic knowledge [39] using medical terminology (beta glucans), and then the pharmacist requests for a clarification about the age of Pt's babv. After Pt states that her baby is one year old, the pharmacist deploys his expertise knowledge using societal expressions (`milk is for pleasure`), and expresses that Pt can give the medicine to her baby. He also elaborates giving beta glucan to 1-year old babies using "`we`" referring to the pharmacy staff and/or pharmacists [29]. This extended demonstration of his expertise knowledge leads to Pt's interrogation about the reasons why this medicine isn't given to them. Then, P donwgrades the certainty of knowledge about using the beta glucan on babies through "can" (`it can be given`), introduces that they won't have any problems as an expert in a community pharmacy interaction (lines 31, 32), and repeats his previous recommendation about the medicine usage (line 33) in an overlapped way with Pt's display of understanding (line 32). Ultimately, this extract closes with thanking sequences of both the pharmacist and the patient.

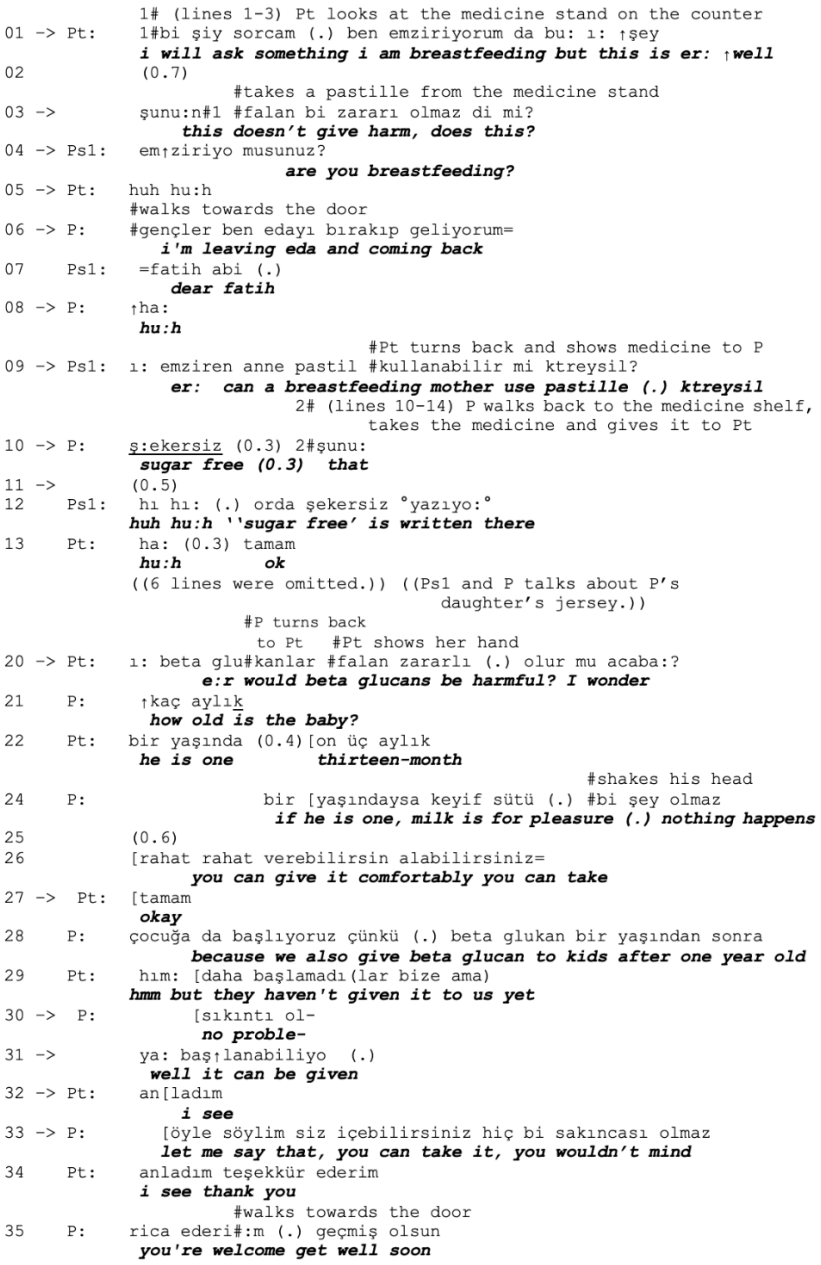

Figure 2. Extract 2-Pastille

In sum, Extract 2 highlights that the pharmacist responds to the patient's question initiated to the pharmacy staff about the medicine usage through various demonstrations of his expertise knowledge (using medical terminology and societal expression, providing extended accounts to Pt's clarification requests). Therefore, he utilizes his own expertise knowledge to make Pt's epistemic status change from less to more knowledgeable.

In a similar way, Figure 3 (Extract 3-Blood pressure) illustrates how the pharmacist provides his expert knowledge while eliciting the patient's history-taking within the pharmacist-initiated interaction. Before Extract 3 starts, the pharmacist has found the prescribed medicines for the patient from the medicine tracking system.

From line 1 to 3, the pharmacist refers to being prescribed a blood pressure medicine, explains the usage of this medicine, and requests for confirmation (`is it ok`) while the patient initiates a Y/N question about when to take the medicine in an overlapped way. After P's repetition of previous

explanation (`take it in the morning`), Pt initiates a request for confirmation on her own medicine taking routine (`taking in the evening`) (line 6). Then, P firstly mentions that she can also take it in the evening regarding her routine, waits for 1.1 seconds of silence, and asks alternative questions to receive more information about the history-taking process of the medicine prescription. After Pt's confirmation of the second alternative with the repetition (`they raised the dose`), and P's minimal acknowledgement, the pharmacist completes the medicine scanning procedure (line 13). In line 14, P displays his expert knowledge by repeating his previous recommendation about taking the medicine in the morning with reference to the effectiveness of the medicine in a mitigated way (`maybe`), and requests for confirmation. Pt displays her alignment with P's suggestion, and shares her friend's explanation about taking the medicine in the evenings, and completes her turn with a laughter which can show her orientation to the problem [40]. The pharmacist initially rejects unprofessional information about the medicine usage, and demonstrates his expertise knowledge thereby explaining when Pt can get the most benefit from the medicine, and how the time of taking medicine has a crucial influence on rising blood pressure through exemplification and evidence-based account. Finally, after Pt's display of her listenership, the pharmacist completes his expertise knowledge-sharing sequence with references to the changing impact of the medicine in a day. Overall, Extract 3 illustrates that the pharmacist produces his expertise knowledge through rejection of unprofessional knowledge, and detailed account provision on the potential results of using the medicine in the morning or evening within the pharmacist-initiated sequence when he realizes the problem about the preferred time for taking the medicine during the history-taking sequence, and provides advice about its preferred usage.

This study showed the displays of the pharmacist's expertise knowledge within three different sequential structures: the patient-, pharmacy staff-, and pharmacist-initiation. Using Conversation Analysis allows for participant-relevant explanations of the diversified practices of the pharmacist's expertise knowledge demonstration in community pharmacy interaction.

In this study, we also documented the epistemic asymmetry between the pharmacist, patients, and pharmacy staff. The interactants negotiate the prescribed expert identity to the pharmacist for the purposes of the expertise transfer as an interactive performance [9,13,14]. On the other hand, the patients were regarded as the non-expert because of their non-access to the professional knowledge. In addition, as demonstrated in the findings (see Extract 2), the pharmacy staff directed the patient's request for information based on the medical knowledge to the pharmacist for the expertise knowledge [10]. Thus, this study indicated that the epistemic asymmetry in the community pharmacy interaction only gives the permission for the pharmacy staff to sell the products, but not providing medical knowledge to the patients.

In this study, we highlighted that the patients initiate Yes/No type questions [27] to request for confirmation and/or clarification through their own demonstrations of knowledge [19]. Therefore, they attempted to manage their epistemic search sequences with the help of the pharmacist as an expert [6]. However, when the pharmacist initiated dispreferred and/or unexpected responses (e.g., suggesting a different medicine in Extract 1) [41-43], the patients requested for more elaboration through their own account provisions (e.g., sharing pain history). As opposed to the non-expert's "recipe" knowledge [44], the pharmacist provided evidence-based accounts using professional and trade knowledge as well as the reference to the epistemic authorities. In addition, the patients frequently expressed their "lack of information" or "doubts" on various medical issues, which indicated what the patient wanted to know or on what subjects they needed an expert opinion [45]. In this study, we explored that the patients also shared their problematic practices with references to other parties' viewpoints with a smiley voice [46] and laughter [40] (see Extract 3). Thus, they displayed their awareness about their doubts and medical problems in talk-in-interaction.

The micro-analytic findings of this study also explored that these various patient practices provided some interactional spaces for the pharmacist to share their expertise knowledge [16] using different practices to respond the patients' questions, and complete the tasks of community pharmacy interaction (e.g., selling the drugs, providing necessary information). As the expert having more professional knowledge, the pharmacist not only produced medical terminology and field-related instances but also referred to the Ministry of Health as an epistemic authority [47]. In line with the

previous literature ([48,49], the pharmacist also provided detailed explanations to avoid the patients' lack of understanding based on the technical medical knowledge.

Figure 3. Extract 3-Blood pressure

All in all, this study explored that the pharmacist demonstrated his expertise knowledge through a wide range of interactional practices such as providing extended explanations and advice, repeating some statements, giving information about the medicine usage instructions, referring to external authority, and using both professional and trade knowledge in response to the patients' requests for clarification and confirmation based on the medical knowledge in the community interactioın. The CA findings of this study also highlighted that the pharmacist's "doing being an expert" [50] resulted in the change of the epistemic asymmetry of the ongoing interaction for the patients from less to more knowledgeable about the medicines and enabled them to buy the medicines. Overall, Conversation

Analysis offered in-depth investigations of displays the pharmacist's expertise knowledge in pharmacy interaction with the diversified expertise knowledge provision practices to manage the patients' epistemic search sequences. We believe that the micro-analytic findings of this study can be utilized in pharmacy education to develop the pharmacy students' and pharmacists' interactional and professional skills to establish more patient-centred communication in an evidence-based way. In this study, we illustrated the displays of one pharmacist's expertise knowledge, but further studies can be carried out with more pharmacist participants in different community pharmacy and country contexts to reach fuller understandings of the pharmacy interaction and provide more recommendations for the community pharmacy communication.

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AUTHOR CONTRIBUTIONS

Concept: E.U.D.; Design: E.U.D., M.B.; Control: M.B.; Sources: E.U.D.; Materials: E.U.D.; Data Collection and/or Processing: E.U.D., M.B.; Analysis and/or Interpretation: E.U.D., M.B.; Literature Review: E.U.D., M.B.; Manuscript Writing: E.U.D., M.B.; Critical Review: M.B.; 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 protocol was approved by the Hacettepe University Senate Ethics Committee.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

A MULTIVARIATE INTERPOLATION APPROACH FOR PREDICTING DRUG LD_{50} VALUE

İLAÇ LD_{50} DEĞERİNİ TAHMİN ETMEK İÇİN ÇOK DEĞİŞKENLİ BİR İNTERPOLASYON YAKLAŞIMI

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ABSTRACT

Objective: *The present study aimed to develop a multivariate interpolation based on the quantitative structure-toxicity relationship (QSTR) that can accurately predict the oral median lethal dose (LD_{50}) values of drugs in mice by considering five different toxicologic endpoints.*
Material and Method: *A mathematical model was created using a comprehensive dataset comprising LD_{50} values from 319 pharmaceuticals belonging to various pharmacological classes. We developed a polynomial model that can predict the range of LD_{50} values for pharmaceuticals. We employed a technique called two-variable polynomial interpolation. This method allowed us to estimate the approximate values of a function at any point within a two-dimensional (2D) space by utilizing a polynomial equation.*
Result and Discussion: *The resulting model demonstrated the ability to predict LD_{50} values for new or untested drugs, rendering it a valuable tool in the early stages of drug development. The Ghose-Crippen-Viswanadhan octanol-water partition coefficient (ALogP) and Molecular Weight (MW) were selected as suitable descriptors for building the best QSAR model. Based on our evaluation, the model achieved an overall success rate of 86.73%. Compared to traditional experimental methods for LD_{50} determination, this innovative approach offers time and cost efficiency while reducing animal testing requirements. Our model can improve drug safety, optimize dosage regimens, and assist decision-making processes during preclinical studies and drug development. This approach provided a reliable and efficient method for preliminary acute toxicity assessments.*
Keywords: *Data analysis, LD_{50}, mathematical toxicology, multivariate interpolation, polynomial interpolation*

ÖZ

Amaç: *Bu çalışmanın amacı, beş farklı toksikolojik sonucu dikkate alarak farelerde ilaçların oral median letal doz (LD_{50}) değerlerini doğru bir şekilde tahmin edebilen, niceliksel yapı-toksisite ilişkisine (QSTR) dayalı çok değişkenli bir interpolasyon yöntemi geliştirmektir.*
Gereç ve Yöntem: *Farklı farmakolojik sınıflara ait 319 ilaca ait LD_{50} değerlerini içeren kapsamlı bir veri seti kullanılarak matematiksel bir model oluşturuldu. Farmasötiklerin LD_{50} değerlerinin aralığını tahmin edebilen bir polinom model geliştirdik. İki değişkenli polinom interpolasyon adı*

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verilen bir teknik kullanarak bunu gerçekleştirdik. Bu yöntem, bir polinom denklemi kullanarak iki boyutlu bir uzayda herhangi bir noktadaki bir fonksiyonun değerlerini tahmin etmemizi sağladı.
Sonuç ve Tartışma: *Elde edilen model, yeni veya denenmemiş ilaçlar için LD_{50} değerlerini tahmin etme yeteneğini gösterdi ve bu nedenle ilaç geliştirme sürecinin erken aşamalarında değerli bir araç olarak kullanılabilir. Değerlendirmemize göre, model genel başarı oranı olarak %86,73 olarak bulundu. LD_{50} değerinin belirlenmesinde kullanılan geleneksel deneysel yöntemlere kıyasla, bu yenilikçi yaklaşım zaman ve maliyet açısından avantajlı olup hayvan deneylerinin gerekliliğini azaltmaktadır. Modelimiz ilaç güvenliğini artırabilir, doz rejimlerini optimize edebilir ve ön klinik çalışmalar ve ilaç geliştirme sürecinde karar verme süreçlerine yardımcı olabilir. Bu yaklaşım, ön akut toksisite değerlendirmeleri için güvenilir ve etkili bir yöntem sunmuştur.*
Anahtar Kelimeler: *Çok değişkenli interpolasyon, LD_{50}, matematiksel toksikoloji, polinom interpolasyonu, veri analizi*

INTRODUCTION

The median lethal dose or concentration (LD_{50}/LC_{50}) serves as a dose indicator for evaluating the acute toxicity of pharmaceuticals/chemicals in risk assessment. This dosage corresponds to the amount resulting in mortality in 50% of the analyzed animal population. The LD_{50}/LC_{50} value is also used for categorizing the toxicity levels of substances, enabling a standardized and systematic approach to toxicological assessments (Table 1) [1].

LD_{50}/LC_{50} tests are conducted in the early stages of drug development to determine the lethal dose of pharmaceuticals. These trials provide a reference point for dose selection in subsequent toxicity studies. The accurate calculation of the LD_{50}/LC_{50} value is essential for ensuring the safe use of medications and predicting potential adverse reactions. Thus, it aids in the establishment of the drug's toxicity profile [2]. Although rats, rabbits, and guinea pigs have traditionally been utilized in such studies, mice are often preferred as a model organism. The LD_{50}/LC_{50} value can be determined through various administration methods, including oral, dermal, or inhalation, depending on the study's design. Regarding ease of use, the oral route is the commonly preferred method of medication delivery. Therefore, oral LD_{50}/LC_{50} data in the literature are widely available compared to other routes of exposure [1].

Table 1. The hazard categories for acute toxicity (based on the LD_{50}/LC_{50} value) according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) guidelines [1]

Exposure Route	Category 1	Category 2	Category 3	Category 4	Category 5
Oral (mg/kg BW)	≤ 5	5-50	50-300	300-2000	2000 <
Dermal (mg/kg BW)	≤ 50	50-200	200-1000	1000-2000	2000 <
Gases (ppmV)	≤ 100	100-500	500-2500	2500-20000	-
Vapors (mg/l)	≤ 0.5	0.5-2	2-10	10-20	-
Dust and Mists (mg/l)	≤ 0.05	0.05-0.5	0.5-1	1-5	-

BW: Body Weight

Due to ethical concerns, regulatory rules have recently restricted the use of laboratory animals in pharmaceutical research [3]. The current legislations promote the adoption of alternative approaches to minimize animal experiments [4]. One of the notable alternative methods involves the quantitative structure-toxicity relationship (QSTR) models, which assist in the rapid and cost-effective assessment of drug toxicity. QSTR modeling has gained recognition as a computational approach in pharmaceutical toxicology. These mathematical-based models establish a relationship between the structural characteristics of pharmaceutical compounds and their potential toxic effects. Various algorithms are employed in QSTR studies to facilitate classification or provide direct quantitative predictions [5]. Several studies have been conducted in the literature to predict the LD_{50}/LC_{50} value of chemical substances using QSTR models, employing datasets of varying sizes [4,6,7]. However, compared to other research areas in QSTR, relatively few studies specifically focused on predicting the LD_{50}/LC_{50}

values of pharmaceuticals [8]. Due to this gap in the literature, we have turned to non-animal-based methods to predict the LD_{50}/LC_{50} values of pharmaceuticals.

The interpolation method is one of the commonly employed approaches. If LD_{50}/LC_{50} values for certain drugs are available at specific doses, interpolation techniques can be used to estimate LD_{50}/LC_{50} values for intermediate doses [9]. Interpolation methods such as linear interpolation, polynomial interpolation, or spline interpolation can be used to approximate LD_{50}/LC_{50} values based on the known data points [4]. Another approach is the regression technique. This technique can be used to estimate LD_{50}/LC_{50} values based on a set of independent variables (predictors) such as drug dosage, administration route, or changing experimental conditions [4]. Various regression techniques like linear, logistic, or nonlinear regression can be applied to fit a model to the available LD_{50}/LC_{50} data and predict LD_{50}/LC_{50} values for newly synthesized drugs or different dosages. QSTR models also aim to establish relationships between the chemical structure or descriptors of drugs and their biological activities. There are critical dose values that play a significant role in the biological activity of a drug. Among these criteria, the LD_{50}/LC_{50} value stands out as an indicator of acute toxicity [4,6]. By analyzing a dataset of drugs with known LD_{50}/LC_{50} values and their corresponding chemical descriptors, QSTR models can be built to predict LD_{50}/LC_{50} values for novel drug molecules based on their structural characteristics. Machine learning techniques, such as decision trees, random forests, support vector machines, or neural networks, can be employed to develop predictive models for LD_{50}/LC_{50} estimation [10,11]. These models learn patterns and relationships from the available LD_{50}/LC_{50} data and can be used to predict LD_{50}/LC_{50} values for novel drugs based on their features or descriptors. The choice of the most appropriate method varies depending on the available data, the nature of the problem, and the specific goals of the analysis.

In this study, we developed a multivariate interpolation-based [12] QSTR model to predict the acute oral LD_{50} values of drugs in mice. This model was formulated based on the impact of critical properties in the chemical structures of pharmaceuticals on the biological response in mice. This approach enables the determination of relationships between the physicochemical properties of pharmaceuticals and their toxicity, allowing for the rapid and effective analysis and interpretation of complex data. This provides a significant advantage in reducing risks and improving safety standards in drug development. Our study was specifically designed to minimize the utilization of experimental animals by narrowing down the range of LD_{50} values. Ultimately, a final LD_{50} value should be established through animal experimentation in the last stage, thus ensuring comprehensive assessment and verification of drug safety. The results obtained from this research contribute to the advancement of drug safety assessment and establish a foundation for future computational toxicology studies.

Figure 1 illustrates the steps to develop a mathematical model capable of predicting the range of LD_{50} values for pharmaceuticals-the initial phase involved data collection. Subsequently, the dataset was pre-processed by eliminating noisy data and establishing the applicability domain. From the dataset, specific drugs were selected for constructing the interpolation polynomial. Two descriptors with the highest efficiency were chosen to represent the two variables, (x and y). Multiple interpolation polynomials were created to assess their success in predicting the LD_{50} value range. If the results were unsatisfactory, the process returned to step 3, where different sets of drugs were selected, and the interpolation process was repeated. Ultimately, the model's performance was evaluated based on the accuracy of correctly classifying the drug ranges of LD_{50} values.

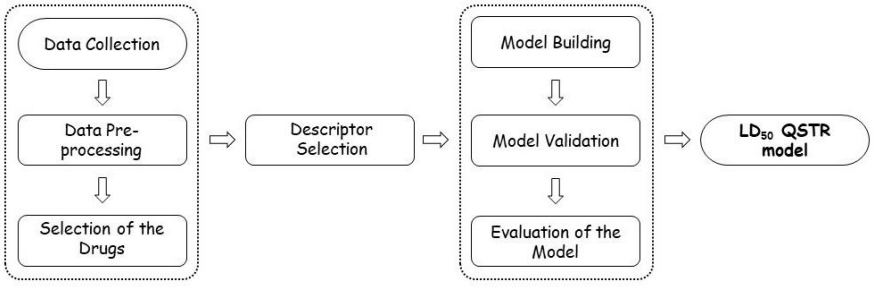

Figure 1. Model development workflow

MATERIAL AND METHOD

Polynomial interpolation, in the context of QSTR, is a mathematical technique used to model the relationship between the chemical structure of a compound and its toxicological activity. QSTR models aim to predict the activity of a chemical based on its structural features. Polynomial interpolation involves fitting a polynomial function to a set of data points, where each data point represents a compound with known structural descriptors and corresponding activity values. The polynomial function is then used to interpolate the activity of compounds based on their structural descriptors.

Estimating the LD_{50} values for drugs solely through interpolating molecular descriptors is challenging and not prevalent. Molecular descriptors alone may not provide sufficient information to predict toxicity levels accurately. Rather than predicting a direct mathematical value, this method yields more successful results in estimating a range. Therefore, in this study, we developed a mathematical model that efficiently predicts the range of LD_{50} values for a pharmaceutical.

Material

This study included a total of 319 drugs, each accompanied by available oral LD50 values [13-15] (Supplementary File 1_Table S1). To ascertain the compounds' chemical structure and physical attributes, we accessed the two-dimensional structural data file (2D SDF) through the PubChem database [16]. Employing the open-source program T.E.S.T. [17], we computed chemical descriptors for these compounds based on the 2D SDF data files.

We diligently cleansed and preprocessed the dataset, ensuring its freedom from any missing values, outliers, or other data quality anomalies. The importance of data quality cannot be understated in the realm of data science and machine learning workflows. Consequently, any corrupted 2D SDF files sourced from PubChem were meticulously eliminated from the dataset, yielding data of exceptional quality and usability.

Subsequently, the remaining SDF files underwent characterization through T.E.S.T. and were then stored in comma-separated files. The "ReplaceMissingValues" tool, an unsupervised attribute filter within the WEKA 3.9.5 (Waikato Environment for Knowledge Analysis) software, was employed to impute the missing descriptive values [23]. Following this step, duplicated data entries were systematically removed from the dataset to ensure its integrity.

The selection of descriptors for accurate LD_{50} estimation depends on the specific characteristics of the drugs being considered. We focused on the two descriptors frequently mentioned in the literature for the interpolation process. Two commonly used and important descriptors for LD_{50} estimation are the Ghose-Crippen-Viswanadhan octanol-water partition coefficient (ALogP) and Molecular Weight (MW). The selection of ALogP and MW as significant descriptors for LD_{50} estimation is based on the following reasons.

ALogP is employed to assess a compound's solubility in nonpolar solvents (e.g., octanol) and polar solvents (e.g., water) [18]. It provides information about the lipophilicity of a drug and its ability to permeate and accumulate in biological membranes. Higher ALogP values are associated with increased toxicity, potentially due to enhanced membrane permeability and the likelihood of accumulating in fatty tissues [5]. Therefore, ALogP is a valuable indicator of a compound's bioavailability and potential to cross biological membranes, making it a significant factor in determining LD_{50} values.

Our other identifier, MW, is a fundamental descriptor used in drug-related QSTR studies, providing information about a drug's size and complexity. MW is a critical parameter in toxicity modeling studies as it directly influences the toxicokinetic of xenobiotics. According to the OECD guidelines, it has been stated that the physicochemical properties of a chemical, such as AlogP and MW, may be helpful for study planning and interpretation of results [19].

Molecular Diversity and Distribution in Chemical Space

Molecular diversity and distribution in chemical space play a crucial role in QSTR modeling. Chemical space refers to the vast multidimensional space that encompasses all possible molecules. The distribution of molecules within this chemical space is important because it affects the coverage and

representativeness of the training data used in QSTR modeling. Molecular diversity measures the variety and heterogeneity of molecules in a dataset. A diverse dataset should cover different regions of chemical space, representing a wide range of structural features and properties. Including diverse molecules in the training set helps capture the full range of interactions and properties that a QSTR model needs to predict accurately [20].

Considering molecular diversity and distribution in chemical space, QSTR models can provide reliable predictions for molecules with similar structural features or properties, even if they were not explicitly included in the training dataset. This enhances the generalization and applicability of QSTR models to guide molecular design, screening, and optimization processes in various fields [20].

Method

In this study, we aimed to develop a polynomial model that can predict the range of oral LD_{50} values for pharmaceuticals. We employed a technique called two-variable polynomial interpolation. This method allows us to estimate the values of a function at any point within a 2D space by utilizing a polynomial equation.

We have a set of data points, each consisting of distinct values $(x_0, y_0), (x_1, y_1), \dots, (x_n, y_n)$, along with their corresponding function values $f(x_i, y_i)$. Our goal was to find a unique multivariate interpolation polynomial, denoted as P(x, y), that satisfies the equation:

$$f(x_i, y_i) = P(x_i, y_i), \tag{1}$$

where, for each $i = 0,1, \dots, n$. This equation should hold true for each i ranging from 0 to n. By constructing such a polynomial, we aimed to accurately predict the function values for any given combination of x and y within the interpolation domain. To accurately predict function values for any combination of x and y within the interpolation domain, we need to create an interpolation matrix. The construction of this matrix can be accomplished using the following procedure.

The polynomial of two variables of the total degree of n is given by

$$P(x, y) = \sum_{i=0}^{n} \sum_{j=0}^{k} a_{j,i}\, x^j y^{i-j}, \tag{2}$$

where, for each $i = 0,1, \dots, n$ and $j = 0,1, \dots, k$ [21]. We used 10 distinct (x, y) values to find a multivariate interpolation polynomial function $P(x, y)$ of the form,

$$\begin{aligned} P(x, y) = {} & a_{0,1} + a_{1,1}x + a_{1,2}y + a_{2,1}x^2 + a_{2,2}xy + a_{2,3}y^2 + a_{3,1}x^3 + a_{3,2}x^2y \\ & + a_{3,3}xy^2 + a_{3,4}y^3 \end{aligned} \tag{3}$$

where $a_{0,1}, a_{1,1}, a_{1,2}, a_{2,1}, a_{2,2}, a_{2,3}, a_{3,1}, a_{3,2}, a_{3,3}$, and $a_{3,4}$ are the coefficients to be determined.

We can construct a system of equations by substituting the data points into the polynomial equation,

$$\begin{aligned} f(x_1, y_1) = {} & a_{0,1} + a_{1,1}x_1 + a_{1,2}y_1 + \cdots + a_{3,1}{x_1}^3 + a_{3,2}{x_1}^2y_1 + a_{3,3}x_1{y_1}^2 \\ & + a_{3,4}{y_1}^3 \\ f(x_2, y_2) = {} & a_{0,1} + a_{1,1}x_2 + a_{1,2}y_2 + \cdots + a_{3,1}{x_2}^3 + a_{3,2}{x_2}^2y_2 + a_{3,3}x_2{y_2}^2 \\ & + a_{3,4}{y_2}^3 \\ & \vdots \\ f(x_{10}, y_{10}) = {} & a_{0,1} + a_{1,1}x_{10} + a_{1,2}y_{10} + \cdots + a_{3,1}{x_{10}}^3 + a_{3,2}{x_{10}}^2y_{10} \\ & + a_{3,3}x_{10}{y_{10}}^2 + a_{3,4}{y_{10}}^3, \end{aligned} \tag{4}$$

where, $a_{0,1}$, $a_{1,1}, \ldots, a_{3,4}$ are the coefficient values that are to be determined to form the interpolation polynomial $P(x, y)$ [22,23]. We can represent this system of equations in matrix form,

$$A\,a\; =\; f, \tag{5}$$

where A is the coefficient matrix, a is the vector of unknown coefficients, and f is the vector of function values. Equation (5) can be expressed as a linear system,

$$Aa = \begin{bmatrix} 1 & x_1 & \cdots & x_1{y_1}^2 & {y_1}^3 \\ 1 & x_2 & & x_2{y_2}^2 & {y_2}^3 \\ \vdots & & \ddots & \vdots & \\ 1 & x_9 & \cdots & x_9{y_9}^2 & {y_9}^3 \\ 1 & x_{10} & & x_{10}{y_{10}}^2 & {y_{10}}^3 \end{bmatrix} \begin{bmatrix} a_{0,1} \\ a_{1,1} \\ \vdots \\ a_{3,3} \\ a_{3,4} \end{bmatrix} = \begin{bmatrix} f(x_1, y_1) \\ f(x_2, y_2) \\ \vdots \\ f(x_9, y_9) \\ f(x_{10}, y_{10}) \end{bmatrix} = f, \tag{6}$$

where, $A \in \mathbb{R}^{10\times10}$, $f \in \mathbb{R}^{10}$, and $a \in \mathbb{R}^{10}$. In this equation, A is a real-valued invertible matrix, f is a vector in the real-valued space, and a is the vector we need to find. Once we have matrix A and vector f, we can solve for vector $a \in \mathbb{R}^{10}$ using matrix operations or linear regression techniques to obtain the coefficients. These coefficients represent the interpolated function. We can then evaluate the interpolated function at new points within the interpolation domain by substituting the input values and obtaining the estimated function values.

The degree of the polynomial can be adjusted based on the targeted level of accuracy and complexity. Higher-degree polynomials can provide a better fit to the data but may also lead to overfitting. Careful consideration of the dataset and the trade-off between accuracy and complexity is critical when choosing the degree of the polynomial for interpolation. In our study, we chose this option since the third-degree polynomial produced the greatest results. The framework of multivariate interpolation (MVI) is shown in Figure 2.

Input: The input points x, y
Output: Corresponding target values $f(x, y)$
function coefficients = findInterpolationCoefficients(x, y, f)
% Create the matrix A
Step 1: $A = \begin{bmatrix} 1 & x_1 & \cdots & x_1{y_1}^2 & {y_1}^3 \\ 1 & x_2 & & x_2{y_2}^2 & {y_2}^3 \\ \vdots & & \ddots & \vdots & \\ 1 & x_9 & \cdots & x_9{y_9}^2 & {y_9}^3 \\ 1 & x_{10} & & x_{10}{y_{10}}^2 & {y_{10}}^3 \end{bmatrix}$;

% Create the vector f
Step 2: $f = \begin{bmatrix} f(x_1, y_1) \\ f(x_2, y_2) \\ \vdots \\ f(x_9, y_9) \\ f(x_{10}, y_{10}) \end{bmatrix}$;
% Solve the linear system $Aa = f$
Step 3: coefficients = $A \,/\, f$;
% Output: Estimated $f(x, y)$
estimated_f = $f(x, y)$;

Figure 2. Algorithm: MVI

Model Validation

Evaluating the performance of the QSTR model on the dataset is crucial to assess its predictive ability across diverse molecules. The test sets should contain molecules that are structurally distinct from the training set, representing novel regions of chemical space. The accuracy of the polynomial was evaluated using the accuracy (ACC) metric. Additionally, a visual analysis of the graph depicting a

polynomial curve is presented.

RESULT AND DISCUSSION

The performance of the multivariate interpolation model was evaluated using various metrics to assess its accuracy and effectiveness in estimating LD_{50} values based on molecular descriptors. We employed the interpolation polynomial $P(x,y)$, utilizing two molecular descriptor values, MW and AlogP, for each drug. The MW values represented the x-axis, while the AlogP values represented the y-axis for constructing the interpolation polynomial $P(x,y)$. For our analysis, we selected a dataset consisting of 10 drugs with their corresponding MW, AlogP, and LD_{50} values. We defined the output function values $f(x_i,y_i)$ based on the LD_{50} values. By inserting the interpolation points (x_i,y_i) into a system of equations, we constructed a coefficient matrix $A \in \mathbb{R}^{10\times10}$, an output vector $f \in \mathbb{R}^{10}$, and an unknown vector $a \in \mathbb{R}^{10}$. The coefficient matrix A had to be non-singular to ensure a unique solution vector $a \in \mathbb{R}^{10}$ [24]. To determine the singularity of A, we computed the number of linearly independent columns, which signifies the columns that are not linear combinations of each other. All 10 columns of A were linearly independent, indicating that matrix A was non-singular or invertible. This guaranteed that the system of equations had a single solution. To calculate the values of $a_{j,i}$, representing the coefficients of the interpolation polynomial $P(x,y)$, we utilized MATLAB, a powerful computational tool commonly used for scientific calculations and data analysis. MATLAB facilitated the efficient solution of the system of equations and enabled us to obtain the coefficients of the interpolation polynomial $P(x,y)$. The coefficient values $a_{j,i}$ for the model $P(x,y)$ with two variables were calculated based on the system (5) and are presented in Table 2.

Table 2. Coefficient values $a_{j,i}$

Coefficient	Calculated Value of Coefficient
$a_{0,1}$	-91.9388
$a_{1,1}$	385.1059
$a_{1,2}$	134.0851
$a_{2,1}$	-526.8917
$a_{2,2}$	-450.4742
$a_{2,3}$	125.3757
$a_{3,1}$	235.0836
$a_{3,2}$	355.8234
$a_{3,3}$	-84.6011
$a_{3,4}$	-165.7975

Once the $a_{j,i}$ values calculated, the interpolation polynomial $P(x,y)$ was determined as follows,

$$P(x,y) = -91.9388 + 385.1059x + 134.0851y - 526.8917x^2 - 450.4742xy + 125.3757y^2 + 235.0836x^3 + 355.8234x^2y - 84.6011xy^2 - 165.7975y^3, \quad (7)$$

where x is MW and y is AlogP descriptor values of the drugs.

Figure 3 illustrates the visual representation of the interpolation polynomial $P(x,y)$, showing the relationship between two variables, MW (Molecular Weight) and AlogP values (partition coefficient), of various drugs. The x-axis corresponds to the MW values, while the y-axis represents the AlogP values. In Figure 3, the interpolation polynomial is visualized as a curve that smoothly passes through the data points. Each data point represents a specific drug, with the MW and AlogP values corresponding to its position on the x and y-axis, respectively.

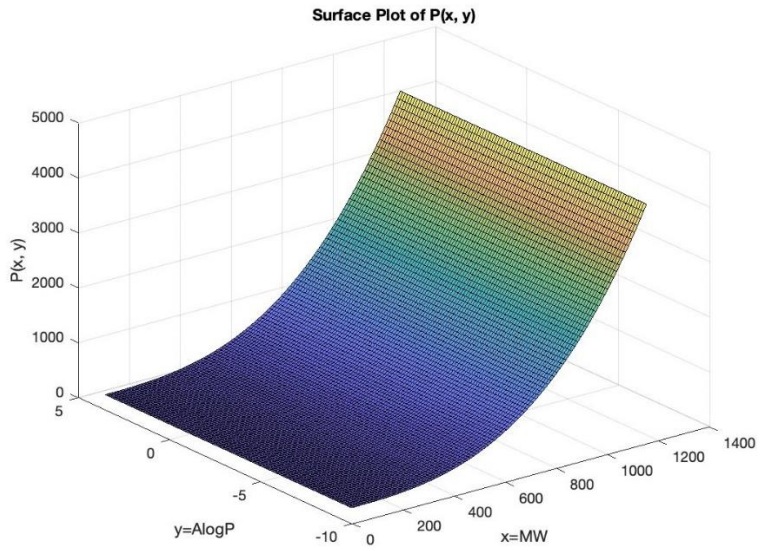

Figure 3. Surface plot of the interpolation polynomial

Model Validation

The model underwent both internal and external validations. Internal validation of a QSTR model involves assessing its accuracy using the molecules employed during its creation. This includes predicting the activities of these molecules and analyzing parameters to determine prediction precision. The accuracy of the multivariate interpolation model during internal validation was assessed by estimating LD_{50} values for the chosen 10 drugs used in creating the polynomial P(x, y). Remarkably, the internal validation achieved a 100% success rate, given that the same drugs were used for both development and testing.

However, the model's ability to predict outcomes for entirely new compounds cannot be reliably determined based solely on internal validation, as it relies on the same compounds utilized in its development. To contend with this matter, external validation becomes instrumental. In this scenario, the dataset is partitioned into training and test sets, comprising 309 and 10 drugs, respectively. The model is constructed using the training set and subsequently validated using the independent test set, ensuring its adaptability to novel compounds. The accuracy of the multivariate interpolation model for the external validation set was evaluated by estimating LD_{50} values for the selected drugs using the input MW and AlogP values. To quantify the accuracy, we employed the ACC metric, which measures the proportion of correct predictions made by the model out of the total number of predictions. Using the interpolation polynomial, we categorized the drugs based on the ranges provided in Table 2. For instance, when estimating the LD_{50} value of a drug, we applied the interpolation polynomial within the appropriate range indicated in Table 2. If the estimated LD_{50} value fell within the correct range, we considered it a correct estimate.

Conversely, if the estimated value corresponded to a different interval, it was considered a false estimate. Based on our evaluation, the model achieved an overall success rate of 86.73%. This means that out of the 309 drugs tested, we correctly predicted the category (range) for 268 drugs. The high success rate indicates the model's proficiency in accurately estimating the LD_{50} values for a significant portion of the tested drugs. Figure 4 presents a comparison between the experimental and calculated LD_{50} values of the drugs. As depicted in the graph, the results obtained through the interpolation polynomial exhibit a notable level of success.

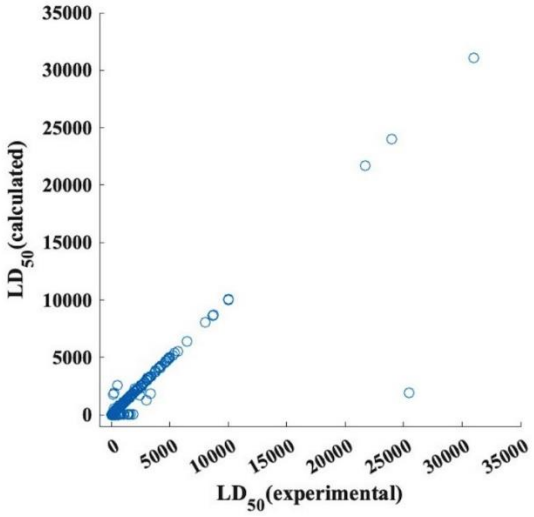

Figure 4. Experimental vs. Calculated LD_{50} values

Our analysis demonstrated that the multivariate interpolation approach yielded accurate LD_{50} predictions, with low errors suggesting a strong correlation between the estimated and actual LD_{50} values. This finding emphasizes the effectiveness of the multivariate interpolation model in estimating acute drug toxicity levels based on the molecular descriptors MW and AlogP. The ACC metric further underscores the model's capability to provide reliable estimations for assessing drug safety. The promising performance of the multivariate interpolation model highlights its potential as a valuable computational tool in drug toxicity assessment and decision-making processes during drug development.

Diversity and Distribution Analysis in Chemical Space

Diversity and distribution analysis in chemical space refers to characterizing the variety and arrangement of molecules within a multidimensional space defined by their structural and chemical properties. It involves assessing the coverage and dispersion of molecules in this space, aiming to understand their relationships, similarities, and differences.

Diversity analysis focuses on measuring and quantifying the variety of molecules in a dataset. The analysis aims to clarify the distribution of a broad range of structural features, functional groups, and physicochemical properties of molecules in a dataset. Different diversity metrics can be used to assess the dissimilarity or similarity between molecules. We used the Tanimoto similarity index for the diversity analysis. The Tanimoto similarity is calculated based on the presence or absence of specific structural features or molecular descriptors in two compounds [25]. By evaluating diversity, we can determine if the dataset adequately represents the chemical space of interest. Upon calculating the similarity value, we obtained a Tanimoto coefficient of 0.174. This result indicates a substantial chemical diversity, as it is closer to 0 within the range of Tanimoto similarity values.

Distribution analysis involves examining the arrangement and clustering of molecules within chemical space. It aims to identify regions that are densely populated with molecules, as well as sparse or unexplored regions. Distribution analysis can be performed using visualization techniques. These methods help visualize the distribution patterns and identify clusters or subgroups of molecules with similar characteristics. We used chemical space mapping for chemical space distribution via molecular MW and AlogP values of each compound. We can explore the relationships between variables and patterns for MW and AlogP values in Figure 5.

The examination of MW reveals that the lowest observed value is 46.04, while the highest value recorded is 1201.84. This wide range of MW values indicates the presence of diverse molecular sizes within the dataset. Additionally, the analysis of AlogP demonstrates a range spanning from -9.3091 to 4.1574. The observed variation in AlogP values signifies a wide diversity of hydrophobicity or

lipophilicity among the molecules. Interestingly, the ranges for MW and AlogP exhibit similar patterns, suggesting that these properties are correlated and within the same chemical domain. The similarity in their ranges indicates that molecules with different MWs also possess a diverse range of AlogP values, implying that their hydrophobic or lipophilic characteristics are not dependent solely on their MW. This finding has significant implications in various scientific domains, particularly in drug discovery. Understanding the relationship between MW and AlogP allowed us to assess the chemical space more comprehensively, enabling the design and selection of compounds with desired molecular properties. Furthermore, this knowledge aids in exploring structure-activity relationships and identifying molecular scaffolds or substructures that contribute to specific MW and AlogP ranges.

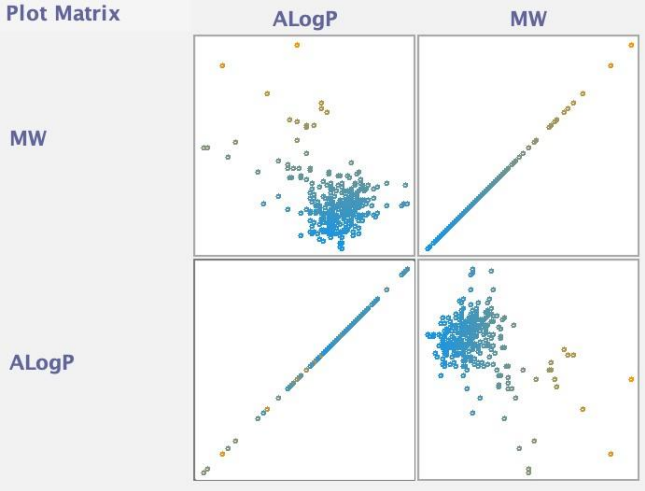

Figure 5. Plot matrix for MW and AlogP

The Comprehensive Data Regarding the Descriptors Chosen for Our Optimal Model

The process of selecting attributes is a crucial stage in machine learning modeling, as it involves identifying the significant descriptors of chemicals to attain optimal performance. Prediction models are created by utilizing various combinations of features in the descriptor pool [26]. We constructed the best-performing model using the identifiers with the highest success rate. In this study, we conducted trials with different combinations of descriptors and developed our most robust model by utilizing two specific descriptors that exhibited the highest predictive capacity. Our top model for predicting the acute oral LD_{50} value range of pharmaceuticals in mice incorporated the identifiers from the Constitutional Descriptors and Molecular Properties classes.

Constitutional Descriptors are commonly employed in QSTR modeling studies [27]. In our mathematical model, the MW is one of the most important attributes among the Constitutional Descriptors. MW represents the mass of a molecule and provides information about its size and structural complexity. The MW of a compound plays a significant role in determining its pharmacokinetic/toxicokinetic properties. As a result, the MW serves as an important factor in comprehending the behavior of a molecule within the biological system [19].

Molecular Properties identifiers have been employed in QSTR modeling studies specifically targeting acute oral toxicity in rodents [28]. Another significant descriptor in our model, the ALogP descriptor, belongs to the Molecular Properties class. ALogP stands for the predicted logarithm of the partition coefficient between octanol and water. The partition coefficient measurement determines a compound's distribution between the hydrophobic and hydrophilic phases. AlogP provides information on the potential of a compound to accumulate in adipose tissue and its permeability across biological barriers [5]. Several studies in the field of QSTR have established a correlation between chemical properties related to solubility in water or lipids and the toxicological effects of compounds [29]. In line with this, descriptive data associated with lipophilicity have been demonstrated to contribute to the

prediction models of acute toxicity. Based on a comprehensive literature analysis and our findings, we claimed that MW and AlogP are fundamental factors significantly contributing to establishing and refining acute oral toxicity models. This relationship could be attributed to processes such as absorption, excretion, and bioaccumulation of chemicals in tissues.

The Strengths and Limitations of the Optimal Model

In conventional acute toxicity studies, various compound doses are administered to experimental subjects before determining the LD_{50}/LC_{50} value of a new drug molecule. Due to the lack of knowledge regarding the toxicological effects of the novel molecule, a broad spectrum of doses can be employed. In this process, animal experiments are performed for each dose until an optimal dose is determined. The Organization for Economic Co-operation and Development (OECD) has established three acute oral toxicity procedures that rely on the utilization of experimental animals. These procedures, known as OECD-420 Fixed Dose Procedure, OECD-423 Acute Toxic Class Method, and OECD-425 Up-and-Down-Procedure, serve as standardized approaches for assessing the acute oral toxicity of substances. The reason for the publication of multiple procedures is to reduce the number of animals used and also to provide a more accurate prediction of acute toxicity. Today, ongoing research aims to minimize the use of animal models in acute toxicity testing. In this context, the OECD has published the "Acute Oral Toxicity: OECD-425 Up-and-Down Procedure" to substitute conventional acute toxicity tests with approaches that involve a reduced number of laboratory animals [30]. Adopting the perspectives of health authorities regarding the reduction of animal experimentation, we aimed to conduct preliminary studies of acute toxicity testing using mathematical models. Before conducting animal experiments to determine the LD_{50}/LC_{50} range, we propose the implementation of preliminary mathematical trials similar to our model for dose adjustment. By employing mathematical methods, dose reduction can be achieved to ensure drug safety, and ultimately, a final LD_{50}/LC_{50} value can be established through animal experiments. As a result, the use of laboratory animals can be significantly reduced while ensuring drug safety.

From a technical point of view, enhanced prediction accuracy is one of the advantages of using the interpolation technique for acute drug toxicity, specifically predicting drug LD_{50}/LC_{50} with computational modeling. Researchers can refine and optimize the model using multivariate interpolation, improving prediction accuracy for drug LD_{50}/LC_{50} values. This enables more precise assessments of a drug's acute toxicity potential, aiding in early-stage drug development and regulatory decision-making. Another advantage we can count on is cost and time efficiency. Computational modeling offers a more time and cost-effective alternative to traditional experimental methods for determining drug LD_{50}/LC_{50}. Our mathematical approach allows researchers to streamline the modeling process, reducing the need for extensive and expensive animal testing, saving resources, and accelerating drug evaluation timelines. One of the most important advantages of using this technique is reduced reliance on animal testing. This technique contributes to the reduction of animal testing in toxicological research. Using computational modeling, researchers can minimize the ethical concerns associated with animal experimentation, promoting more humane research practices while maintaining scientific rigor.

There are also disadvantages besides the advantages of using an interpolation technique for acute toxicity. The greatest challenge in applying mathematical modeling is the complexity and expertise requirements. Multivariate interpolation for drug LD_{50}/LC_{50} prediction involves complex mathematical modeling techniques. It requires expertise in computational modeling and statistical analysis, which may limit accessibility for researchers without the necessary skills or resources. Continuous model improvement is another point to note. Using a mathematical equation necessitates ongoing efforts to improve and validate the model. This includes incorporating new data, refining the model's parameters, and accounting for evolving scientific knowledge. Sustaining a robust and up-to-date model requires continuous research and resource allocation.

Our model is specifically designed to evaluate acute toxicity through oral administration in mice. However, since LD_{50} values can vary for the same molecule across different exposure routes, such as dermal or inhalation [13], the applicability of our model is limited in those situations. Furthermore, considering the species-specific toxicity variations, separate model scenarios should be developed for guinea pigs, rabbits, rats, or other experimental animal species.

Limiting our study to drug molecules presents both advantages and disadvantages. The presence of a well-balanced dataset, encompassing compounds with diverse physicochemical properties, reduces the occurrence of molecules outside the AD, consequently enhancing the model's predictive performance. By exclusively focusing on pharmaceuticals, we ensured a dataset with homogeneity. Nevertheless, our model overlooked the evaluation of non-pharmaceutical substances. QSTR models discussed in the literature regarding acute toxicity encompass a broad range of chemicals [31]. In contrast, our model is specifically designed for pharmaceutical molecules, which sets it apart in scope and focus.

The primary objective of modeling studies is to construct a dataset encompassing a wide range of molecules, maximizing its inclusiveness [4]. While we acknowledge the validity of this approach, we argue that it is equally important for molecules to belong to specific chemical groups to establish a reliable prediction model. The selection of descriptors based on specific chemical groups can pave the way for future molecule development studies. Considering that there are studies in the literature evaluating various chemicals for determining LD_{50}/LC_{50} values, our study, which solely focuses on the LD_{50}/LC_{50} values of drugs, takes an innovative approach.

Acute toxicity effects are complex processes arising from various biokinetic, cellular, and molecular events. Attempting to condense the intricate physiological phenomena associated with acute toxicity into a single numerical value may result in the loss of valuable information. Moreover, available data on LD_{50}/LC_{50} values exhibit significant variability due to variations in experimental protocols, animal species, strains, and laboratories. This variability undermines the reliability and reproducibility of acute toxicity measurements. Consequently, these challenges complicate the modeling process and lead to a relatively limited number of QSTR models for predicting acute oral toxicity compared to other endpoints [8]. However, the disadvantage mentioned in this section applies not only to mathematical modeling studies but also to animal experiments, where the LD_{50}/LC_{50} value is traditionally determined. LD_{50}/LC_{50} values have been used to initially assess relative toxicity among chemicals [4]. This issue can be addressed by integrating non-animal-based prediction models and diverse animal models and incorporating various exposure scenarios. It is worth noting that the dataset we used for our study lacks inorganic chemicals and salt structures, which could be an area for improvement in future research. As a result, our models could not provide predictions for these substances. The substances currently utilized as active drug ingredients were excluded from the evaluation.

In conclusion, the LD_{50}/LC_{50} value, representing the dosage at which 50% of specific test subjects experience fatality, is critical for assessing acute toxicity during drug development. The LD_{50}/LC_{50} test assesses the toxic effects of drugs on human health, establishing appropriate dosage regimens and ensuring their safe usage. Due to ethical considerations, traditional animal-based methods in acute toxicology studies are being replaced by mathematically based approaches. Our model has successfully predicted the five toxicologic endpoints of regulatory significance related to the acute oral toxicity of pharmaceuticals in mice. The endpoints are critical to regulatory regimes since it serves as the foundation for chemical toxicological categorization. We have argued that the current mathematical approach holds promise in assessing the LD_{50}/LC_{50} value of drug candidates during the early stages of drug development. This means new pharmaceuticals can be synthesized more cost-effective, timely, and safely. Cutting-edge models, such as ours, have the remarkable potential to significantly reduce the necessity for animal testing in toxicological research, thereby addressing ethical concerns. Reliable and validated *in silico* techniques can be utilized as an initial step in calculating the LD_{50}/LC_{50} range of drugs, serving as a valuable tool in early toxicity assessment. In conclusion, the presented mathematical model offers a reliable and practical means for estimating the LD_{50}/LC_{50} values of drugs in mice.

AUTHOR CONTRIBUTIONS

Concept: G.K., F.K.Ç.; Design: G.K., F.K.Ç.; Control: G.K., F.K.Ç.; Sources: G.K., F.K.Ç.; Materials: G.K., F.K.Ç.; Data Collection and/or Processing: F.K.Ç.; Analysis and/or Interpretation: G.K., F.K.Ç.; Literature Review: G.K., F.K.Ç.; Manuscript Writing: G.K., F.K.Ç.; Critical Review: G.K., F.K.Ç.; Other: F.K.Ç.

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

TRADITIONAL USES OF MEDICINAL PLANTS IN ERZİNCAN PROVINCE, TÜRKİYE

ERZİNCAN (TÜRKİYE) İLİNDEKİ TIBBİ BİTKİLERİN GELENEKSEL KULLANIMLARI

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ABSTRACT

Objective: *This research was carried out to record the usage of plants, parts of this plant used, and methods of preparation by people living in 10 districts and 58 villages of Erzincan province.*
Material and Method: *The medicinal plant species used by the local people for treatment, was collected and determined. All traditionally used information was collected; herbarium materials were prepared, They are deposited in Erzincan Binali Yıldırım University herbarium (EBYU), Erzincan Binali Yıldırım University.*
Result and Discussion: *A total of 100 medical plants taxa pertaining to 39 families were defined in this research. Out of these, 88 species grew naturally, while 12 species were cultivated. The most widespread plant families were Asteraceae (14), Lamiaceae (8), and Rosaceae (14). Infusion was the most widely used preparation method. The utilization of traditional medicine was still extensive among the people in Erzincan. However, through increscent health service facilities in region, herbal medicine seemed to be more related to health care and illness prevention than curation. There is also the loss of traditional knowledge as it receives new immigrants. There is a gradual loss of traditional knowledge on the use of medicinal plants, both in younger generations and due to migration.*
Keywords: *Erzincan, ethnobotany, ethnopharmacology, medicinal plants, Türkiye*

ÖZ

Amaç: *Bu araştırma, Erzincan ilinin 10 ilçesi ve 58 köyünde yaşayan halkın bitkinin kullanım alanlarını, bu bitkinin kullanılan kısımlarını ve hazırlanma yöntemlerini kayıt altına almak amacıyla yapılmıştır.*
Gereç ve Yöntem: *Yöre halkının tedavi amacıyla kullandığı şifalı bitki türleri toplanarak, belirlenmiştir. Geleneksel olarak kullanılan tüm bilgiler toplanmış, herbaryum materyalleri hazırlanmış, Erzincan Binali Yıldırım Üniversitesi herbaryumunda (EBYU) depolanmıştır.*
Sonuç ve Tartışma: *Bu araştırmada 39 familyaya ait toplam 100 tıbbi bitki taksonu tanımlanmıştır. Bunlardan 88 türün doğal, 12 türün ise kültür bitkisi olduğu tespit edilmiştir. En yaygın bitki familyaları Asteraceae (14), Lamiaceae (8), Rosaceae (14) olarak gözlenmiştir. İnfüzyon en yaygın kullanılan hazırlama yöntemidir. Erzincan'da halk arasında geleneksel tıbbın kullanımı hâlâ*

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yaygındır. Bununla birlikte, bölgede artan sağlık tesisleri sayesinde, bitkisel ilaç, iyileştirmeden ziyade sağlık bakımı ve hastalıkları önlemede kullanıldığı tespit edilmiştir. Bölgede yeni göç ve genç nüfus arttığı gözlenmiştir. Hem genç nesillerde hem de göç nedeniyle şifalı bitkilerin kullanımına ilişkin geleneksel bilgide kademeli bir kayıp tespit edilmiştir.
Anahtar Kelimeler: *Erzincan, etnobotani, etnofarmakoloji, tıbbi bitkiler, Türkiye*

INTRODUCTION

Plants are a resource that has been used as both a protective/therapeutic and a tool throughout human history [1]. In Türkiye, one of the world's more important biodiversity, more than 30% of the about 12000 vascular plant taxa are endemic (about 4000), which is more than the number of endemic species in European countries (1352) [2-4]. Türkiye provides a variety of ecosystems due to its geographical location, climate, geology, soil and water resources, and ecological benefits such as being on bird migration routes [5-7]. Due to the diversity of the flora and fauna, the Anatolian people have had a rich source of medical plants and animal remedies for a long time, and as a result, valuable folk medicine knowledge has been acquired in the district and countryside [8].

Eastern Anatolian flora also shows diversity due to its different ecological regions, geographical differences and different climates. Erzincan Province, located in the transitional zone among the Eastern Black Sea, Eastern Anatolia, and Central Anatolia regions, is considered to be one of the most significant centers of genetic diversity and endemism in Türkiye [9]. The number of ethnobotanical studies in Erzincan province is very few. These studies have been completed in a specific region or local markets, not the general of Erzincan province (Üzümlü, Tercan, Kemah, and İliç districts) [9-12,27]. The current study was conducted to document the utilization of medicinal plants, plant parts used, and methods of preparation by individuals living in Kemah (10 villages), Üzümlü (5 villages), Tercan (6 villages), Çayırlı (4 villages), Otlukbeli (2 villages), Refahiye (9 villages), İliç (8 villages), Kemaliye (9 villages) and the center (5 villages) of Erzincan province.

MATERIAL AND METHOD

The Study Area

Erzincan province is located in Türkiye's Eastern Anatolia Region and is grouped into the B7 square. It is part of the Iran-Turanian Plant Geography Region. It is an eastern Anatolian province with a population of 239.223 and a surface area of 11.903 km² in 2023 [13-15]. Erzurum to the east, Bayburt to the north, Gümüşhane to the northwest, Tunceli to the south, and Sivas to the west surround Erzincan province (Figure 1).

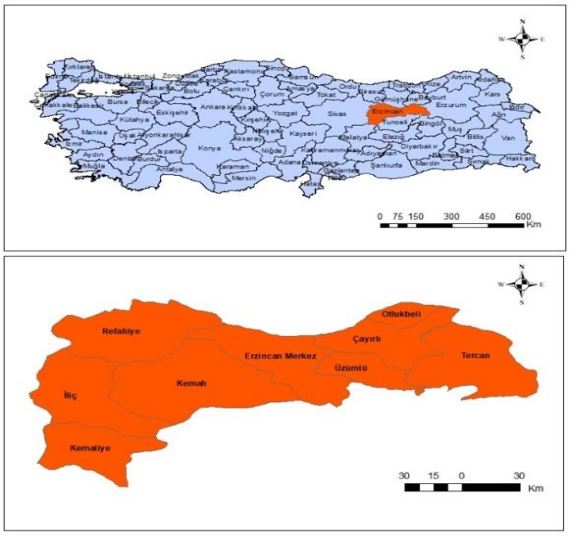

Figure 1. Geographical location of the investigation region

Data Collection

The ethnopharmacological information was gathered in the field by conducting structured and semistructured interviews with all competent people from 58 villages, including Harmankaya (1), Gözaydın (2), Apçaağa (3), Ocak (4), Yuvalı (5), Başbağlar (6), Yeşilyamaç (7), Kocaçimen (8), Salihli (9), Akdoğu (10), Uluyamaç (11), Büyükköy (12), Sabırlı (13), Ortatepe (14), Ağıldere (15), Büyükarmutlu (16), Balkaya (17), Kureyşli sarıkaya (18), Sansa (19), Pelitli (20), Bayırbağ (21), Yuvalı (22), Kemerçam (23), Ağören (24), Üçpınar (25), Konarlı (26), Yaylım (27), Başköy (28), Yaylakent (29), Yaylalar (30), Cennetpınarı (31), Baltaşı (32), Dereköy (33), Sarıyazı (34), Beşikli (35), Aktaş (36), Karacalar (37), Ayranpınarı (38), Yahşiler (39), Kayabaşı (40), Eriç (41), Muratboynu (42), Küçükotlukbeli (43) Avcıçayırı (44), Gemecik (45), Sarıkoç (46), Dıştaş (47), Yurtbaşı (48), Gümüşakar (49), Kürelik (50), Gazipınarı (51), Kılıçkaya (52), Mecidiye (53), Başpınar (54), Kalecik (55), Geyikli (56), Akarsu (57), Şahaloğlu (58). Because this is a Ministry of Agriculture and Forestry project, The settlements were chosen by the Ministry of Agriculture and Forestry. settlements. A total of 150 people were interviewed face-to-face, including midwives, shepherds, foresters, farmers, healers, beekeepers, housewives, teachers, headmen, and plant collectors. While 46 of the informants were female (30.67%), the remaining 104 were male (69.33%). A questionnaire was filled out with the participants, and video photographs and audio recordings were taken during the interview, again with the permission of the participants. The interviews with the participants were randomly selected (tea houses, mosque garden, house, field, plateau etc.). During the study, the local name of the plant, the therapeutic effect of the plant, the part(s) of the plant used, and the preparation/application methods were learned from the participants.

Plant Materials

In 2022 and 2023, the plant samples were collected from the villages. The authors, Mustafa KORKMAZ and Sercan ÇORLU, pressed and described scientific names of the collected samples using the Flora of Turkey and the East Aegean Islands, the Turkish Plants List (Vineacious Plants), the Flora of the USSR, and Flora Europaea [16-19]. The scientific names of plant species are given with reference to the plant list [20]. Voucher specimens were stored in Erzincan Binali Yıldırım University Herbarium (EBYU).

Ethnobotanical Index

The UV (use value of a species) index was calculated using the formula UV=ΣUi/N, where Ui is the number of use reports stated by significant percentage for a taxon and N is the number of sources [21-23].

RESULT AND DISCUSSION

Interviews were used to capture the demographic characteristics of the participants in the field research. A total of 150 individuals from diverse backgrounds, including academicians, beekeepers, farmers, retirees, religious officers, engineers, teachers, housewives, shepherds, and individuals engaged in the collection of medicinal plants, were subjected to face-to-face interviews. Among the informants, 104 were female (69.33%), while the remaining 46 were male (30.67%). A total of 150 people (46 women and 104 men) were contacted. 2 under the age of 19, 10 between the ages of 19-35, 16 between the ages of 36-49, 84 between the ages of 50-70, and 38 above the age of 70. 13 of the participants had never attended a formal educational institution (Table 1). All the informants are native of and still living in Tercan, Otlukbeli, Çayırlı, Üzümlü, Kemah, Refahiye, İliç, and Kemaliye districts (Erzincan, Türkiye). It has been observed that most of the people who use medicinal plants are in the age range of 50-70 years. Again, it has been determined that most of the users of medicinal plants are men with primary school education and below (Table 1). In the ethnobotanical study conducted in Erzurum, a province characterized by substantial cultural and socioeconomic interaction with Erzincan, it was observed that individuals aged 50-70 derived the greatest benefits from medicinal plants [21,32]. In addition, a similar situation was reported in ethnobotanical studies conducted on Bayburt, Gümüşhane, and Sivas provinces, which are neighboring provinces of Erzincan [24-26]. It is understood that women

attach more importance to the use of medicinal plants in Erzincan, as in other provinces of Eastern Anatolia (Erzurum, Van, Elazığ, Bingöl and Tunceli) and Eastern Black Sea region (Bayburt, Gümüşhane, and Trabzon) [21,24-28,32-34].

Table 1. The demographic profile of the participants

Demographic Characteristics	Number
Age Range	
Below 19	2
19-35	10
36-49	16
50-70	84
70 and above	38
Sex	
Women	46
Men	104
Educational Levels	
Illiterate	13
Literate	6
Primary school	80
Secondary school	22
High school	19
University	8
PhD	2

A total of 100 medicinal plant taxa from 39 plant families were gathered in the Erzincan province of Türkiye (Table 2). The most common medicinal plant families were Asteraceae (14), Rosaceae (14), Lamiaceae (8), Apiaceae (5), Adoxaceae (5), Malvaceae (5), Amaryllidaceae (4), and Polygonaceae (4). A total of 100 medicinal plant taxa were collected in Erzincan and they belong to 39 different plant families. There are 88 wild species and 12 cultivated plants among them. Table 2 list the 100 herbs defined in the area, organized alphabetically by family and botanical name. According to our results, the most used taxa are *Helichrysum arenarium*, *Cephalaria procera*, *Juglans regia*, *Origanum acutidens*, *Malva neglecta*, *Tilia tomentosa*, *Thymus pseudopulegioides*, *Morus nigra*, *Plantago major*, *Pinus nigra*, *Rumex pationtia*, *Rumex ponticus*, *Rheum ribes*, *Crataegus monogyna*, *Solanum tuberosum*, and *Urtica dioica*. In the fields of ethnobotany and ethnopharmacology, various studies have been conducted in different regions to document the rich diversity of plant species and their traditional uses. In the Bayburt province, Kadıoğlu et al. conducted a comprehensive ethnobotanical study, identifying a total of 92 taxa from 36 families [24]. Similarly, in the Gümüşhane province, Akbulut and Zengin undertook ethnobotanical research, documenting 74 taxa that represented 38 distinct families [25]. Furthermore, in Sivas, another survey revealed the presence of 100 taxa spanning 38 different families [26]. Likewise, in the Erzurum province, an ethnobotanical study recorded 99 taxa belonging to 38 distinct families [21]. On the other hand, when studies in the Eastern Anatolia and Eastern Black Sea 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 [21,24-28, 32-34].

The most widely utilized plant organs to prepare remedies were the aerial parts (52), leaves (30), fruits (29), roots (5), seeds (9), latex (7), bulbus (6), and Bark (3), Resina (3), and Rhizoma (3) although bark, flowers, pix liquida, tuber, and stylus were also utilized in some remedies. Similarly, in studies conducted in the surrounding provinces of Erzincan (Erzurum, Bayburt, Gümüşhane, and Sivas), it was recorded that the above-ground parts, leaves and fruits of plants used for medicinal purposes were mostly used [21,24-26]. On occasion, local people also utilized other components, such as butter, and milk to prepare remedies. The major methods for preparing remedies were infusion (55), decoction (30), raw

(26), heating (11), crushing (6), and boiling (4) (Table 2). Remedies were mostly taken internally (57%). The dosage of the medicinal preparations was often not accurate (e.g., one "pinch", one spoon).

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

Family	Plant species	Local name and Herbarium Number	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Adoxaceae	*Sambucus ebulus* L.	Mürver, Patrik (EBYU- 4643)	Lea	Inf	Int	Nausea, Immunostimulant, Catarrh,	0.007
Adoxaceae	*Sambucus nigra* L.	Kara mürver, Patrik (EBYU-4575)	Lea	Inf	Int	Immunostimulant, Venous insufficiency	0.020
Adoxaceae	*Viburnum lantana* L.	Gilaburi, Gilaburin, Girabur (EBYU- 4574)	Fru	Row	Eat	Renal calculi	0.007
Adoxaceae	*Viburnum opulus* L.	Gilaburi, Gilaburin, Girabur (EBYU- 4593)	Fru	Row	Eat	Renal calculi	0.013
Adoxaceae	*Centaurea carduiformis* DC.	Galagan (EBYU-4572)	See	Row	Eat	Venous insufficiency	0.007
Amaranthaceae	*Beta vulgaris* var. *vulgaris* L.	Pancar, Kızılca (EBYU- 4476)	Aer	Inf	Int	Constipation	0.013
Amaryllidaceae	*Allium vineale* L.	Çayır sarımsağı (EBYU- 4504)	Bul	Inf	Int	Tonsillitis	0.013
Amaryllidaceae	*Allium tuncelianum (Kollmann)* Özhatay, B.Mathew & Şiraneci	Dağ Sarımsağı (EBYU- 4480)	Bul	Inf	Int	Antidiabetic, Antihypertensive	0.027
			Bul	Cru	Ext	Otitis, Fruncle	0.020
Amaryllidaceae	**Allium cepa* L.	Soğan, Pizaf (EBYU- 4511)	Bul	Raw	Eat	Antihypertensive, Toothache	0.027
			Bul	Raw	Ext	Wounds	0.027
			Bul	Hea	Ext	Fruncle	0.020
			Bul	Inf	Int	Tonsillitis, Catarrh, Galactagogue,Antibacterial	0.033
Amaryllidaceae	**Allium sativum* L.	Sarımsak (EBYU- 4680)	Bul	Raw	Eat	Hypertension	0.027
			Bul	Cru	Ext	Scorpion Sting	0.007
Apiaceae	*Prangos ferulacea* (L.) Lindl.	Çaşur, Çarşır, Çakşır (EBYU- 4487)	Roo	Dec	Int	Antidiabetic	0.027
			Aer	Inf	Int	Antihelmintic, Galactagogue	0.027
			Aer	Dec	Int	Laxative, Immunostimulant,	0.007
Apiaceae	*Daucus carota* L.	Ezelteri (EBYU- 4578)	Aer	Inf	Int	Headache	0.007
Apiaceae	*Anethum graveolens* L.	Samid, Dere otu (EBYU- 4639)	Aer	Inf	Int	Hemorrhoid	0.013
Apiaceae	*Eryngium billardierei* F.Delaroche	Çahır dikeni (EBYU- 4493)	Fru	Dec	Int	Heart failure	0.007
Apiaceae	*Pimpinella anisum* L.	Anason (EBYU- 4545)	Fru	Inf	Int	Flatulence	0.033
Asteraceae	*Anthemis cretica* L.	Papatya (EBYU-4566)	Aer	Dec	Ext	hair dye	0.013
			Aer	Inf	Int	Antidepressant, Diuretic, Sinusitis	0.040
Asteraceae	*Gundelia tournefortii* L.	Kengel, Kenger (EBYU- 4641)	Lat	Raw	Che	Flatulence, Immunostimulant, Sedative	0.010
			Lat	Dec	Int	Antidiabetic,	0.007
			Lat	Dec	Ext	Wounds	0.033
Asteraceae	*Helichrysum arenarium* subsp. *aucheri* Boiss.	Ölmez otu, Ana fatma Çiçeği (EBYU- 4642)	Aer	Inf	Int	Immunostimulant, Antidiabetic, Laxative, Diuretic, Peptic ulcer, Renal Calculi, Antitussive	0.087
			Aer	Inf	Ext	Aphta	0.047
Asteraceae	*Tragopogon dubius* Scop.	Yemlik (EBYU- 4494)	Lea	Raw	Eat	Laxative	0.047

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

Family	Plant species	Local name and Herbarium Number	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Asteraceae	*Achillea biebersteinii* Afan.	Civan perçemi (EBYU- 4526)	Aer	Dec	Int	Flatulence, Amenorrhea, Dysmenorrhea	0.020
Asteraceae	*Achillea millefolium* L.	Kılıç otu, Hılıç otu (EBYU-4563)	Lea	Raw	Ext	Hemostatic	0.013
Asteraceae	*Tripleurospermum monticolum* (Boiss. & A.Huet) Bornm.	Yabani papatya (EBYU- 4496)	Aer	Inf	Ext	Conjunctivitis	0.013
Asteraceae	*Scorzonera tomentosa* L.	Alven (EBYU- 4539)	Lat	Raw	Ext	Wounds	0.007
Asteraceae	*Tagetes patula* L.	Kadife Çiçeği (EBYU- 4586)	Aer	Raw	Ext	Wounds	0.007
Asteraceae	*Artemisia absinthium* L.	Süpürge otu, Yeşil ot (EBYU- 4586)	Aer	Raw	Ext	İnsecticide	0.007
			Aer	Inf	Int	Antidiabetic	0.007
Asteraceae	*Taraxacum officinale* Weber ex Wiggers.	Karahindiba, Sarı çiçek (EBYU- 4516)	Aer	Inf	Int	Immunostimulant	0.007
			Roo	Dec	Int	Hepatoprotective	0.047
Asteraceae	*Taraxacum buttleri* Soest	Karahindiba, Sarı çiçek (EBYU- 4577)	Lea	Raw	Che	Aphta	0.007
Asteraceae	*Cichorium intybus* L.	Beyazhindiba (EBYU- 4588)	Aer	Inf	Int	Immunostimulant	0.013
Asteraceae	*Cota tinctoria* (L.) J. Gay	Sarı papatya (EBYU- 4621)	Aer	Inf	Int	Expectorant	0.007
Asteraceae	*Tussilago farfara* L.	Öksürük otu (EBYU- 4620)	Aer	Inf	Int	Anti-İnflammatory, Expectorant	0.070
Berberidaceae	*Berberis crataegina* DC.	Karamuk (EBYU- 4674)	Lea	Dec	Int	Antidiabetic, Antiparasitic	0.070
			Roo	Dec	Int	Antiparasitic	0.013
			Fru	Inf	Int	Asthma, Antidiabetic,	0.047
Boraginaceae	*Alkanna tinctoria* (L.) Tausch.	Havaciva otu, Kök boya (EBYU- 4510)	Roo	Boi	Ext	Wounds	0.020
Brassicaceae	**Brassica oleracea* L.	Lahana	Lea	Boi	Ext	Plantar fasciitis	0.027
Caprifoliaceae	*Cephalaria procera* Fisch. Et Lall.	Guling, Gulinga, Pelemir (EBYU- 4581)	Aer	Raw	Ext	Hemostatic	0.093
Cornaceae	*Cornus mas* L.	Kızılcık (EBYU- 4607)	Fru	Inf	Int	Immunostimulant, Antidiabetic, Nausea, Antidiarrheal	0.033
Cucurbitaceae	**Cucumis sativus* L.	Salatalık	Fru	Raw	Ext	Wounds	0.007
Cupressaceae	*Juniperus foetidissima* Willd.	Ardıç (EBYU- 4534)	See	Raw	Int	Asthma, Hepatoprotective	0.040
			Lea	Inf	Int	Antihypertensive	0.033
			Lat	Hea	Ext	Furuncle	0.040
Cupressaceae	*Juniperus excelsa* M. Bieb.	Ardıç (EBYU- 4628)	See	Dec	Int	Antidiarrheal, Asthma	0.027
Elaeagnaceae	*Elaeagnus angustifolia* L.	İğde (EBYU- 4529)	Lea	Inf	Int	Antidiabetic, Tonsillitis	0.013
Equisetaceae	*Equisetum arvense* L.	Kırkkilit otu, boğumotu (EBYU-4571)	Aer	Inf	Ext	Lumbal hernia	0.013
Euphorbiaceae	*Euphorbia macroclada* Boiss.	Sütlegen, Sütleğen (EBYU- 4584)	Aer	Dec	Ext	Wounds	0.007
Euphorbiaceae	*Euphorbia seguieriana* Necker	Sütlegen, Sütleğen (EBYU- 4484)	Lat	Hea	Ext	Wounds, Verruca vulgaris, Bee sting	0.047
Fabaceae	*Coronilla orientalis* Miller var. *orien talis* (All.) Vitman	Sarı çiçek (EBYU- 4592)	Aer	Raw	Ext	Antibacterial	0.007
Fagaceae	*Glycyrrhiza glabra* L.	Bayam kökü, meyan kökü (EBYU- 4626)	Roo	Dec	Int	Renal Calculi, Peptic ulcer, Nausea	0.020
Fagaceae	*Querceus pubescens* Willd.	Meşe (EBYU-4569)	Bar	Dec	Int	Antidiarrheal	0.020
			Bar	Hea	Ext	Otitis	0.013

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

Family	Plant species	Local name and Herbarium Number	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Gentianaceae	*Centaurium erythraea* Rafn.	Kırmızı Kantaron (EBYU- 4482)	Aer	Inf	Int	Peptic ulcer	0.033
Hypericaceae	*Hypericum scabrum* L.	Sarı Kantaron (EBYU- 4528)	Aer	Mas with Olive Oil	Ext	Wound, Rheumatic pain	0.067
			Aer	Raw	Ext	Hemostatic	0.033
			Aer	Mas with Olive Oil	Int	Peptic ulcer, Sedative, Hipnotic	0.067
			Aer	Inf	Int	Nausea	0.013
Juglandaceae	**Juglans regia* L.	Ceviz	Fru	Row	Ext	Scorpion Sting	0.080
Lamiaceae	*Melissa officinalis* L.	Oğul otu, Melisa (EBYU- 4530)	Aer	Inf	Int	Sedative, Hipnotic	0.013
Lamiaceae	*Mentha longifolia* (L.) Hudson subsp. *longifolia*	Nane (EBYU- 4549)	Aer	Inf	Int	Gastroesophageal reflux	0.020
Lamiaceae	*Origanum acutidens* (Hand.-Mazz.) Ietswaart	Annuk, Zahter (EBYU- 4546)	Aer	Inf	Int	Catarrh	0.14
Lamiaceae	*Salvia argentea* L.	Dadırgan (EBYU- 4604)	Aer	Raw	Int	Erectile dysfunction	0.013
Lamiaceae	*Salvia sclarea* L.	Potporik, Tortum (EBYU- 4521)	Aer	Inf	Int	Antidiarrheal	0.07
Lamiaceae	*Salvia spinosa* L.	Adaçayı (EBYU- 4644)	Aer	Inf	Int	Nausea	0.07
Lamiaceae	*Teucrium polium* L. subsp.*polium*	Bitotu (EBYU-4561)	Aer	Inf	Int	Headache, Asthma	0.013
Lamiaceae	*Thymus pseudopulegioides* Klokov & Des.-Shost.	Geven (EBYU- 4602)	Aer	Inf	Int	Immunostimulant	0.200
Malvaceae	**Abelmoschus esculentus* (L.) Moench	Bamya	See	Hea	Ext	Rheumatic pain	0.020
			See	Cru	Ext	Hemostatic	0.007
Malvaceae	*Alcea apterocarpa* (Fenzl) Boiss.	Hatmi çiçeği (EBYU-4554)	Lea	Inf	Int	Nausea, Catarrh	0.007
Malvaceae	*Althaea armeniaca* Ten.	Gülfatma Çiçeği (EBYU- 4666)	Aer	Boi	Ext	Fruncle	0.067
Malvaceae	*Malva neglecta* Wallr.	Ebe gömeci, Ebe gümeci (EBYU- 4475)	Lea	Raw	Ext	Urticaria, Hemostatic	0.107
			Lea	Boi	Ext	Rheumatic Pain, Lumbal hernia	0.060
			Lea	Inf	Int	Expectorant, Urinary tract infection	0.113
Malvaceae	*Tilia tomentosa* Moench	Ihlamur (EBYU-4552)	Flo	Inf	Int	Expectorant, Nausea, Catarrh	0.167
Moraceae	*Morus alba* L.	Beyaz dut (EBYU- 4503)	Fru	Raw	Eat	Tonsilitis, Tuberculose	0.087
			Fru	Dec	Int	Tonsilitis	0.080
			Fru	Raw	Ext	Eczema	0.067
			Fru	Inf	Int	Eczema, Galactagogue	0.033
			Lea	Inf	Int	Immunostimulant, Asthma	0.067
Moraceae	*Morus nigra* L.	Kara dut (EBYU-4553)	Fru	Cru	Gar	Aphta	0.113
Paeoniceae	*Paeonia mascula* subsp. *mascula* (L.) Miller	Ağgül, Ayıgülü (EBYU- 4519)	Aer	Inf	Int	Antidiabetic, Laxative	0.067
Papaveraceae	*Papaver rhoeas* L.	Gelincik (EBYU- 4518)	Flo	Inf	Int	Antitussive	0.080
Pinaceae	*Pinus nigra* L.	Karaçam (EBYU- 4591)	Fru	Dec	Int	Nausea, Catarrh	0.120
			Pix	Hea	Ext	Wound	0.080
			Res	Hea	Int	Peptic ulcer	0.033

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

Family	Plant species	Local name and Herbarium Number	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Pinaceae	*Pinus brutia* L.	Sarıçam (EBYU -4501)	Res	Hea	Int	Tuberculose, Wounds	0.060
			Fru	Dec	Int	Antidiabetic, Asthma	0.033
Plantaginaceae	*Plantago major* L. subsp. *major*	Sinirli ot, Keçi dili, Hayş, Damarlı ot (EBYU- 4591)	Lea	Raw	Ext	Fruncle, Rheumatic pain, Gingivitis	0.167
			Lea	Inf	Int	Antidiabetic	0.100
Plantaginaceae	*Platanus orientalis* L.	Çınar (EBYU- 4481)	Lea	Inf	Int	Rheumatic pain	0.033
Poaceae	*Cynodon dactylon* (L.) Pers.	Ayrık otu (EBYU- 4630)	Aer	Inf	Int	Psoriasis	0.060
Poaceae	**Zea mays* L.	Mısır	Sty	Inf	Int	Diuretic	0.047
Poaceae	**Triticum aestivum* L.	Kırmızı buğday	Aer	Inf	Ext	Conjuctivitis	0.033
Polygonaceae	*Rumex pationtia* L.	Labada, Evelik (EBYU- 4512)	Lea	Cru	Ext	Fruncle	
			Lea	Inf	Int	Laxative	0.147
Polygonaceae	*Rumex ponticus* E.H.L.Krause	Tırşo, Tırşik, Evelik (EBYU- 4522)	Lea	Raw	Eat	Antidiabetic	0.133
Polygonaceae	*Polygonum cognatum* Meissn.	Madımak, Kuş ekmeği, kuş epeleği (EBYU- 4474)	Aer	Inf	Int	Antidiabetic, Urinary Tract İnfection	0.060
Polygonaceae	*Rheum ribes* L.	Eşgın, Işgın, Ribes (EBYU- 4502)	Roo	Dec	Int	Antidiabetic, Prostatic hyperplasia, Antiasthmatic	0.267
			Aer	Raw	Int	Psoriasis, Antihypertensive	0.253
Portulacaceae	*Portulaca oleracea* L.	Semiz otu, Soğukluk, Pirpirim (EBYU- 4670)	Aer	Inf	Int	Renal calculi	0.033
Ranunculaceae	*Ranunculus repens* L:	Sarı çiçek (EBYU- 4515)	Aer	Cru	Ext	Fruncle	0.033
Rosaceae	*Rosa agrestis* Savi	Kuşburnu (EBYU- 4479)	Fru	Dec	Int	Laxative, Nausea, Catarrh	0.113
Rosaceae	*Rosa canina* L.	Kuşburnu (EBYU- 4671)	Fru	Dec	Int	Nausea, Catarrh	0.047
Rosaceae	*Rosa micrantha* Borrer ex. Sm.	Kuşburnu (EBYU- 4507)	Fru	Dec	Int	Antiasthmatic, Venous stasis	0.060
Rosaceae	*Rosa dumalis* Bechst. subsp. *boissieri* (Crepin) Ö. Nilsson var. *boissieri*	Kuşburnu (EBYU- 4540)	Fru	Dec	Int	Laxative, Nausea, Catarrh	0.047
Rosaceae	*Rubus caesius* L.	Böğürtlen, Ahududu (EBYU-4514)	Fru	Raw	Int	Tonsilitis	0.047
			Roo	Dec	Int	Renal calculi	0.033
Rosaceae	*Crataegus monogyna* Jacq. subsp. *monogyna* Jacq.	Alıç (EBYU- 4492)	Fru	Dec	Int	Nausea, Catarrh, Rheumatic pain, Antiasthmatic, Antihyperlipidemic, Heart failure, Urinary Tract İnfection	0.180
Rosaceae	*Cerasus avium* L. Moench.	Mekhem, Yabani kiraz (EBYU- 4524)	Fru	Inf	Int	Rheumatic pain, Urinary Tract İnfection, Diüretic,	0.047
Rosaceae	*Cerasus mahaleb* (L.) Miller	Mehlem, mahlep (EBYU- 4523)	Rhi	Dec	Int	Immunostimulant, Asthma	0.033
Rosaceae	*Sarcopoterium spinosum* (L.) Spach	Dadaş otu (EBYU-4559)	Aer	Dec	Int	Urinary Tract İnfection	0.007
Rosaceae	**Prunus avium* L.	Kiraz	Fru	İnf	Int	Antitussive, Urinary Tract İnfection	0.020
Rosaceae	*Prunus spinosa L.*	Dağ eriği (EBYU- 4601)	Fru	Dec	Int	Antidiabetic	0.020
Rosaceae	*Cydonia oblonga* L.	Ayva (EBYU- 4627)	Lea	Inf	Int	Expectorant	0.027
			See	Mas	Ext	Wounds	0.007
Rosaceae	*Amygdalus communis* L. var. *amara* DC.	Acı badem (EBYU- 4669)	See	Raw	Eat	Antidiabetic	0.007
			Res	Hea	Ext	Wounds	0.007
Rosaceae	**Armenica vulgaris* Lam.	Kayısı	Fru	Inf	Int	Laxative	

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

Family	Plant species	Local name and Herbarium Number	Used part of the plant[a]	Prep.[b]	Adm.[c]	Use	UV
Santalaceae	*Viscum album* L.	Ökse otu (EBYU- 4654)	Lea, Bar	Dec	Int	Antidiabetic	0.013
Scrophulariaceae	*Verbascum trichostylum* Hub.-Mor.	Sığır kuyruğu (EBYU- 4490)	Aer	Inf	Int	Antitussive, Tonsilitis	0.067
			Aer	Mas	Ext	Hyperpigmentation	0.060
Solanaceae	*Hyoscyamus niger* L.	Delipatpat, Banotu (EBYU- 4614)	Aer	Hea	Inh	Antiasthmatic	0.013
Solanaceae	**Solanum tuberosum* L.	Patates	Rhi	Raw	Ext	Wounds, Rheumatic pain	0.013
			Rhi	Coo	Int	Antidiarrheal	0.200
Urticaceae	*Urtica dioica* L. subsp. *dioica*	Isırgan, Gezgezk (EBYU- 4491)	Aer	Inf	Int	Antidiabetic, Antiasthmatic, Immunostimulant, Anticarcinogen, Urinary Tract İnfection, Antihypertensive	0.247
			Aer	Dec	Ext	Alopesi areata	0.100
			Aer	Raw	Ext	Rheumatic pain	0.200
			See	Dec	Int	Diuretic, Prostatic hyperplasia, Immunostimulant	0.267
Vitaceae	**Vitis vinifera* L.	Asma, Üzüm (EBYU-4567)	Lea	Inf	Ext	Alopesi areata	0.013
Xanthorrhoeaceae	*Eremurus spectabilis* M. Bieb.	Kiriş, Çiriş (EBYU-4568)	Aer	Inf	Int	Antidiabetic	0.120
Zygophyllaceae	*Peganum harmala* L.	Üzerlik	Aer	Hea	Inh	Sedative, Hipnotic	0.053
Zygophyllaceae	*Triblus terrestiris* L.	Demir diken, Çoban çökerten (EBYU- 4655)	Aer	Inf	Int	Renal calculi	0.007

[a] Plant part(s) used: Aer: Aerial parts; Bar: Bark; Bul: Bulbus; Flo: Flowers; Fru: Fruits; Lat: Latex; Lea: Leaves; Ole: Oleum; Res: Resin; Rhi: Rhizoma; Roo: Roots; See: Seeds; Sty: Stylus; Per: Pericarp; Pix: Pis liquida; Tub: Tuber; Who: Whole plant.
[b] Preparations: Boi: Boiled; Cooked: Coo; Cru: Crushed; Dec: Decoction; Hea: Heated; Inf: Infusion; Che: Chewable; Mas:Maseration
[c] Adm.: Administration; Int: Internal use; Ext: External use; Eat: Eaten as meal; Gar: Gargle; Inh: Inhalation, Che: Chewing
*Cultivated plants

We also documented the local names of the plants indicated by the informants. In some instances, the same vernacular name was used for more than one plant species, which might lead to misunderstanding and possibly minimize safe plant use. In other cases, the same plant had more than one vernacular name (e.g., *Cephalaria procera*: guling, gulinga, pelemir; *Plantajo major*: Sinirli ot, Keçi dili, Hayş, Damarlı ot). Although most of the plant names are of Turkish origin, Kurdish names have been identified [21-23].

The authors compared their findings to those of previous comprehensive ethnobotanical research carried out in the region of Erzincan (Üzümlü, Tercan, Kemah, and İliç districts) [9-12,27]. The most frequently used medicinal plant species in Erzincan were identified as *Urtica dioica*, *Rheum ribes*, *Rumex pationtia*, *Plantago major*, *Peganum harmala*, *Morus nigra*, *Malva neglecta*, *Cephalaria procera*, and *Helichrysum arenarium.* These plants were also recorded in ethnobotanical studies conducted in certain districts (Üzümlü, Tercan, Kemah, and İliç districts) of Erzincan [9-12,27]. In addition to this information, all of the previous studies were carried out only in certain regions of Erzincan [9-12,27]. Moreover, the use of these plants for medicinal purposes has been similarly recorded in studies conducted in many provinces of Eastern Anatolia and Eastern Black Sea region [12,21,24,25].

Helichrysum arenarium (0,087), *Cephalaria procera* (0,093), *Juglans regia* (0,080), *Origanum acutidens* (0,140), *Malva neglecta* (0,113), *Tilia tomentosa* (0,167*), Thymus pseudopulegioides* (0,200), *Morus nigra* (0,113), *Plantago major* (0,167), *Pinus nigra* (0,120), *Rumex pationtia* (0,147), *Rumex ponticus* (0,133), *Rheum ribes* (0,267), *Crataegus monogyna* (0,180), *Solanum tuberosum* (0,200), and *Urtica dioica* (0,267), had the highest UVs (Table 2). The informants utilized medical plants mainly for the treatment of diabetes, immun systems, wounds and asthma. It has been determined that the number

of plants used for cardiovascular problems, prostatic hyperplasia, erectile dysfunction, and bacterial infection are the lowest. Other studies in Eastern Anatolia have observed that medicinal plants are mostly used in skin disorders and gastro-intestinal disorders [12,21,24-26].

Helichrysum arenarium is traditionally used in Erzincan in the treatment of diabetes and gastrointestinal disorders and also as an immunostimulant. Local people living in Erzincan province reported that this plant was being used in the form of infusion. Again, local people reported that this plant is also used externally for aphta in the mouth. In previous studies, it was determined that the *Helichrysum arenarium* plant has antidiabetic, antibacterial and antifungal activity [35-37]. It has also been reported that the *Helichrysum arenarium* species is used in folk medicine in Sivas and Bayburt [24,26]. It has been reported that *Cephalaria procera* Fisch. Et Lall Erzincan was used externally as a hemostatic agent in Tercan, Çayırlı, Kemah, and Otlukbeli districts. In the *in vitro* study conducted in this direction, it was determined that *Cephalaria procera* Fisch. Et Lall showed hemostatatic activity [38]. It has also been reported to be used as a hemocytatic agent in ethnobotanical studies conducted in Erzurum province [21,32]. *Urtica dioica, Plantago major, Tilia tomentosa, Rheum ribes,* and *Crataegus* monogyna are plants which are used in Turkey and across the World [24-33,39,40].

Our study was carried out on the whole of Erzincan and compared to the previous ethbotanical studies on the province of Erzincan (Üzümlü, Tercan, Kemah, and İliç districts), *Allium vineale, Scorzonera tomentosa, Tagetes patula, Coronilla orientalis, Teucrium polium, Sarcopoterium spinosum* were recorded for the first time in the province of Erzincan [9-11,27], but it is known that these plants are used as folk medicine in other regions of Anatolia [12,24-31].

It has been determined that the local people do not value traditional knowledge as much as they used to, and those who refuse the methods of modern medicine want to benefit from this information today. In addition, the use of medicinal plants has decreased as the local people's access to the doctor-pharmacist has become easier with the developing technology. In addition to these, there are also villages evacuated due to terrorist incidents in the region where the study was conducted. These villages continue to receive immigration from other regions. New settlers do not use or know this ancient information. Due to these risks, the possibility of loss of traditional knowledge has become very high. This study can be an important and meaningful resource for Erzincan, which will prevent the loss of ethnopharmacological information, and which has severe geographical conditions and some local problems.

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AUTHOR CONTRIBUTIONS

Concept: S.G., M.K., S.Ç.; Design: S.G., M.K, S.Ç., S.T.; Control: S.G., M.K.; Sources: S.G., M.K., S.Ç.; Materials: S.G., M.K., S.Ç.; Data Collection and/or Processing: S.G., M.K., S.Ç., S.T.; Analysis and/or Interpretation: S.G., M.K., S.Ç.; Literature Review: S.G., M.K., S.Ç.; Manuscript Writing: S.G., M.K.; Critical Review: S.G., M.K., S.Ç., S.T.; 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

INVESTIGATION OF CYTOTOXIC AND APOPTOTIC EFFECTS OF *PRANGOS HEYNIAE* H. DUMAN & M. F. WATSON EXTRACTS ON HEPG2 CELLS

PRANGOS HEYNIAE H. DUMAN & M. F. WATSON EKSTRELERİNİN HEPG2 HÜCRELERİNDEKİ SİTOTOKSİK VE APOPTOTİK ETKİLERİNİN ARAŞTIRILMASI

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ABSTRACT

Objective: *This study aims to investigate the anticancer potential of Prangos Heyniae H. Duman & M. F. Watson root extracts against human hepatoma cells, and examine the molecular mechanisms potentially involved in extract-induced cytotoxicity.*
Material and Method: *HepG2 cells were treated with chloroform, n-hexane, or methanol extracts from roots of P. heyniae to investigate the possible effects on cell viability. Following the determination of IC_{50} values by the MTT test, n-hexane, and methanol extracts were excluded because of their selectivity indices. The chemical characterization of chloroform extract was performed by HPLC to understand the chemical composition-bioactivity relationship. Alterations induced by chloroform extract on mitochondrial membrane potential and caspase-3 activation were further investigated. In addition, cell viability was measured in the presence of different selective inhibitors of pathways to define the type of cell death pathway contributing to cytotoxicity.*
Result and Discussion: *Chloroform extract but not n-hexane or methanol extracts led to strong and selective inhibition of cell viability on HepG2 cells. In addition, cytotoxicity increased by chloroform extract was only restored in the presence of a pan-caspase apoptosis inhibitor. Also, treatment of HepG2 cells with chloroform extract impaired mitochondrial membrane potential and led to significant caspase-3 activation. Oxypeucedanin, isoimperatorin, and osthole were detected as the major components of the chloroform extract. These results represent that apoptosis may be involved in the anticancer effect of coumarin and furanocoumarin derivatives in chloroform extract.*
Keywords: *Anticancer effect, apoptosis, liver cancer, Prangos heyniae*

ÖZ

Amaç: *Bu çalışmanın amacı; Prangos Heyniae H. Duman & M. F. Watson kök ekstrelerinin insan*

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karaciğer kanseri hücrelerindeki antikanser potansiyellerini araştırmak ve ekstre ile indüklenen sitotoksisitede rol oynayan moleküler mekanizmaları değerlendirmektir.
Gereç ve Yöntem: *P. heyniae kök ekstrelerin HepG2 hücrelerinin canlılığına olan olası etkilerini araştırmak amacıyla hücreler kloroform, n-hekzan ya da metanol ekstreleri ile inkübe edildi. MTT testi ile IC_{50} değerlerinin belirlenmesi sonrası, selektivite indeksleri nedeniyle n-hekzan ve metanol ekstreleri ile çalışmaya devam edilmedi. Yapı-biyolojik aktivite ilişkisini kurabilmek amacıyla kloroform ekstresinin kimyasal karakterizasyonu HPLC analizi ile gerçekleştirildi. Kloroform ekstresinin mitokondriyal membran potansiyeli ve kaspaz-3 aktivasyonu üzerindeki etkileri ileri deneylerle araştırıldı. Ayrıca, sitotoksisitede rol oynayan hücre ölüm yolunun belirlenmesi amacıyla selektif inhibitörler varlığında hücre canlılığı ölçüldü.*
Sonuç ve Tartışma: *Kloroform ekstresi, HepG2 hücrelerinde canlılığın güçlü ve selektif inhibisyonuna neden oldu. Benzer sitotoksik etki n-hekzan ya da metanol ekstreleri ile saptanmadı. Kloroform ekstresi tarafından indüklenen sitotoksisite pan-kaspaz apoptoz inhibitörü varlığında önlendi. Ayrıca, HepG2 hücrelerinin kloroform ekstresi ile inkübasyonu, mitokondriyal membran potansiyelinde hasara ve kaspaz-3 aktivasyonuna neden oldu. Kloroform ekstresinin ana bileşenleri olarak oxypeucedanin, isoimperatorin ve osthole tespit edildi. Bu sonuçlar, kloroform ekstresindeki kumarin ve furanokumarin türevlerinin antikanser etki mekanizmasında apoptozun rol oynayabileceğini göstermektedir.*
Anahtar Kelimeler: *Antikanser etki, apoptoz, karaciğer kanseri, Prangos heyniae*

INTRODUCTION

Hepatocellular carcinoma is one of the most serious primary malignant tumors [1]. The limitations of current therapeutic approaches highlight the necessity of discovering more effective, selective, and less toxic novel drugs for cancer therapy. In recent years, the investigation of new and effective therapeutic strategies, including detecting molecular targets and searching for anticancer drugs, is one of the most essential types of research areas [2]. Based on this strategy, natural compounds are seemingly promising remarkable anticancer drug candidates. Depending on their various chemical structures, natural compounds may exert cytotoxic and apoptotic activities on different cancer cell lines through various molecular mechanisms. Numerous studies investigating natural compounds and the molecular mechanisms of their anticancer effects contribute to understanding carcinogenesis pathways and provide less toxic, more effective treatment protocols [3]. Especially, the secondary metabolites were isolated from the plant extracts, and further studies were carried out to identify novel therapeutic agents for cancer therapy [4].

The genus *Prangos* Lindl., a significant genus of the family Apiaceae, is widely used traditionally for various diseases, including hemorrhoids, intestinal diseases, dyspepsia, diabetes, and hypertension. The distribution of *Prangos* species has been reported from Europe to Tibet, mainly located in Iran and Türkiye [5-7]. Coumarin glycosides, simple phenolics, flavonoids, terpenic compounds, γ-pyron derivatives, fatty acids, phytosterols, and essential oils were isolated from *Prangos* species [7], and several pharmacological activities of *Prangos* species including antidiabetic [8], anticholinesterase, antityrosinase [9], anti-inflammatory and anti-microbial [10] activities were evaluated via especially *in vitro* assessments.

In recent years, the cytotoxicity potential of *Prangos* species is gaining more attention owing to their marker compounds, coumarin derivatives. The cytotoxic and cytostatic activities of *P. asperula, P. uloptera*, *P. turcica*, and *P. ferulacea* extracts containing coumarin derivatives were demonstrated in Vero [11], Hela [12], PC-3 [13], and HT29 [14] cell models, respectively. Thus, it is valuable to investigate and reveal the cytotoxicity and anti-proliferative profile of other species of the genus.

Prangos heyniae H. Duman & M. F. Watson is an endemic plant of Türkiye, and the investigations on the anticancer potential of this species are extremely limited. The biological activities of *P. heyniae,* including antityrosinase, anticholinesterase, antioxidant, and cytotoxic activities, were reported previously [9,15,16], however, the cytotoxicity against hepatocellular carcinoma and the response of liver cancer cells to different extracts of this plant have not been studied yet. In our previous study, the cytotoxicity of the extracts of the plant and the isolated coumarin derivatives on different cells were evaluated [16]. However, molecular mechanisms of observed cytotoxic effects and anticancer

pathways induced by *P. heyniae* root extracts have not been studied yet. For that reason, the present study aimed to discover the cytotoxic and apoptotic potential of different extracts prepared from *P. heyniae* H. Duman & M.F. Watson on HepG2 cells. Moreover, we aimed to clarify the chemical characterization of the extract in order to establish a correlation between the chemical composition and its pro-apoptotic activities.

MATERIAL AND METHOD

Preparation of Root Extracts

The plant roots were dug up from Hadim-Korualan road, roadside, Konya province, Türkiye, in June 2016. The roots of *Prangos heyniae* H. Duman & M. F. Watson were authenticated and deposited at the Herbarium of Ege University, Faculty of Pharmacy. The plant material was air-dried and grinded before the extraction process. The extracts were prepared with one of the three solvents; *n*-hexane, chloroform ($CHCl_3$), or methanol. The process was repeated three times at room temperature. After filtration, the yielded extracts were prepared for bioactivity studies. The preparation of extracts and detailed description of methods were mentioned in our previous study [16].

Cell Culture and Chemicals

Human liver cancer cell line HepG2 (ATCC, HB-8065, USA) and mouse embryonic fibroblast cell line NIH/3T3 (ATCC, CRL-1658, USA) cells were proliferated in DMEM (Thermo Scientific, Waltham, USA) enriched with 10% FBS and 1% pen/strep (Santa Cruz Texas, USA). Cells were kept and grown in the required conditions (37°C, 5% CO_2). JC-1 dye and caspase-3 activity kit, MTT, Q-VD-Oph, and other chemicals were provided by Sigma-Aldrich (Darmstadt, Germany).

MTT Assay

For *in vitro* incubations, 10 mg/ml solutions of extracts were prepared in dimethyl sulfoxide (DMSO). Then, stock solutions were diluted with appropriate amounts of medium to incubate the cells with desired final concentrations (0-600 µg/ml) for 48h. The maximum concentration of DMSO was 1% (v/v). Effects of *n*-hexane, $CHCl_3$, and methanol root extracts on the viability of HepG2 and NIH/3T3 cell lines were evaluated by MTT assay [17].

The cells (6×10^3 cells/well) were exposed to the extracts at increasing concentrations (0-600 µg/ml). The treatment period was 48 hours. Cells were maintained at the required conditions, as mentioned before. Subsequently, MTT solution was added to the wells. Before the measurement, crystals were dissolved using 120µL DMSO. The absorbance values of purple crystals were measured by a microplate reader at 540 nm [17]. Cells exposed to DMSO (1%, v/v), and Triton-X (1%, v/v) were taken as positive and solvent control, respectively [17].

"% cell viability" was determined by calculating the ratio of the average absorbance of the treated cells to that of the solvent control (the cell viability of the solvent control was considered 100%). The cytotoxic concentration values that killed cells by 50% (IC_{50}) were calculated from the response versus concentration curve. In addition, we have determined the IC_{10} and IC_{75} values of the $CHCl_3$ extract. The selectivity index (SI), indicating cytotoxic selectivity, was calculated using the ratio of IC_{50} in NIH/3T3 cells to IC_{50} in HepG2 cells.

Chemical Characterization of the $CHCl_3$ Extract Using HPLC-DAD

$CHCl_3$ was selected for HPLC analysis due to its selective cytotoxic effect. HPLC studies were conducted in order to assess whether the activity of $CHCl_3$ extract depends on the presence of major compounds, including oxypeucedanin (OXY), isoimperatorin (ISO), and osthole (OST), which are the most common bioactive molecules isolated and characterized from *P. heyniae*. HPLC analyses were applied as described previously [18]. In brief, separations of 10 µl of 7.5 ppm $CHCl_3$ extract or 100 ppm standard molecules (OXY, ISO, and OST) were performed by using ACE 5-C18 column (250x4.6 mm; particle size 5 µm) with gradient elution of A (Water, 0.5% acetic acid) and B (Methanol). The running time was 8.35 min, and the flow rate was 1 ml/min in the wavelength range of 200-400 nm [18].

MTT Assay in the Presence of Selective Inhibitors of Different Cell Death Pathways

$CHCl_3$ extract was evaluated for the potential molecular and apoptotic effects through cell death analysis, mitochondrial membrane potential (MMP), and caspase-3 activity.

Selective inhibitors can be used to determine the role of different types of cell death pathways in the anticancer effect of drugs/drug candidates [19]. Therefore, the cell viability was measured in the presence or absence of selective inhibitors of apoptosis, necroptosis, autophagy, or ferroptosis to assess the form of the cell death pathway induced by $CHCl_3$ extract in HepG2 cells. In this experiment, cells ($6x10^3$ cell/well) were pre-incubated with selective inhibitors of apoptosis (Q-VD-Oph; final concentration: 25 µM), necroptosis (necrostatin-1; final concentration: 20 µM), autophagy (chloroquine; final concentration: 12.5 µM), and ferroptosis (ferrostatin-1; final concentration: 2.5 µM) for 1 h [19]. Then, $CHCl_3$ extract was added into wells at IC_{50} concentration. In parallel experiments, cells were treated with the indicated final concentrations of inhibitors without extract to assess their cytotoxic effects. Following the incubation period (48 h), MTT assay was performed, as mentioned previously.

Measurement of MMP

JC-1 was used to determine the effect of $CHCl_3$ extract on MMP in HepG2 cells. Briefly, HepG2 cells were seeded on a black 96-well plate. $CHCl_3$ extract was incubated with cells for 48 h at three concentration levels (IC_{10}, IC_{50}, and IC_{75}). Cells that were incubated with 25 µM rotenone [20] were utilized as a positive control. After the incubation time, cells were exposed to 5 µg/ml dye for 10 min. MMP was measured at ex: 490 nm, em: 520 nm [21].

Measurement of Caspase-3 Activity

In HepG2 cells, caspase-3 activity induced by $CHCl_3$ extract was measured using a commercial kit (Thermo Fisher Scientific, E13183). Cells ($6x10^3$ cells/well) were incubated with $CHCl_3$ extract at various concentrations (IC_{10}, IC_{50}, and IC_{75}). After 48 h, the reaction mix (50 µM) was added into wells. Then, 2mM DEVD-pNA (10 µl, 200µM final concentration) was added to each control and sample well. Absorbance was measured at 405nm by a multi-plate reader. The activity of caspase-3 in each well was presented as the fold of the solvent control group (DMSO %1) [22].

Statistical Analysis

GraphPad Prism® version 8 (GraphPad Software, San Diego California, USA) was assessed for statistical analyses. Experiments were conducted three times, and performed in triplicate. All data were expressed as the mean± standard deviation (SD). Following the evaluation normality of the data by Kolmogorov-Smirnov, statistical differences were determined by one-way ANOVA and then Dunnett's post hoc test. $p < 0.05$ was accepted as statistically significant for differences.

RESULT AND DISCUSSION

The mortality rate of patients diagnosed with hepatocellular carcinoma remains at very high levels due to poor selectivity and adverse effects of chemotherapeutic treatments, or drug resistance [23]. Therefore, numerous studies have been conducted to develop more effective and safe therapeutic strategies for liver cancer treatment [24]. Within the concept of these potential strategies, natural products have also been recommended as valuable anticancer drug candidates because of their anti-proliferative properties [25]. Thus, continuing investigations for anticancer agents from natural sources play a fundamental role in drug discovery [26].

Cytotoxicity by MTT Assay

Isolated compounds from different *Prangos* species, such as *P. turcica, P. ferulacea, and P. pabularia* were shown to exhibit strong anti-proliferative effects on various cancer cells [11-14]. Although studies investigating *P. heyniae* are limited, we previously reported that $CHCl_3$ extract of *P. heyniae* selectively reduced the viability of A549, HK-2, and SH-SY-5Y cells [16]. In that study, isolation and characterization steps were explained in detail; however, the mechanisms of cytotoxic and anti-proliferative activities were not revealed.

In the present study, we assessed the cytotoxic effects of *P. heyniae* root extracts on HepG2 and NIH/3T3 cells by MTT assay (Fig. 1A-F). In accordance with the results, $CHCl_3$ and *n*-hexane extracts were shown to be potent against liver cancer cell viability. IC_{50} values of $CHCl_3$ and *n*-hexane extracts on HepG2 cells were 15.50±1.632 µg/ml (Fig. 1A) and 34.09 ± 1.114 µg/ml (Fig. 1C), respectively. As revealed in Fig. 1D, methanol fraction exhibited weaker cytotoxic activity (IC_{50}=188.3 ± 1.214 µg/ml) against HepG2 cells in contrast to other extracts.

It is also critical whether the extract or compound is selectively toxic to cancer cells [26]. Hence, MTT assay was also performed on NIH/3T3 cells, and selectivity indices were calculated for each extract. As can be seen in Fig. 1B, 1D, and 1E, IC_{50} values of $CHCl_3$, *n*-hexane, and methanol extracts on NIH/3T3 cells were 198.9±1.607, 37.41±1.431, and 180.7±1.479 µg/ml, respectively. $CHCl_3$ extract demonstrated higher selectivity to HepG2 cells than non-malignant cell lines (SI=12.8). Nonetheless, the same selective toxic effect was not found in the results of *n*-hexane (SI=1.05) and methanol (SI=0.95) fractions. Therefore, *n*-hexane and methanol extracts were excluded from further evaluation. The IC_{10} (2.045 µg/ml) and IC_{75} (36.564 µg/ml) values of $CHCl_3$ extract were also calculated for further experiments.

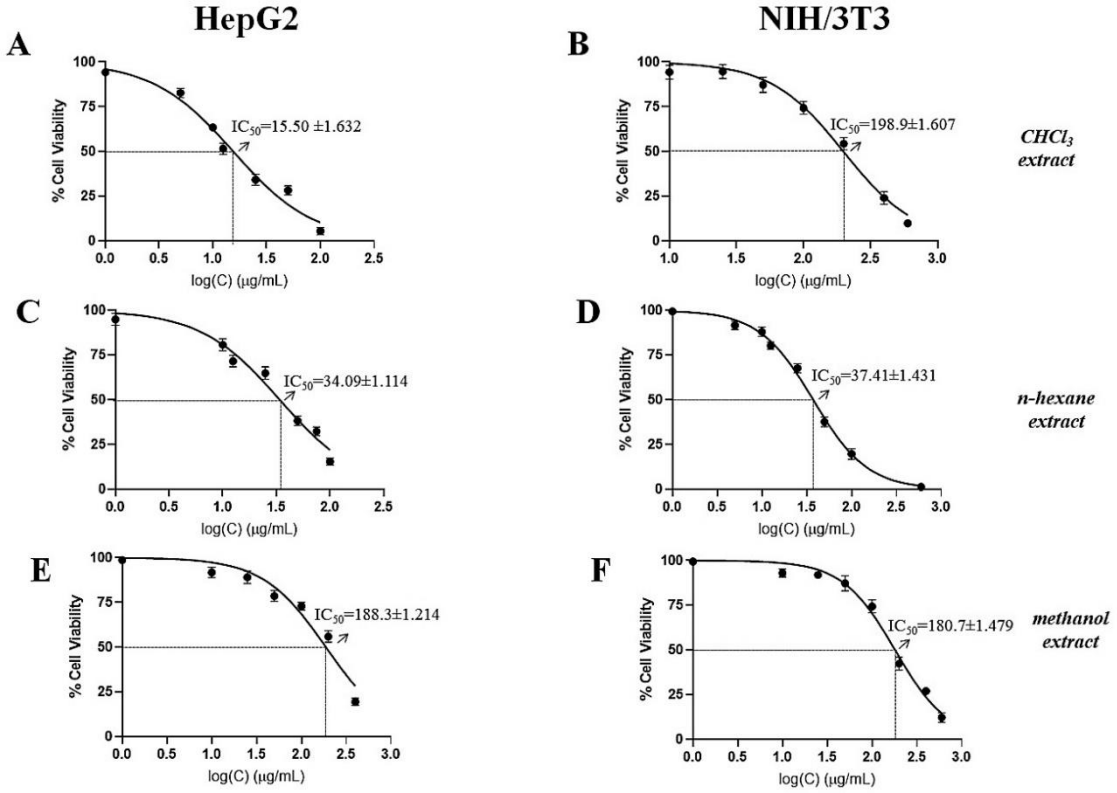

Figure 1. Cytotoxicity of $CHCl_3$, *n*-hexane, and methanol extracts on HepG2 and NIH/3T3 cells for 48 h. "% Cell viability" was calculated as a percentage of solvent control (DMSO-1%, v/v). Graphs represent % cell viability versus log (concentration). Values are represented as mean ± SD. **A,** Cytotoxicity of $CHCl_3$ extract on HepG2 cells; **B,** Cytotoxicity of $CHCl_3$ extract on NIH/3T3 cells, **C,** Cytotoxicity of *n*-hexane extract on HepG2 cells; **D,** Cytotoxicity of *n*-hexane extract on NIH/3T3 cells, **E,** Cytotoxicity of methanol extract on HepG2 cells; **F,** Cytotoxicity of methanol extract on NIH/3T3 cells

HPLC-DAD Analysis of $CHCl_3$ Extract

The chemical characterization of the $CHCl_3$ extract was determined by HPLC-DAD system. OXY, ISO, and OST were chosen as standards in accordance with our previous study [16].

The lower chromatogram indicates the retention times for standard molecules. Retention times for OXY, ISO, and OST at 100 ppm were 19.94, 13.82, and 14.23 min, respectively. The higher chromatogram displays the major peaks of molecules in 7.5 ppm $CHCl_3$ extract. Retention times for major peaks were 10.99, 13.82, and 14.24 min, respectively. A comparison of both chromatograms showed that $CHCl_3$ extract contained all three molecules, and these molecules were the major compounds in the wavelength range of 200-400 nm (Fig. 2).

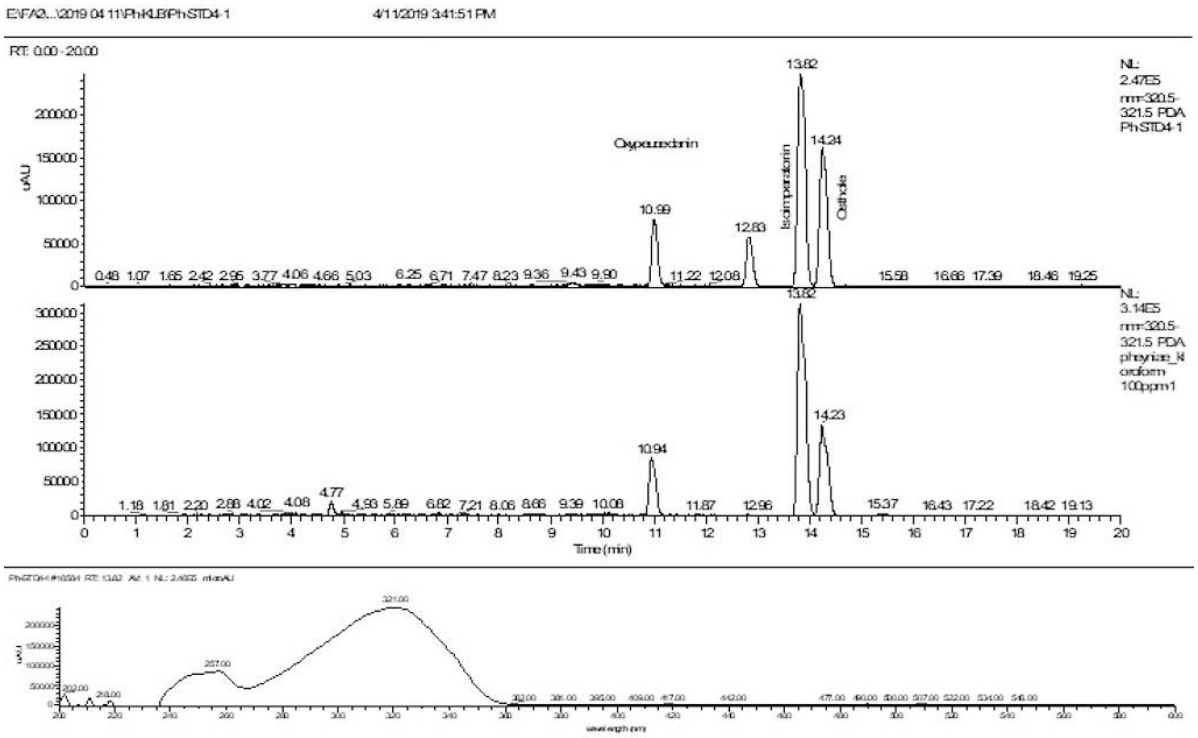

Figure 2. Chemical characterization of $CHCl_3$ extract by HPLC-DAD

Evaluation of Cell Death Pathway Potentially Involved in $CHCl_3$ Extract-Induced Cytotoxicity

According to the MTT assay, $CHCl_3$ extract significantly diminished the number of viable HepG2 cells (Fig. 1A). However, this assay alone is inadequate to reveal data about cell death induction. Therefore, the results need to be supported by additional experiments [19].

Selective inhibitors of apoptosis, necroptosis, ferroptosis, and autophagy were used to assess which mode of cell death pathway was induced by $CHCl_3$ extract [19]. HepG2 cells were treated with $CHCl_3$ extract with or without cell death pathway inhibitors. Additionally, cells were exposed solely to selective inhibitors in order to determine their own cytotoxicity. However, no significant decrease was observed (data not shown).

As seen in Fig. 3, remarkable cell viability recovery was only observed with Q-VD-Oph. Pre-treatment with a pan-caspase inhibitor dramatically reversed the cytotoxic effect of the extract. Cell viability was increased with Q-VD-Oph from 50% to 95%. In contrast, all other selective inhibitors were not able to restore the number of living HepG2 cells. Adding necrostatin-1, ferrostatin-1, or chloroquine did not lead to a significant change in the cytotoxicity (Fig. 3). These results suggest that apoptosis may contribute to cell death induced by $CHCl_3$ extract treatment. However, this conclusion should be supported by apoptosis determination assays or markers.

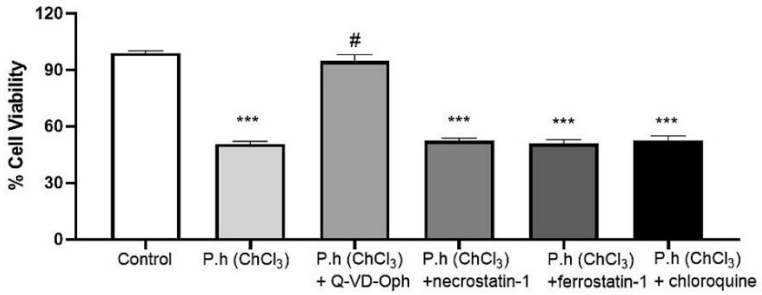

Figure 3. % Cell viability after $CHCl_3$ extract from roots of *P. heyniae* (P. h ($CHCl_3$)) treatment in combination with selective inhibitors of different cell death pathways. HepG2 cells were treated with P. h ($CHCl_3$) at IC_{50} concentration in the presence or absence of inhibitors for 48 h. Q-VD-Oph (25 µM, pan-caspase inhibitor), necrostatin-1 (20 µM, inhibitor of necroptosis), ferrostatin-1 (2.5 µM, inhibitor of ferroptosis), and chloroquine (12.5 µM, inhibitor of autophagy) were used as selective inhibitors. % viability results were normalized to solvent control (DMSO %1). The lines indicate mean ± SD. ***, significantly different ($p < 0.0001$) than control; #, significantly different ($p < 0.0001$) than P. h ($CHCl_3$); cells treated only with $CHCl_3$ extract

Determination of MMP

The intrinsic apoptosis pathway results in oxidative stress, mitochondrial dysfunction, and caspase activation [27]. Hence, MMP is an important aspect of the intrinsic apoptotic pathway [28]. We investigated whether $CHCl_3$ extract impairs the MMP of HepG2 cells and the role of mitochondrial dysfunction in cytotoxicity. Rotenone (positive control) decreased MMP by 63% as expected. IC_{1O} concentration of the $CHCl_3$ extract did not lead to impairment of the mitochondrial membrane. However, the higher doses of the extract significantly decreased the potential compared to the control. MMP was decreased by 16%, and 22% at IC_{5O} and IC_{75} concentrations, respectively (Fig. 4).

We demonstrated that $CHCl_3$ extract seems to impair mitochondrial structure and functions and induces intrinsic apoptosis in HepG2 cells. Previous studies showed that OST led to activation of Bax, and disruption of MMP in different cancer cell lines [29-31]. However, only a few studies have studied the effects of OXY and ISO on MMP. The present study revealed data about the mitochondrial effects of *P. heyniae*. In our further studies, we aimed to conduct incubations with three main compounds of $CHCl_3$ extract to reveal their individual contributions.

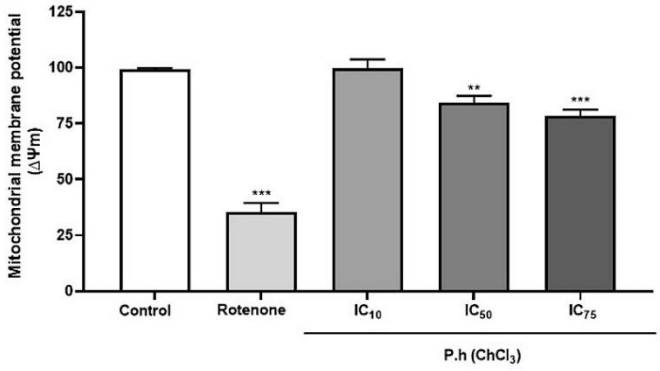

Figure 4. $CHCl_3$ extract decreased MMP in HepG2 cells. After the incubation with increasing doses of extract (IC_{10}: 2.045, IC_{50}: 15.50, IC_{75}: 36.564 mg/ml) for 48 h, the fluorescence of JC-1 dye was directly measured. Rotenone (25 µM) was used as a positive control. Results (mean ± SD) were calculated as the percent of the control signal (DMSO, 1%). ** $P < 0.001$, *** $P < 0.0001$ considered as significantly different from solvent control

Caspase-3 Activity

Caspase-3, the main effector caspase of apoptosis, is an effective marker to assess the mechanism of cell death. The result of significant cytotoxicity induced by $CHCl_3$ extract was prevented in the presence of Q-VD-Oph, which led us to confirm apoptotic death by caspase-3 activity [32]. It is unknown whether caspase-3 activation and apoptosis were induced by *P. heyniae* in HepG2 cells. Our findings indicate that caspase-3 activation was triggered by the treatment of $CHCl_3$ extract in a dose-dependent manner. The signals of control and cells treated with IC_{10} of $CHCl_3$ extract were similar. At the IC_{50} level, there was a significant increase in caspase-3 activity, which was three times higher than the control. This increase was gradually amplified at the IC_{75} level (3.6-fold over control) (Fig. 5).

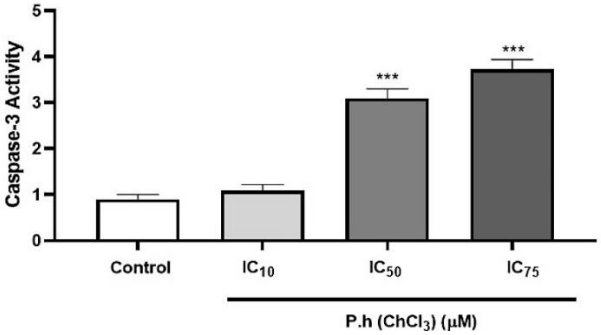

Figure 5. Caspase-3 activity induced $CHCl_3$ extract of *P. heyniae*. Values indicate the fold change relative to the solvent control (DMSO 1%). Lines were expressed as mean ± SD. *** P<0.001 vs. solvent control

Caspase-3 activity is one of the main hallmarks of apoptosis [32]. Previous studies suggested that OXY, OST, and ISO led to anticancer activities and induced apoptosis by activating caspase-3, -8, and -9 proteins in various types of human cancer cells, including DU145 [33], CD133 [34], PC-3, H1299 [35]. In contrast, one study revealed that OXY and ISO decrease caspase-3 activity, increase MMP, and demonstrate a protective effect against doxorubicin-induced apoptosis and neurotoxicity in PC-12 cells [36]. As seen in Fig. 5, $CHCl_3$ extract treatment induced caspase-3 activity in HepG2 cells. This result is consistent with our other findings (Fig 3-5).

The present study assessed the anticancer and apoptotic effects of $CHCl_3$ extract on HepG2 cells. According to our data, $CHCl_3$ extract treatment principally results in intrinsic apoptosis. Nevertheless, this conclusion should be supported by Annexin V, cleavage of PARP1, and other studies. In our laboratory, further mechanistic studies are planned to investigate the mechanism of the cell death pathway.

AUTHOR CONTRIBUTIONS

Concept: E.A.; Design: E.A., G.A., A.E.; Control: E.A., G.A., A.E., E.A., İ.T., Ş.B.; Sources: E.A., G.A., A.E., E.A., İ.T., Ş.B.; Materials: E.A., G.A., A.E., E.A., İ.T., Ş.B.; Data Collection and/or Processing: E.A., G.A., A.E., E.A., İ.T., Ş.B.; Analysis and/or Interpretation: E.A., G.A., A.E., E.A., İ.T., Ş.B.; Literature Review: E.A., G.A., E.A., A.E., İ.T., Ş.B.; Manuscript Writing: E.A., G.A., E.A., A.E., İ.T., Ş.B.; Critical Review: E.A., G.A., A.E., E.A., İ.T., Ş.B.; 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 ethics committee approval is not required for this study.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

AN *IN SILICO* PHARMACOKINETIC INVESTIGATION OF ORGANIC LUMINOGENS: UNDERSTANDING THE NIR AIEGENS AND THEIR INTERACTIONS WITH SERUM ALBUMINS

ORGANİK LUMİNOJENLERİN İN SİLİKO FARMAKOKİNETİK İNCELENMESİ: NIR AIEJENLERİ VE SERUM ALBÜMİNLERİ İLE ETKİLEŞİMLERİNİ ANLAMAK

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ABSTRACT

Objective: *Fluorescence imaging (FLI) is accepted as a highly effective method for visualizing bioanalytics directly and gaining insight into complicated biological structures and processes. In this context, newly tailored organic molecules, which have the potential to be used in FLI, especially near-infrared (NIR) regions supported by aggregation-induced emission luminogens (AIEgens), are a rapidly developing area of study. Herein, using ADMET and molecular docking analyses, we examined the pharmacokinetic properties of both model (D_2-A_2-D_2) and newly designed (D_n-A_n-D_n) organic luminogens to interact with blood proteins, namely bovine serum albumin (BSA) and human serum albumin (HSA), which have emerged as a versatile carrier of several therapeutic agents against preliminary cancer and infectious diseases.*

Material and Method: *The structural properties of the examined luminogens were computed using the Gaussian 09 software package. The DFT/B3LYP/6-31G(d,p) level was then utilized for geometry optimization and accurately determining electronic structures and molecular properties. Lipinski's rule of five was applied to predict the drugability of the compounds using the SwissADME web tool. Molinspiration was used for further validation of these properties and additional bioactivity parameters. Toxicity parameters were evaluated with OSIRIS Property Explorer (v.4.5.1). Molecular docking simulations of the luminogen-albumin complexes were performed using SAMSON 2022 R2 modeling platform and implemented Autodock-vina extension. The X-ray crystal structures of bovine serum albumin (BSA, PDB ID: 4F5S) and human serum albumin (HSA, PDB ID: 4L9Q) were obtained from the Protein Data Bank. Visualization of the docking interactions was conducted using Discovery Studio Visualizer 2021.*

Result and Discussion: *The compounds D_1-A_1-D_1 and D_1-A_4-D_1 stood out concerning molecular weight (MW) and Clog$P_{o/w}$ values, making them promising candidates for drug design. An analysis of lipophilicity revealed that these two compounds displayed high miLogP values, indicating a high degree of lipophilicity, which is generally beneficial for drug delivery. They also exhibited moderate bioactivity based on GPCR ligand and protease inhibitor (PI) parameters. On the other hand, D_4-A_3-D_4 showcased paramount interaction with bovine serum albumin (BSA), while D_5-A_3-D_5 demonstrated the highest binding affinity with human serum albumin (HSA).*

Keywords: *ADMET, AIEgen, BSA, HSA, molecular docking*

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ÖZ

Amaç: *Floresans görüntüleme (FLI), biyoanalitikleri doğrudan görselleştirme ve karmaşık biyolojik yapıları ve süreçleri anlamak için son derece etkili bir yöntem olarak kabul edilir. Bu bağlamda, özellikle agregasyon-indüklü emisyon luminojenleri (AIEjen) tarafından desteklenen ve yakın kızılötesi (NIR) bölgede kullanılma potansiyeli olan yeni özelleştirilmiş organik moleküller, hızla gelişen bir çalışma alanıdır. Bu noktada, ADMET ve moleküler kenetlenme analizlerini kullanarak, hem model (D_2-A_2-D_2) hem de yeni tasarlanmış (D_n-A_n-D_n) organik luminogenlerin kan proteinleri ile etkileşme özelliklerini farmakokinetik açıdan inceledik. Bu kan proteinleri, özellikle sığır serum albumini (BSA) ve insan serum albumini (HSA), erken kanser ve bulaşıcı hastalıklarla mücadelede çeşitli terapötik ajanların taşıyıcısı olarak öne çıkmıştır.*

Gereç ve Yöntem: *İncelenen luminojenlerin yapısal özellikleri Gaussian 09 yazılım paketi kullanılarak hesaplandı. Daha sonra DFT/B3LYP/6-31G(d,p) seviyesi, geometri optimizasyonu ve elektronik yapıların ve moleküler özelliklerin doğru bir şekilde belirlenmesi için kullanıldı. Bileşiklerin ilaçlaştırılabilirliğini tahmin etmek için Lipinski'nin beşli kuralı SwissADME web aracı kullanılarak uygulandı. Bu özelliklerin ve ek biyoaktivite parametrelerinin daha fazla doğrulanması için Molinspiration kullanıldı. Toksisite parametreleri OSIRIS Property Explorer (v.4.5.1) ile değerlendirildi. Luminojen-albümin komplekslerinin moleküler kenetlenme simülasyonları SAMSON 2022 R2 modelleme platformu ve Autodock-vina uzantısı kullanılarak gerçekleştirildi. Sığır serum albümininin (BSA, PDB ID: 4F5S) ve insan serum albümininin (HSA, PDB ID: 4L9Q) X-ışını kristal yapıları Protein Data Bank'tan alındı. Bağlanma etkileşimlerinin görselleştirilmesi Discovery Studio Visualizer 2021 kullanılarak gerçekleştirildi.*

Sonuç ve Tartışma: *D_1-A_1-D_1 ve D_1-A_4-D_1 bileşikleri, moleküler ağırlık (MA) ve $ClogP_{o/w}$ değerleri açısından öne çıkarak onları ilaç tasarımı için umut verici adaylar haline getirdi. Lipofilisite analizi, bu iki bileşiğin yüksek miLogP değerleri gösterdiğini ortaya çıkardı ki bu genellikle ilaç taşınım için istenen yüksek derecede lipofilikliğe işaret etmektedir. Ayrıca bu bileşikler, GPCR ligandı ve proteaz inhibitörü (PI) parametrelerine dayalı olarak da orta düzeyde biyoaktivite sergilediler. Öte yandan D_4-A_3-D_4, sığır serum albümini (BSA) ile olağanüstü etkileşim sergilerken, D_5-A_3-D_5, insan serum albümini (HSA) ile en yüksek bağlanma afinitesini gösterdi.*

Anahtar Kelimeler: *ADMET, AIEjen, BSA, HSA, moleküler kenetlenme*

INTRODUCTION

Cancer, characterized by the abnormal and uncontrolled growth of human cells, encompasses the development of tumors. The process of metastasis, whereby cancer cells spread to distant locations within the body, is a well-known feature of the advanced stages of the disease [1]. Metastatic cancer has increasingly posed a grave threat to public health, constituting a significant ailment affecting humankind [2]. Medicine has developed and implemented various diagnostic and treatment techniques to tackle cancer's many forms and stages. These include surgical procedures, chemotherapy, radiation treatment, immunotherapy, and hormone therapy [3-5]. While these cancer treatments offer potential benefits, it is crucial to recognize that they can also lead to significant side effects, potentially causing harm to healthy tissues or organs [6-8].

For this reason, fluorescence imaging (FLI)-assisted photodynamic therapy (PDT) has gained significant attention as a promising alternative to conventional treatment methods. This approach offers several advantages, including minimizing long-lasting side effects, precise monitoring of drug distribution, tumor visualization, spatial and temporal specificity, and minimally invasive treatment [9-12]. PDT operates through a mechanism wherein specially formulated photoactive materials, referred to as photosensitizers (PSs), are photoexcited by light of a specific wavelength. These PSs can follow two distinct photodynamic reaction pathways, namely type I and type II, resulting in the production of highly cytotoxic reactive oxygen species (ROS) or singlet oxygen ($^1O_2$), respectively [13,14]. The induction of either apoptotic (programmed) or necrotic (non-programmed) cell death in malignantly proliferating cells is a consequence of the cytotoxic species generated through these reaction pathways [15-17].

Photosensitizers (PSs) are activated through photochemical processes, allowing them to enter an excited state. This activation can be achieved using different light sources, including those within the

visible and near-infrared (NIR) spectrum, which is particularly desirable for PS activation [18,19]. Hence, the current focus lies in advancing photosensitizers that exhibit emission in the near-infrared (NIR) region. This choice of wavelength facilitates improved penetration efficiency, especially in deep tissues, thereby enabling efficient eradication of tumor cells [20]. It is worth highlighting that conventional imaging techniques, operating within the 400-700 nm emission range, exhibit a limited tissue penetration depth. Furthermore, the imaging quality of deep tissues is often considered inadequate in the first near-infrared (NIR-I) region, spanning 700-900 nm. As a result, recent research endeavors have concentrated on developing photosensitizers that emit in the second near-infrared (NIR-II) region, spanning 1000 to 1700 nm.

The utilization of the NIR-II region offers an enhanced depth of penetration and improved spatial-temporal resolution, leading to superior monitoring quality in biological imaging [21-24]. The design of an appropriate photosensitizer for photodynamic therapy requires meticulous consideration of the compound's structure. One crucial aspect is attaining a high photoluminescence quantum yield (PLQY) to enable substantial emission within the desired region of the electromagnetic spectrum [25]. Challenges arise in the design of photosensitizers, notably due to the occurrence of aggregation-caused quenching (ACQ) effect arising from strong intermolecular π-π interactions. This effect significantly reduces the PLQY of the photosensitizer, ultimately impacting tissue penetration efficiency.

Researchers have focused on various luminogens that exhibit aggregation-induced emission (AIE) properties to tackle these challenges. Furthermore, several types of fluorophores capable of generating near-infrared (NIR-II) emissions have also garnered interest. These include quantum dots (QDs), carbon nanotubes, rare earth materials, and organic fluorophores [26]. Organic fluorophores possess outstanding designability regarding their physical and optical characteristics, minimal biotoxicity, *in vivo* biocompatibility, and biodegradability. These attributes render them the optimal choice with immense potential for clinical translation, making them highly suitable for various biomedical applications [27,28]. Recently, attention has turned to using aggregation-induced luminogens (AIEgens) as a potential solution. AIEgens employ twisted structures that effectively minimize intermolecular π-π interactions, enhancing photoluminescence properties [29-32].

Cheng's group has made remarkable contributions to the exploration of donor-acceptor-donor (D-A-D) type AIEgens, which have gained prominence as a notable source of near-infrared (NIR-II) emissions [33]. The design strategy employed for D-A-D luminogens is based on the premise that a reduced energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) leads to emission at longer wavelengths. Thus, extending the π-conjugation length in photosensitizers is a favorable approach to decreasing the band gap value [34]. Carrying out this strategy for developing organic fluorophores in the NIR-II region presents more significant challenges than visible light emitters.

The alternative solution involves constructing electron donors (D) to elevate the HOMO level and electron acceptors (A) to reduce the LUMO level. Consequently, the majority of NIR-II AIEgens are constructed by reinforcing the donor and acceptor units [35]. P. Xu et al. successfully synthesized a novel near-infrared (NIR-II) emitter exhibiting AIE characteristics. The design of the emitter involved incorporating a triphenylamine fragment as the electron donor (D), along with tetraphenylethene as a molecular rotor, onto a benzobisthiadiazole (BBT) unit serving as the electron acceptor (A). It is worth noting that BBT is a commonly utilized building block for NIR dyes [36].

Extensive scientific efforts have been dedicated to studying the complex interactions between human serum albumin (HSA) and bovine serum albumin (BSA) with a variety of dye molecules. An example of this is the triphenylmethane dye, Brilliant Green, which demonstrated a remarkable 300-fold increase in fluorescence when it was bound to Bovine Serum Albumin (BSA) in the presence of the macrocyclic host cucurbituril (CB7). This observation highlights the cooperative binding behavior between the dye and BSA, as well as the crucial role played by CB7 in enhancing the binding affinity [37].

Anees et al. conducted a notable investigation on the interaction between NIR-I dye molecules and HSA and BSA, demonstrating their potential utility as protein sensors [38]. The study emphasized the phenomenon of self-assembly shown by near-infrared (NIR-I) dye molecules, which resulted in the formation of nanoparticles. This self-assembly process occurred in the presence of human serum

albumin (HSA) or bovine serum albumin (BSA), resulting to an increase in fluorescence emission and the ability to selectively bind to proteins. The accurate detection and quantification of proteins can be achieved by the subsequent changes in fluorescence characteristics that occur upon dye attachment. This highlights the promise of near-infrared dye-protein interactions for applications in protein sensing.

Additionally, the study conducted by Jameson et al. investigated the interaction between near-infrared II (NIR-II) dye molecules and human serum albumin (HSA) and bovine serum albumin (BSA) for the purpose of bioimaging applications [39]. The findings of this work demonstrate the successful encapsulating of near-infrared-II (NIR-II) dye molecules within nanoparticles that are biocompatible. These nanoparticles were then coupled to human serum albumin (HSA) or bovine serum albumin (BSA). The dye-protein complexes that were obtained exhibited heightened fluorescence emission in the near-infrared II (NIR-II) region, hence opening up possibilities for the advancement of deep tissue imaging. The enhanced stability and pharmacokinetic properties of these dye nanoparticles were ascribed to their interaction with proteins, highlighting the promise of near-infrared II (NIR-II) dye-protein interactions in the field of advanced bioimaging and theragnostic applications.

Furthermore, the researchers investigated the interaction between NIR-I dye molecules and human serum albumin (HSA) and bovine serum albumin (BSA) in order to assess their potential as photothermal agents in the field of cancer therapy [40]. The present study showcased the creation of enduring dye-protein complexes that, when subjected to near-infrared (NIR-I) laser radiation, displayed effective conversion of light into heat energy. Consequently, this phenomenon resulted in the generation of localized heat, ultimately leading to the specific eradication of tumor cells. This highlights the promising prospects of near-infrared dye-protein interactions in the development of groundbreaking photothermal therapies.

In conclusion, the literature provides an in-depth understanding of the multifaceted interactions between dye molecules and serum albumins, elucidating the underlying binding mechanisms, fluorescence enhancement phenomena, and potential applications of these interactions in a variety of scientific domains ranging from chemistry and medicine to diagnostics and therapeutics.

In this study, a detailed quantum chemical, ADMET and molecular docking investigation was conducted to reveal the drug-likeness properties and binding potentials of both model (D_2-A_2-D_2) [41–43] and newly designed (D_n-A_n-D_n) organic luminogens (Figure 1) with blood proteins, namely bovine serum albumin (BSA) and human serum albumin (HSA), which have emerged as a carrier for a variety of anticancer and anti-infectious drug molecules.

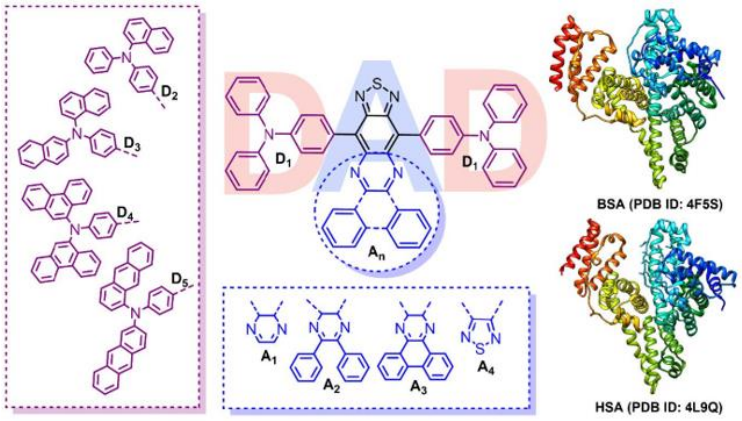


Figure 1. Scope of the investigated organic luminogens and target serum albumins (HSA and BSA)

For each docking procedure involving BSA and HSA, the conformation with the highest binding score was chosen. Their binding poses, residue interactions, and docking scores were shown in the relevant parts of the manuscript. These results provide valuable insights into the specific binding interactions between the proteins and the ligands, shedding light on their potential therapeutic

applications. Detailed information about the remaining ligands and macromolecule complexes was provided in the Electronic Supplementary Information (ESI†).

MATERIAL AND METHOD

Density Functional Theory (DFT) Calculations

The Gaussian 09 package was used to analyze several quantum chemical properties of our investigated luminogens. Accordingly, the target molecules were initially modeled and constructed using GaussView (v. 5.0.8) software, and their initial geometries were predicted with a potential energy surface (PES) scan utilizing the PM6 method. Then, the corresponding geometry optimization calculations were done using Density Functional Theory (DFT) at the B3LYP level of theory and the 6-31G(d,p) basis set. The DFT calculations were carried out to obtain accurate electronic structures and molecular properties of the luminogens. Additionally, the B3LYP functional was selected as it has been widely used for studying organic molecules and has shown good performance in predicting their properties, and the 6-31G(d,p) basis set was chosen to ensure a good balance between computational cost and accuracy.

In Silico (ADMET/Drug-likeness) Analyses

Lipinski's rule is a crucial guideline used to determine the potential drugability of a compound. It considers various essential factors such as blood-brain barrier permeability (BBB), gastrointestinal absorption (GI), the number of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), as well as the compound's lipophilicity measured by the logPo/w partition coefficient between n-octanol and water.

The optimal BBB permeability and GI absorption depend on the compound's molecular weight (MW). The MW should be below or equal to 500 g/mol for adequate oral bioavailability. The values of HBA and HBD significantly impact the compound's ability to bind to macromolecules. Recommended thresholds for HBA and HBD are 10 and 5, respectively. Several mathematical models, such as iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT, can be employed to assess the lipophilicity of potential drug candidates. The Consensus logPo/w, obtained by averaging the predictions from these five models, offers a robust indicator, with values ideally below 5 [44,45]. When considering the drug-likeness of a potential compound, various descriptive parameters come into play, including the number of rotatable bonds (nROTB), topological polar surface area (TPSA), solubility, and saturation. The nROTB, which significantly influences the molecule's flexibility, should be kept below 9 for optimal drug-like characteristics.

Moreover, the topological polar surface area (TPSA) represents an additional parameter exclusively based on the fragmental system developed by Ertl et al. Its incorporation has proven instrumental as a descriptor in various models and rules, facilitating the quick estimation of ADME properties. Notably, TPSA exhibits particular relevance in predicting the crossing of compounds through biological barriers, especially in absorption and brain permeability. The recommended TPSA value typically varies from 20 to 130$Å^2$.

Additionally, a soluble compound offers numerous advantages in drug development, primarily ease of handling and formulation. Notably, for drugs intended for parenteral administration, high water solubility is essential to ensure the effective delivery of an adequate amount of the active ingredient within the limited volume of the pharmaceutical dosage. The decimal logarithm of the molar solubility in water (logS) is employed to calculate predicted values, whose optimal logS value should be less than 6. Furthermore, saturation is a vital factor that influences the physicochemical characteristics of a potential drug molecule. It quantifies the ratio of sp^3 hybridized carbons to the total carbon count in the molecule. It is advised that the estimated saturation value should be no less than 0.25 [46,47].

In this scope, the SwissADME web tool was employed to assess the drug-likeness and pharmacological behavior of the prospective drug molecules. Additionally, bioavailability radar representations were generated to visually represent molecular descriptors such as lipophilicity (XLOGP3), size (MW), polarity (TPSA), solubility (logS), saturation, and flexibility (nROTB). Furthermore, these properties were also calculated and validated along with the additional analysis of

bioactivity parameters using Molinspiration. OSIRIS Property Explorer (v.4.5.1) revealed several toxicity parameters, including mutagenicity, tumorigenicity, irritation, and reproductive effect.

Molecular Docking Studies

The 2D structures of the ligands were obtained using ChemDraw. As indicated, the ligands were minimized and optimized using Gaussian 09 software (B3LYP/6-31G(d,p); refer to ESI† for further details) before initiating the molecular docking simulations.

Molecular docking analyses were performed on the dye-albumin complexes SAMSON 2022 R2 modeling platform and Autodock-vina extension. The resulting docking interactions were visualized using Discovery Studio Visualizer 2021 (client version; Accelrys Software Inc., San Diego, CA, USA). The X-ray crystal structures of Bovine Serum Albumin (BSA, PDB ID: 4F5S) and Human Serum Albumin (HSA, PDB ID: 4L9Q) were obtained from the Protein Data Bank (www.rcsb.org). Preprocessing steps were carried out, including the removal of water molecules and any existing ligands' addition of charges and hydrogens to the structures of HSA and BSA for docking purposes. Chain B of both HSA and BSA structures was eliminated, and Chain A was selected for the subsequent molecular docking process. The grid box size was set at 75.7 x 73.2 x 113.8 $Å^3$ with a grid point spacing of 0.375 Å, and the center coordinates of the grid box were defined as x = 2.2, y = -1.6, and z = 28.2.

RESULT AND DISCUSSION

Prediction of Drug-likeness and Pharmacokinetic

SwissADME was employed to predict pharmacological properties to investigate the druggability of our tested ligands initially. In this scope, molecular weight (MW), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), consensus logP ($ClogP_{o/w}$), number of rotatable bonds (nROTBs), topological polar surface area (TPSA), gastrointestinal absorption (GI abs.), blood-brain barrier (BBB), and solubility (S) were determined and listed in Table 1.

Table 1. ADME prediction of the investigated dye molecules

Ligands	MW[a]	HBA[b]	HBD[c]	nROTB[d]	TPSA[e]	GI abs.[f]	BBB[g]	CLog $P_{o/w}$[h]	S[i]
Model Dye	927	4	0	10	86.28	L	No	12.96	Ins
D_1-A_1-D_1	675	4	0	8	86.28	L	No	8.72	Ins
D_1-A_2-D_1	827	4	0	10	86.28	L	No	11.25	Ins
D_1-A_3-D_1	825	4	0	8	86.28	L	No	11.53	Ins
D_1-A_4-D_1	681	4	0	8	114.52	L	No	6.9	Ins
D_2-A_1-D_2	775	4	0	8	86.28	L	No	10.46	Ins
D_2-A_3-D_2	925	4	0	8	86.28	L	No	13.19	Ins
D_2-A_4-D_2	781	4	0	8	114.52	L	No	8.46	Ins
D_3-A_1-D_3	875	4	0	8	86.28	L	No	12.18	Ins
D_3-A_2-D_3	1027	4	0	10	86.28	L	No	14.66	Ins
D_3-A_3-D_3	1025	4	0	8	86.28	L	No	14.85	Ins
D_3-A_4-D_3	881	4	0	8	114.52	L	No	10	Ins
D_4-A_1-D_4	1075	4	0	8	86.28	L	No	15.38	Ins
D_4-A_2-D_4	1228	4	0	10	86.28	L	No	17.76	Ins
D_4-A_3-D_4	1226	4	0	8	86.28	L	No	18.16	Ins
D_4-A_4-D_4	1081	4	0	8	114.52	L	No	13.05	Ins
D_5-A_1-D_5	1075	4	0	8	86.28	L	No	15.41	Ins
D_5-A_2-D_5	1228	4	0	10	86.28	L	No	17.88	Ins
D_5-A_3-D_5	1226	4	0	8	86.28	L	No	18.08	Ins
D_5-A_4-D_5	1081	4	0	8	114.52	L	No	13	Ins

Rules: [a]MW≤500 g/mol, [b]HBA≤5, [c]HBD≤10, [d]nROTB≤9, 20≤[e]TPSA≤130$Å^2$, [h]CLog$P_{o/w}$≤5

Abbreviations: [f]GI abs: Gastrointestinal absorption, [g]BBB: Blood-brain barrier, $CLogP_{o/w}$: Consensus $logP_{o/w}$ [i]S: Solubility
L: Low, Ins: Insoluble

According to the results, the size of our ligands has crossed the threshold (MW≤500 g/mol), and it is deduced that none of the molecules meet the criteria to cross the blood-brain barrier (BBB) and gastrointestinal absorption (GI abs.) for these ligands could not be possible. Another key chemical factor for determining the oral bioavailability of small compounds is the amount of hydrogen bond acceptors (HBA) and donors (HBD). These standards are believed to affect passive diffusion across cell membranes, a critical process during medication absorption and distribution [48].

Our results showed that 4 hydrogen bond acceptors were present in the entire set of our ligands; however, no HBDs were detected. These numbers fell within the intended range and satisfied the criterion. Moreover, the consensus $logP_{o/w}$ parameter, denoting the partition coefficient between n-octanol and water, is a pivotal descriptor employed to evaluate the lipophilicity of a drug molecule. It serves as a crucial criterion in adherence to Lipinski's rule, which stipulates that the $logP_{o/w}$ value of a candidate drug should ideally fall below 5. This requirement ensures optimal lipophilicity for efficient transmembrane permeation, as higher lipophilicities may impede cellular uptake.

The consensus $logP_{(o/w)}$ parameter also represents the average value derived from the five models discussed above: iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT. These models are utilized to evaluate the lipophilicity of a drug molecule, providing a comprehensive assessment from multiple perspectives. The findings from Table 1 revealed a consistent pattern among the analyzed prospective pharmaceuticals, as they all exhibited lipophilic characteristics that exceeded the desired target range. Specifically, D_4-A_1-D_4 stands out with a relatively lower $ClogP_{o/w}$ value of 6.9 compared to model dye and other ligands, which exhibited a significantly higher value of 12.96. As a consequence of their extreme lipophilicity, all of these compounds were determined to be water-insoluble. The number of rotatable bonds (nROTBs) serves as a significant parameter to assess the flexibility of a drug molecule and its ability to bind to macromolecules. Potent drugs are expected to possess an appropriate number of rotatable bonds (nROTBs) within the upper limit of 9, ensuring sufficient flexibility for effective binding to target macromolecules. Table 1 shows that D_1-A_2-D_1, D_3-A_2-D_3, D_4-A_2-D_4, and D_5-A_2-D_5 exceeded the upper limit of the nROTBs. Notably, these compounds belong to a series of candidate drug molecules composed of various donor and acceptor fragments. Upon closer analysis, it becomes apparent that the excessive number of rotatable bonds is primarily attributed to the A_2 unit, which comprises a 5,6-diphenyl-2,3-dihydropyrazine backbone. This acceptor fragment consists of two rotatable phenyl rings bonded to a dihydropyrazine ring.

Consequently, the prospective photosensitizers containing the A_2 fragment exhibit two additional nROTBs, resulting in a total of 10 and surpassing the upper limit. In contrast, the remaining ligands in the series possess 8 nROTBs, which fall within the desired limit. It is noteworthy that model dye exceeded the optimal limit for nROTBs. Furthermore, polar surface area (PSA) has also emerged as a widely utilized molecular descriptor in the investigation of drug transport via GI absorption and BBB permeation. It represents the cumulative contribution of polar atoms, including oxygen (O), nitrogen (N), and their associated hydrogen atoms, to the molecular surface area, typically in terms of van der Waals interactions. To address the challenge of complex PSA calculations and enable rapid virtual bioavailability screening of large compound libraries, Ertl et al. developed an efficient additive fragment-based method for PSA computation. The predicted optimal value for TPSA is generally considered to be less than 130 Å². Analyzing the TPSA data presented in Table 1, it is evident that all of the computed ligands fall within the desired range, indicating that the polarity of our prospective drug molecules is adequate. It is worth noting that the ligands containing the 1,2,5-thiadiazole acceptor scaffold (A4) exhibit a higher TPSA value of 114.52 Å², while the TPSA values of the other ligands are 86.28 Å². This disparity can be attributed to the presence of two nitrogen atoms and one sulfur atom in the thiadiazole unit, contributing to increased polarity and, consequently, a higher TPSA value.

Bioavailability radars serve as valuable tools for visualizing the calculated physicochemical data of candidate drug molecules within the context of oral drug-like property space, providing insights into their potential oral bioavailability. In Figure 2, radar plots showcasing the studied molecules with the highest binding affinities to BSA and HSA are depicted, along with model dye. The radar representations of the other investigated luminogens could be accessed in the supplementary material (ESI). The examined parameters, such as lipophilicity, size, solubility, and saturation, as presented in the radar plots, exhibited significant deviations from the hexagonal pink area. However, as expected, the polarity

of our series, as determined by TPSA values, fell within the optimal range. Similarly, the ligands demonstrated optimal flexibility, as derived from nROTBs, except for those containing the 5,6-diphenyl-2,3-dihydropyrazine acceptor unit (A_2), surpassing the previously discussed nROTB limit.

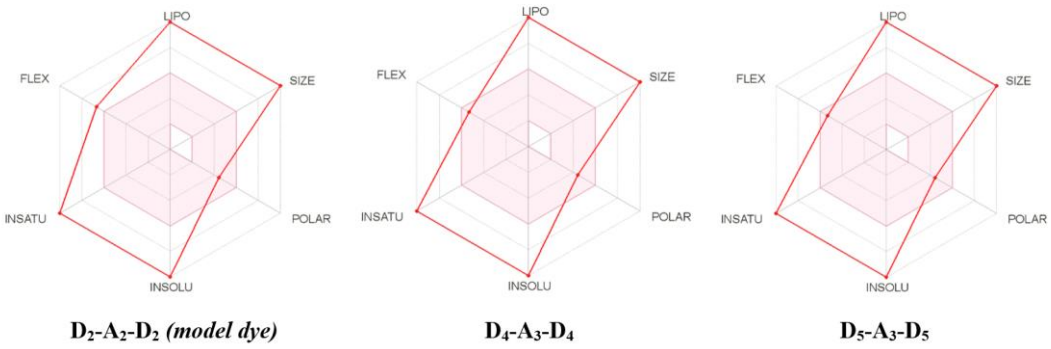


Figure 2. Bioavailability radar plots of the studied ligands

To validate the drug-likeness findings obtained from SwissADME, we utilized the Molinspiration Property Calculation tool. Table 2 presents the results of this analysis, specifically the miLogP parameter, which offers a mathematical approach to determine the lipophilicity of potent pharmaceuticals. The computed miLogP values for our ligands (D_n-A_n-D_n) fell within the range of $9.65<(D_n\text{-}A_n\text{-}D_n)<10.88$, indicating a high level of lipophilicity for our investigated photosensitizers. It is noteworthy that while the miLogP value for our model dye was found to be 10.39, D_1-A_1-D_1 and D_1-A_4-D_1 exhibited relatively lower miLogP values of 9.65 and 9.75, respectively, positioning them closer to the desired range in comparison to the model dye.

Table 2. Drug-likeness data for the candidate drug molecules

Ligands	miLogP[a]	TPSA[b]	nAtoms[c]	nON[d] (HBA)	nOHNH[e] (HBD)	Violation	nROTB[f]	Volume
Model Dye	10.39	58.04	71	6	0	2	10	830.93
D_1-A_1-D_1	9.65	58.04	51	6	0	2	8	600.13
D_1-A_2-D_1	10.15	58.04	63	6	0	2	10	742.95
D_1-A_3-D_1	10.2	58.04	63	6	0	2	8	732.11
D_1-A_4-D_1	9.75	56.99	50	6	0	2	8	590.84
D_2-A_1-D_2	10.02	58.04	59	6	0	2	8	688.12
D_2-A_3-D_2	10.43	58.04	71	6	0	2	8	820.09
D_2-A_4-D_2	10.09	56.99	58	6	0	2	8	678.83
D_3-A_1-D_3	10.29	58.04	67	6	0	2	8	776.1
D_3-A_2-D_3	10.58	58.04	79	6	0	2	10	918.92
D_3-A_3-D_3	10.62	58.04	79	6	0	2	8	908.08
D_3-A_4-D_3	10.35	56.99	66	6	0	2	8	766.81
D_4-A_1-D_4	10.67	58.04	83	6	0	2	8	952.07
D_4-A_2-D_4	10.88	58.04	95	6	0	2	10	1094.88
D_4-A_3-D_4	10.9	58.04	95	6	0	2	8	1084.04
D_4-A_4-D_4	10.7	56.99	82	6	0	2	8	942.78
D_5-A_1-D_5	10.67	58.04	83	6	0	2	8	952.07
D_5-A_2-D_5	10.88	58.04	95	6	0	2	10	1094.88
D_5-A_3-D_5	10.9	58.04	95	6	0	2	8	1084.04
D_5-A_4-D_5	10.71	56.99	82	6	0	2	8	942.78

[a]miLogP: Partition coefficient between n-octanol and water ($logP_{o/w}$), [b]TPSA: Topological polar surface area, [c]nAtoms: Number of atoms, [d]nON: Number of hydrogen bond acceptors (HBA), [e]nOHNH: Number of hydrogen bond donors (HBD), [f]nROTB: Number of rotatable bonds

Furthermore, the TPSA values of the potent drugs analyzed and listed in the table indicate that the ligands fall below the upper limit of ≤130 $Å^2$, ranging from 56.99 to 58.04 $Å^2$. As mentioned previously, Molinspiration and SwissADME calculations confirm that the polarity, represented by the TPSA value, is sufficient for our investigated potent drugs. According to the Molinspiration analysis, the ligands demonstrated 6 HBAs and 0 HBDs, which fall within the acceptable ranges for these parameters. The number of rotatable bonds (nROTBs) obtained from the analysis agreed with the results from the SwissADME analysis, providing further evidence of the influence of the A_2 acceptor fragment (5,6-diphenyl-2,3-dihydropyrazine) on the number of rotatable bonds. Hence, it can be concluded that ligands that do not contain the A_2 unit possess an optimal number of rotatable bonds.

Table 3. Bioactivity score for the candidate drug molecules

Ligands	GPCRL[a]	ICM[b]	KI[c]	NRL[d]	PI[e]	EI[f]
Model Dye	-3.63	-3.78	-3.74	-3.77	-3.57	-3.7
D_1-A_1-D_1	-0.73	-1.75	-1.26	-1.38	-0.53	-1.16
D_1-A_2-D_1	-2.94	-3.61	-3.51	-3.56	-2.48	-3.32
D_1-A_3-D_1	-2.96	-3.59	-3.51	-3.57	-2.49	-3.29
D_1-A_4-D_1	-0.64	-1.62	-1.23	-1.23	-0.47	-1.08
D_2-A_1-D_2	-2.11	-3.27	-2.91	-3.06	-1.62	-2.68
D_2-A_3-D_2	-3.63	-3.77	-3.75	-3.78	-3.57	-3.69
D_2-A_4-D_2	-1.93	-3.16	-2.83	-2.88	-1.47	-2.52
D_3-A_1-D_3	-3.45	-3.70	-3.65	-3.68	-3.14	-3.59
D_3-A_2-D_3	-3.78	-3.87	-3.85	-3.87	-3.76	-3.82
D_3-A_3-D_3	-3.79	-3.87	-3.85	-3.88	-3.76	-3.81
D_3-A_4-D_3	-3.36	-3.68	-3.65	-3.66	-3.01	-3.57
D_4-A_1-D_4	-3.83	-3.90	-3.89	-3.9	-3.81	-3.86
D_4-A_2-D_4	-3.93	-3.98	-3.97	-3.98	-3.92	-3.95
D_4-A_3-D_4	-3.93	-3.97	-3.97	-3.99	-3.92	-3.94
D_4-A_4-D_4	-3.83	-3.90	-3.89	-3.9	-3.80	-3.86
D_5-A_1-D_5	-3.83	-3.90	-3.89	-3.91	-3.8	-3.86
D_5-A_2-D_5	-3.93	-3.98	-3.97	-3.98	-3.92	-3.95
D_5-A_3-D_5	-3.93	-3.98	-3.97	-3.99	-3.92	-3.94
D_5-A_4-D_5	-3.83	-3.90	-3.90	-3.90	-3.79	-3.86

[a]GPCRL: G protein-coupled receptor ligands Ligands, [b]ICM: Ion Channel Modulator, [c]KI: Kinase Inhibitor, [d]NRL: Nuclear Receptor Ligand, [e]PI: Protease Inhibitor, [f]EI: Enzyme Inhibitor

The utilization of the Molinspiration platform enables the calculation of diverse bioactivity descriptors. These descriptors include GPCR Ligands (GPCRL), Ion Channel Modulator (ICM), Nuclear Receptor Ligand (NRL), Protease Inhibitor (PI), and Enzyme Inhibitor (EI). For a compound to be designated as a potential drug, it is imperative for it to exhibit a bioactivity score surpassing 0.0, denoting a significant level of bioactivity. Conversely, bioactivity scores ranging from -0.50 to 0.0 indicate moderate activity, whereas below -0.50 indicates a lack of bioactivity [49]. GPCR ligands, specifically G protein-coupled receptor ligands, are integral members of a diverse family of signaling proteins responsible for cellular responses to hormones, metabolites, cytokines, and neurotransmitters. Their exceptional druggability and pivotal role in major diseases render them highly attractive drug discovery and development targets. In parallel, ion channels are the foundation for numerous physiological processes, including rapid cell reconfigurations, cardiac and muscular contractions, neuronal activity, and tumor cell proliferation.

Various chemical compounds such as antibodies, peptides, small molecules, or ions function as ion-channel modulators through interactions with membrane proteins, exerting a crucial influence on ion-channel function [50]. The kinase family has been widely researched as a therapeutic target for 30 years. In addition to metastatic cancer, the deregulation of kinase activity has been demonstrated to

contribute to immune, inflammatory, degenerative, metabolic, cardiovascular, and viral diseases. Due to their proven druggability and the clinical safety profile of approved kinase inhibitors, kinases are attractive targets for small molecules [51]. Nuclear Receptors (NRs) are ligand-induced transcription factors that translocate to the nucleus and directly control gene transcription. They play a crucial role in vital physiological processes such as cell growth, development, immunity, metabolism, and reproduction of cancer cells [52]. Table 3 presents the outcomes of the calculated parameters for our investigated ligands, indicating that none met the criteria to be classified as bioactive compounds. Specifically, upon evaluating the GPCRL scores, it is evident that D_1-A_1-D_1 and D_1-A_4-D_1 ligands possessed moderate bioactivity with -0.73 and -0.64, respectively. The remaining part of our models exhibited inactivity, ranging from -3.93 to -0.64.

Similarly, none of our ligands displayed bioactivity for Ion Channel Modulator (ICM) scores, ranging from -3.98 to -1.62. Kinase Inhibitor (KI) scores further revealed that our potent drugs exhibited values below the optimal bioactivity threshold, ranging from -3.97 to -1.26. Furthermore, the analysis of Nuclear Receptor Ligand (NRL) characteristics indicated that our ligands fell below the desired limit. Likewise, the scores for Protease Inhibitors (PIs) and Enzyme Inhibitors (EIs), which play significant roles in physiological processes, demonstrated that the majority of our ligands did not meet the criteria to be considered bioactive compounds. However, it is noteworthy to mention that D_1-A_4-D_1 and D_1-A_1-D_1 exhibited moderate protease inhibitory activity, with scores of -0.47 and -0.53, respectively. These findings highlight the potential of these compounds for the treatment of certain cancers associated with protease enzymes. Based on these results, it is essential to pursue various lead optimization strategies to achieve bioactivity within the desired range.

The toxicity risk factors of the luminogens, such as mutagenicity, tumorigenicity, skin irritability, and reproductive consequences, were evaluated using OSIRIS software. To support the previous findings, solubility and lipophilicity were also determined. Additionally, the studied photosensitizers' drug-likeness and drug scores were assessed to further assess the druggability of the compounds. The results are illustrated in Table 4.

Table 4. Druglikeness and toxicity risk data of the investigated ligands

Ligands	cLogp	Solubility	Druglikeness	Drug Score	Mutagenic	Tumorigenic	Irritant	Reproductive Effective
Model Dye	16.01	-17.35	0.36	0.04	🔴	🔴	🟢	🟢
D_1-A_1-D_1	10.12	-10.58	-0.27	0.06	🔴	🟢	🟢	🟢
D_1-A_2-D_1	13.62	-14.13	-2.84	0.04	🔴	🟢	🟢	🟢
D_1-A_3-D_1	13.95	-15.19	-4.63	0.04	🔴	🟢	🟢	🟢
D_1-A_4-D_1	11.20	-7.40	-0.67	0.06	🔴	🟢	🟢	🟢
D_2-A_1-D_2	12.51	-13.79	2.94	0.05	🔴	🔴	🟢	🟢
D_2-A_3-D_2	16.34	-18.4	-1.38	0.03	🔴	🔴	🟢	🟢
D_2-A_4-D_2	13.59	-10.62	2.55	0.04	🔴	🔴	🟢	🟢
D_3-A_1-D_3	14.90	-17.00	-0.07	0.03	🔴	🔴	🟡	🟢
D_3-A_2-D_3	18.40	-20.56	-2.64	0.02	🔴	🔴	🟡	🟢
D_3-A_3-D_3	18.73	-21.6	-4.42	0.02	🔴	🔴	🟡	🟢
D_3-A_4-D_3	15.98	-13.83	-0.49	0.03	🔴	🔴	🟡	🟢
D_4-A_1-D_4	19.68	-23.43	2.48	0.03	🔴	🔴	🟡	🟢
D_4-A_2-D_4	23.18	-26.93	-0.10	0.03	🔴	🔴	🟡	🟢
D_4-A_3-D_4	23.50	-28.03	-1.92	0.02	🔴	🔴	🟡	🟢
D_4-A_4-D_4	20.76	-20.25	2.05	0.03	🔴	🔴	🟡	🟢
D_5-A_1-D_5	19.68	-23.43	-1.64	0.02	🔴	🔴	🔴	🟢
D_5-A_2-D_5	23.18	-26.93	-4.23	0.01	🔴	🔴	🔴	🟢
D_5-A_3-D_5	23.50	-28.03	-6.01	0.01	🔴	🔴	🔴	🟢
D_5-A_4-D_5	20.76	-20.25	-2.06	0.02	🔴	🔴	🔴	🟢

🔴: High toxicity, 🟡: Moderate toxicity, 🟢: No detectable toxicity

The lipophilicity and solubility results obtained from the SwissADME and Molinspiration analyses were in line with the findings from the OSIRIS analysis. Notably, these two parameters provide complementary information, as lipophilicity and water solubility are inversely proportional to each other. Consistent with expectations, the OSIRIS analysis indicated that our luminogens exhibited considerably high lipophilicity, ranging from 10.12 to 23.50. In contrast, the water solubility of the ligands varied between -28.03 and -7.40. Consequently, to comprehend the pharmacological properties of our ligands, drug-likeness and drug score factors were analyzed [53]. Equation 1 calculates the drug-likeness (d), where *Vi* indicates scores of molecular fragments and n denotes the number of molecular fragments.

$$d = \frac{\sum Vi}{\sqrt{n}} \qquad \text{Equation 1}$$

Equation 2 is used to obtain the drug score (ds) of the examined sensitizers, where the s_i parameter represents the contributions calculated directly from cLogP, logS, molecular weight, and drug-likeness, and t_i represents the contribution taken from the four toxicity risk classes.

$$ds = \pi\left(\frac{1}{2} + \frac{1}{2}si\right).\pi\text{ti} \qquad \text{Equation 2}$$

The data showed that D_2-A_n-D_2 and D_4-A_n-D_4 series (including model dye, denoted as D_2-A_2-D_2) have better drug-likeness (d) potential. More specifically, D_2-A_1-D_2 and D2-A4-D2 have 2.94 and 2.55 drug-likeness values, respectively, and D_4-A_1-D_4 and D_4-A_4-D_4 possess 2.48 and 2.05 drug-likeness values, respectively. These scores are found to be much higher in comparison with model dye possessing a 0.36 drug-likeness value. Subsequently, considering the drug scores, the D_1-A_n-D_1 series have been determined to be more prominent than other series. Specifically, D_1-A_1-D_1 and D_1-A_4-D_1 have the same drug score (ds) of 0.06, and in the case of D_2-A_1-D_2, this value was found to be 0.05, three of which ligands have higher drug scores when compared to model dye having 0.04 ds value. In addition, the assessment of potential toxicity risk parameters, as presented in Table 4, has provided valuable insights. Our findings indicate that the studied ligands exhibit a high level of mutagenicity, raising concerns about their potential to induce genetic mutations.

Additionally, all investigated ligands, except for the D_1-A_n-D_1 series, display a high tumorigenicity profile. These characteristics are highly undesirable in drug molecules as they can contribute to cancer development by promoting the malignant proliferation of cells. Furthermore, evaluating irritancy, skin irritation, and reproductive effectiveness parameters is crucial in assessing the potential toxic effects of compounds. Ligands with irritancy properties can harm human skin, leading to reactions such as burning sensations, stinging, and redness. Our analysis reveals that the D_1-A_n-D_1 and D_2-A_n-D_2 ligands do not exhibit skin irritation characteristics, while the D_3-A_n-D_3 and D_4-A_n-D_4 series display a moderate irritancy property. Notably, the D_5-A_n-D_5 series of ligands demonstrate a considerably high level of irritation, which is undesirable in a drug molecule. Moreover, assessing reproductive effects is important in determining whether a candidate drug molecule impacts the reproductive system of the human body. According to the data obtained from OSIRIS, none of the investigated photosensitizers exhibit any reproductive effects.

Molecular Docking Study

Molecular docking is a popular computational strategy for drug development that enables the evaluation of the potential for interaction between ligands and macromolecules like proteins. It allows for detecting new pharmaceuticals and the molecular-level prediction of interactions between ligands and their target molecules. Furthermore, docking facilitates the exploration of structure-activity correlations (SAR) without prior knowledge of the chemical structure of the target modulators. While initially developed to study the molecular recognition mechanisms between small and large molecules, the application of docking in drug development has evolved significantly in recent years.

The primary objective of this study is to evaluate the binding capabilities of newly designed luminogens, specifically the D_n-A_n-D_n series, with albumin proteins, including bovine serum albumin (BSA) and human serum albumin (HSA). These albumin proteins have emerged as robust carriers for

various pharmaceuticals in treating cancer and infectious diseases. Therefore, a comprehensive molecular docking strategy has been employed. Initially, a series of well-known NIR-I and NIR-II AIEgens were investigated, serving as model dyes commonly used in photodynamic therapy (PDT). Subsequently, modifications were made to these model dyes' acceptor and donor units to enhance their selectivity and binding affinities with BSA (PDB ID: 4F5S) and HSA (PDB ID: 4L9Q). These albumin proteins have shown to be effective pharmacological carriers for various drugs used to treat cancer and infectious disorders. The investigation began with several NIR-I and NIR-II AIEgens, a widely known luminogen family frequently employed in photodynamic treatment (PDT). These model dyes' acceptor and donor units were then altered to improve their selectivity and binding affinities with BSA (PDB ID: 4F5S) and HSA (PDB ID: 4L9Q).

The molecular docking simulations were performed for 20 newly designed photosensitizers, and the resulting binding scores and binding domains are presented in Table 5. Detailed information regarding the residue category, type of interactions, residue distance from the binding domain, and specific residue information can be found in the electronic supplementary information (ESI†). This comprehensive approach provides insights into the binding affinities and interactions between the investigated ligands and albumin proteins, contributing to our understanding of their potential as therapeutic agents. BSA and HSA are well-known for their distinct structural compositions, consisting of three main domains (Domain I, Domain II, and Domain III), which comprise two subdomains each (IA, IB, IIA, IIB, IIIA, and IIIB).

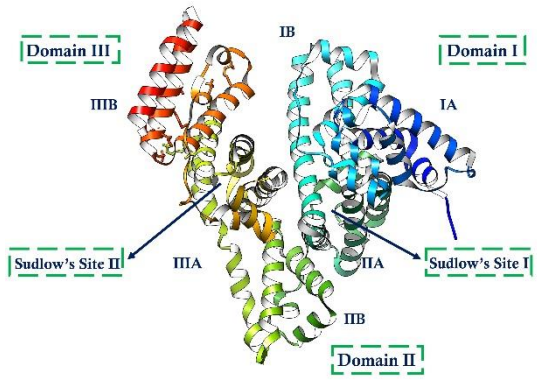
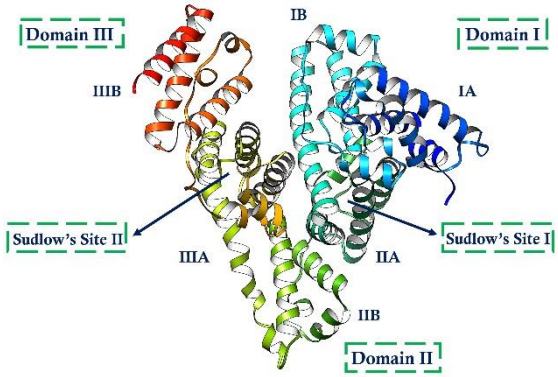
Crystal structural investigations have revealed that the primary ligand binding sites in BSA and HSA are located in hydrophobic voids within subdomains IIA and IIIA, known as Sudlow's site I and Sudlow's site II, respectively. Table 5 demonstrates the binding interactions between our investigated organic luminogens and the ligand-binding subdomains. Interestingly, the D_1-A_n-D_1 series exhibited interactions with different subdomains compared to the other studied luminogens. Specifically, D_1-A_1-D_1 bonded to the IIIB subdomain, while D_1-A_2-D_1 and D_1-A_3-D_1 interacted with IB, IIA, and IIIA subdomains. D_1-A_4-D_1 targeted both IIIA and IIIB subdomains. The remaining series of luminogens, including D_2-A_n-D_2, D_3-A_n-D_3, D_4-A_n-D_4, and D_5-A_n-D_5, selected IA and IB subdomains as their binding sites. Furthermore, the binding interactions between the ligands and HSA were more complex than the ligand-BSA complexes. The majority of the ligands were in co-interaction with IB, IIA, and IIIA sub-domains. This binding interaction was observed in D_1-A_3-D_1, D_2-A_3-D_2, D_3-$A_{(1,2,3)}$-D_3, D_4-A_n-D_4 and D_5-$A_{(2,3)}$-D_5. In addition, several ligands, including D_2-A_4-D_2, D_3-A_4-D_3, D_5-A_1-D_5, and D_5-A_4-D_5, selected IA and IB sub-domains as binding targets. The remaining luminogens selected different binding sites other than the rest of the ligands. Specifically, D_1-A_1-D_1 & IA, D_1-A_2-D_1 & IIB and IIA, D_1-A_4-D_1 & IIA, and IIIA and D_2-A_1-D_2 & IB binding interactions were observed. It is noteworthy that model dye is bound to IA and IB sub-domains of the BSA and IB, IIA, and IIIA sub-domains of the HSA.

Most ligands exhibited binding interactions with IB, IIA, and IIIA subdomains. This pattern was observed in ligands such as D_1-A_3-D_1, D_2-A_3-D_2, D_3-$A_{(1,2,3)}$-D_3, D_4-A_n-D_4, and D_5-$A_{(2,3)}$-D_5. Conversely, other ligands, including D_2-A_4-D_2, D_3-A_4-D_3, D_5-A_1-D_5, and D_5-A_4-D_5, targeted IA and IB subdomains as their binding sites. The remaining luminogens displayed unique binding interactions distinct from the rest. For instance, D_1-A_1-D_1 bound to IA subdomain, D_1-A_2-D_1 interacted with IIB and IIA subdomains, D_1-A_4-D_1 targeted IIA and IIIA subdomains, and D_2-A_1-D_2 bound to IB subdomain. It is worth noting that the model dye exhibited binding to IA and IB subdomains of BSA, whereas IB, IIA, and IIIA subdomains of HSA. The binding poses of the ligand-BSA, and ligand-HSA complexes and their 2D/3D residue interaction representations were illustrated in Table 6 and Table 7, respectively. The electronic supplementary information file provides the complementary binding poses and 2D/3D residue interactions (ESI†).

The docking scores of the ligands, as shown in Table 5, provided insights into their binding affinities. The D_1-A_n-D_1 series exhibited binding affinities ranging from -8.5 kcal/mol to -10.3 kcal/mol, indicating relatively weaker binding compared to the other luminogens, including the model dye with a binding affinity of -10.7 kcal/mol. D_2-A_n-D_2 and D_3-A_n-D_3 series displayed relatively higher docking scores, ranging from -10.5 kcal/mol to -12.7 kcal/mol and -11.9 kcal/mol to -13.2 kcal/mol, respectively. D_4-A_n-D_4 and D_5-A_n-D_5 series, excluding D_5-A_2-D_5, exhibited the highest docking scores, with the D_4-

A_n-D_4 series ranging from -13.8 kcal/mol to -15.1 kcal/mol and the D_5-A_n-D_5 series ranging from -12.3 kcal/mol to -14.0 kcal/mol. Notably, the ligand with the highest docking score in the D_4-A_n-D_4 series, D_4-A_3-D_4, contained two D_4 donor fragments [N-(phenanthrene-9-yl)-N-phenylphenanthren-9-amine] with an A_3 acceptor unit (2,3-dihydrodibenzo[f,h]quinoxaline) fragment bonded to benzothiadiazole (BTD), which is commonly used in various organic NIR-I and NIR-II luminogens (Figure 3). The docking scores of our investigated luminogens interacting with HSA, listed in decreasing order, are as follows: D_4-A_3-D_4 (-15.1) > D_5-A_1-D_5 (-14.0) > D_4-A_1-D_4 (-13.9) = D_4-A_2-D_4 (-14.3) > D_4-A_4-D_4 (-13.8) > D_5-A_4-D_5 (-13.7) > D_3-A_4-D_3 (-13.2) > D_5-A_3-D_5 (-13.2) > D_3-A_1-D_3 (-12.7) > D_3-A_3-D_3 (-12.6) > D_5-A_2-D_5 (-12.3) > D_3-A_2-D_3 (-11.9) > D_2-A_3-D_2 (-11.8) > D_2-A_1-D_2 (-11.3) > model dye D_2-A_2-D_2 (-10.7)) > D_2-A_4-D_2 (-10.5) > D_1-A_2-D_1 (-10.3) > D_1-A_3-D_1 (-9.6) > D_1-A_1-D_1 = D_1-A_4-D_1 (-8.5).

Table 5. Docking scores and binding domain data of the studied ligands with BSA and HSA

BSA	HSA
	

Ligands	Binding score		Binding domain	
	BSA	HSA	BSA	HSA
Model Dye	-10.7	-11.8	IA & IB	IB & IIA & IIIA
D_1-A_1-D_1	-8.5	-10.0	IIIB	IA
D_1-A_2-D_1	-10.3	-12.6	IB & IIA & IIIA	IIB & IIIA
D_1-A_3-D_1	-9.6	-12.7	IB & IIA & IIIA	IB & IIA & IIIA
D_1-A_4-D_1	-8.5	-10.1	IIIA & IIIB	IIA & IIIA
D_2-A_1-D_2	-11.3	-12.3	IA & IB	IB
D_2-A_3-D_2	-11.8	-12.8	IA & IB	IB & IIA & IIIA
D_2-A_4-D_2	-10.5	-12.3	IA & IB	IA & IB
D_3-A_1-D_3	-12.7	-13.9	IA & IB	IB & IIA & IIIA
D_3-A_2-D_3	-11.9	-13.2	IA & IB	IB & IIA & IIIA
D_3-A_3-D_3	-12.6	-14.3	IA & IB	IB & IIA & IIIA
D_3-A_4-D_3	-13.2	-13.7	IA & IB	IA & IB
D_4-A_1-D_4	-13.9	-12.6	IA & IB	IB & IIA & IIIA
D_4-A_2-D_4	-13.9	-12.9	IA & IB	IB & IIA & IIIA
D_4-A_3-D_4	-15.1	-13.5	IA & IB	IB & IIA & IIIA
D_4-A_4-D_4	-13.8	-13.1	IA & IB	IB & IIA & IIIA
D_5-A_1-D_5	-14.0	-14.4	IA & IB	IA & IB
D_5-A_2-D_5	-12.3	-14.0	IA & IB	IB & IIA & IIIA
D_5-A_3-D_5	-13.2	-15.9	IA & IB	IB & IIA & IIIA
D_5-A_4-D_5	-13.7	-14.7	IA & IB	IA & IB

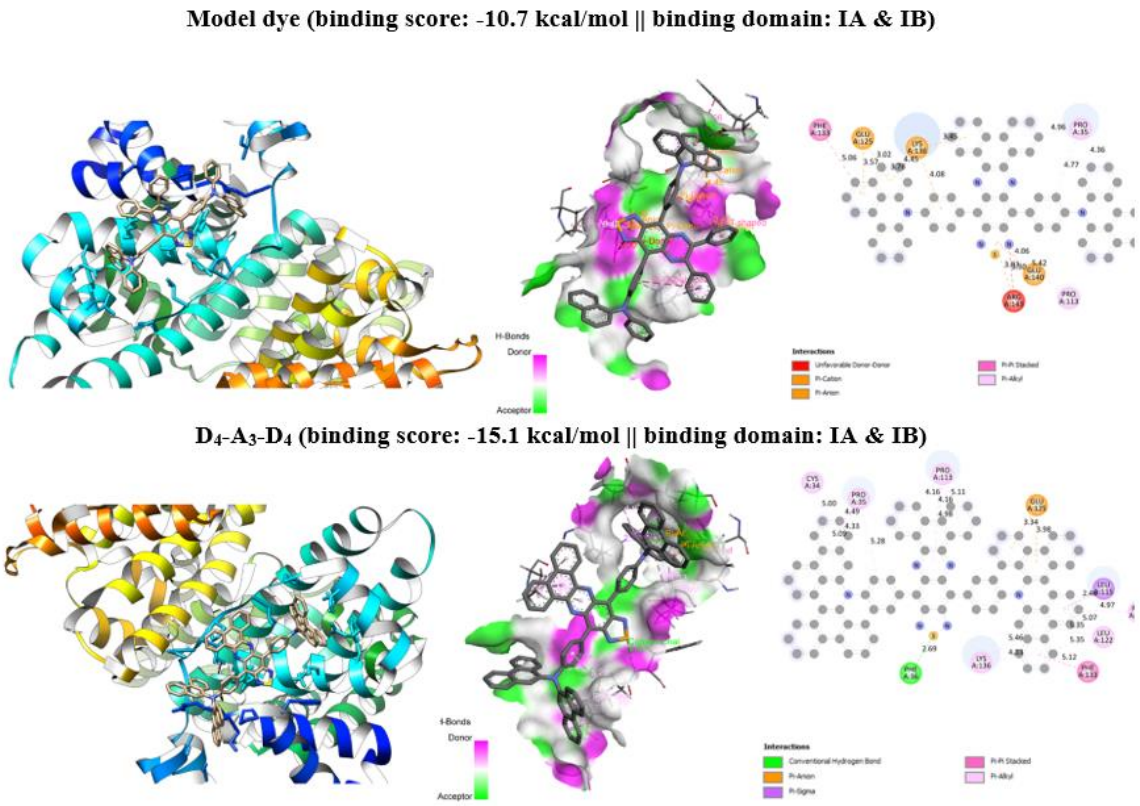


Figure 3. Binding poses and residue interactions of the model dye and D_4-A_3-D_4 with BSA

The D_1-A_n-D_1 series exhibited binding affinities ranging from -10 to -12.7 kcal/mol, indicating lower scores than the other ligands. Except for D_1-A_2-D_1 and D_1-A_3-D_1, which displayed docking scores of -12.6 kcal/mol and -12.7 kcal/mol, respectively, the ligands in this series had lower scores than the model dye (-11.8 kcal/mol). Notably, the D_2-A_n-D_2 series demonstrated higher docking scores, ranging from -12.3 to -12.8 kcal/mol. Among the investigated sensitizers, the D_3-A_n-D_3, D_4-A_n-D_4 (excluding D_4-A_1-D_4 with relatively lower scores), and D_5-A_n-D_5 series exhibited the highest docking scores. The docking scores for the D_3-A_n-D_3 series ranged from -13.2 to -14.3 kcal/mol, while those for the D4-An-D4 ligands ranged from -12.6 to -13.5 kcal/mol and for the D_5-A_n-D_5 ligands, the scores ranged from -14.4 to -15.9 kcal/mol. Notably, D_5-A_3-D_5 achieved the highest docking score of -15.9 kcal/mol among all the investigated potential drug molecules (Figure 4). When examining the structure of D_5-A_3-D_5, which displayed the best docking score, it consisted of two D_5 donor fragments [N-(anthracene-2-yl)-N-phenylanthracen-1-amine] with an A_3 acceptor unit (2,3-dihydrodibenzo[f,h]quinoxaline) fragment bonded to a benzothiadiazole (BTD) backbone. The docking scores of our investigated luminogens interacting with BSA can be ranked in descending order as follows: D_5-A_3-D_5 (-15.9) > D_5-A_4-D_5 (-14.7) > D_5-A_1-D_5 (-14.4) > D_3-A_3-D_3 (-14.3) > D_5-A_2-D_5 (-14.0) > D_3-A_1-D_3 (-13.9) > D_3-A_4-D_3 (-13.7) > D_4-A_3-D_4 (-13.5) > D_3-A_2-D_3 (-13.2) > D_4-A_4-D_4 (-13.1) > D_4-A_2-D_4 (-12.9) > D_2-A_3-D_2 (-12.8) > D_1-A_3-D_1 (-12.7) > D_4-A_1-D_4 = D_1-A_2-D_1 (-12.6) > D_2-A_4-D_2 = D_2-A_1-D_2 (-12.3) > model dye {D_2-A_2-D_2} (-11.8) > D_1-A_4-D_1 (-10.1) > D_1-A_1-D_1 (-10.0).

Generally, there is an increasing trend of binding affinities observed from the D_1-A_n-D_1 to D_5-A_n-D_5 series, with some exceptions. In ligand-HSA complexes, more complex residue interactions were observed. It was found that many ligands interacted electrostatically with ARG218 and hydrophobically with LYS195 amino acid residues. Other prominent interactions involved electrostatic interactions with ARG186, GLU294 and hydrophobic interactions with LYS444, PRO447, and TYR452. Notably, the ligand D5-A3-D5, which exhibited the highest binding affinity, interacted electrostatically with

ARG218, GLU292, GLU294, LYS274, LYS444, and hydrophobically with ALA191 and PRO447 amino acid residues.

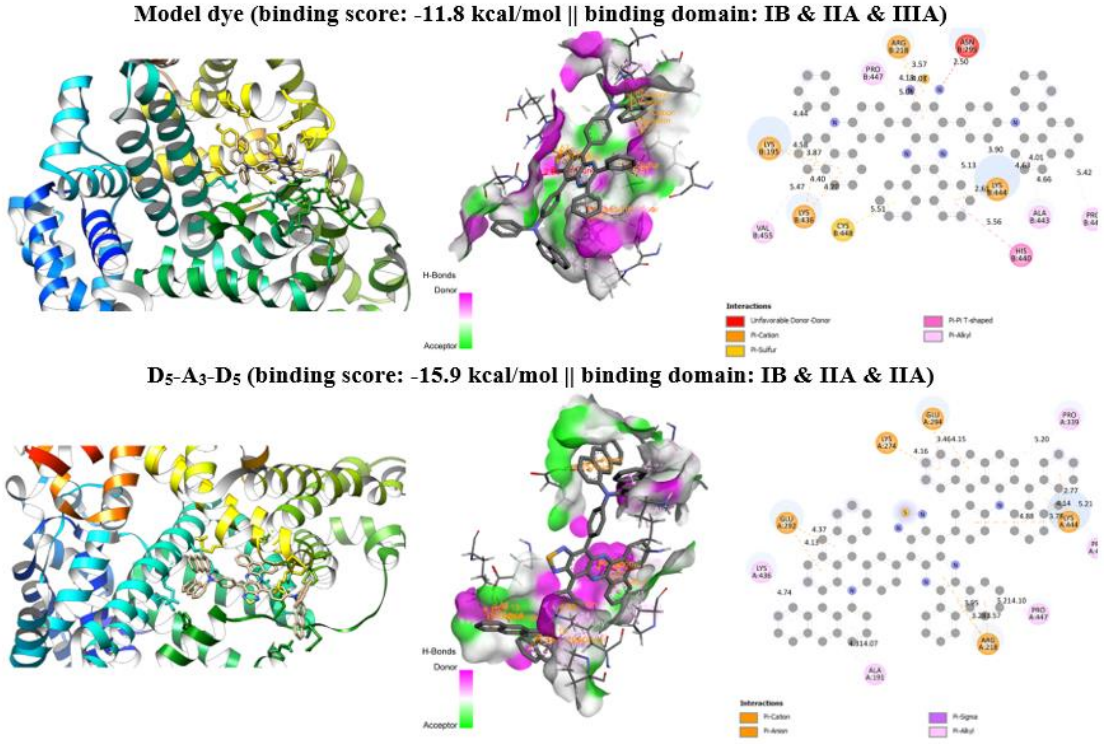


Figure 4. Binding poses and residue interactions of the model dye and D_5-A_3-D_5 with HSA

The main objective of this study was to investigate the docking performance of twenty novel organic NIR-I and NIR-II luminogens. Target ligands' drug-likeness and pharmacokinetic properties were assessed with SwissADME, Molinspiration, and OSIRIS to achieve this goal. Molecular docking simulations were also conducted, and the results were analyzed using UCSF-Chimera (v.2021-06-26) and Discovery Studio Visualizer 2021 software. Based on the drug-likeness assessments conducted using SwissADME and Molinspiration, it was found that D_1-A_1-D_1 and D_1-A_4-D_1 exhibited relatively favorable characteristics regarding MW and $ClogP_{o/w}$ values. Specifically, D_1-A_1-D_1 had an MW of 675 g/mol and a $ClogP_{o/w}$ value of 8.72, while D_1-A_4-D_1 had an MW of 681 g/mol and a $ClogP_{o/w}$ value of 6.9.

Furthermore, the Molinspiration study highlighted the lipophilicity (with miLogP approach), and it was observed that D_1-A_1-D_1 and D_1-A_4-D_1 displayed notable miLogP values of 9.65 and 9.75, respectively. These findings indicate that D_1-A_1-D_1 and D_1-A_4-D_1 possessed favorable drug-likeness properties compared to the other investigated luminogens and the model dye. Overall bioactivity scores of our investigated luminogens also showed that D_1-A_1-D_1 and D_1-A_4-D_1 demonstrated moderate bioactivity regarding GPCRL and PI parameters. Moreover, the drug-likeness and drug scores of our luminogens were also validated with OSIRIS. D_2-A_1-D_2 and D_2-A_4-D_2 have a drug-likeness value of 2.94 and 2.55, respectively, whereas D_4-A_1-D_4 and D_4-A_4-D_4 have a value of 2.48 and 2.05, respectively. These values are significantly higher than those for model dye, with a 0.36 score.

Regarding drug scores (ds), the D_1-A_n-D_1 series was more prevalent than the other series. Specifically, D_1-A_1-D_1 and D_1-A_4-D_1 both have a drug score of 0.06, and D_2-A_1-D_2 has a drug score of 0.05, three of which outperformed model dye, which has a 0.04 ds value. In addition, the drug-likeness and drug scores of our luminogens were further comprehended and validated through OSIRIS. Specifically, D_2-A_1-D_2 and D_2-A_4-D_2 demonstrated notable drug-likeness values of 2.94 and 2.55,

respectively, and these values were shifted to 2.48 and 2.04 for D_4-A_1-D_4 and D_4-A_4-D_4, respectively. These values exhibit a significant increase compared to the drug-likeness value of model dye, which stands at a mere 0.36.

Furthermore, regarding drug scores, the D1-An-D1 series displayed a higher prevalence than the other series. Notably, both D_1-A_1-D_1 and D_1-A_4-D_1 achieved a drug score of 0.06, while D_2-A_1-D_2 obtained a slightly lower drug score of 0.05. Remarkably, all three of these ligands surpassed the model dye at 0.04 ds value. OSIRIS analysis further confirmed the consistency of the lipophilicity and water solubility levels of our ligands with the findings from SwissADME and Molinspiration.

Additionally, the assessment of toxicity risks revealed that the entire set of our luminogens exhibited high mutagenic properties. It was also repeated with tumorigenic characteristics, except for the D_1-$A_{(1-4)}$-D_1 series, which proved safe. Notably, the D_1-A_n-D_1 and D_2-A_n-D_2 series did not exhibit any skin irritation, whereas the D_3-A_n-D_3 and D_4-A_n-D_4 series showed moderate irritancy. On the other hand, the D_5-A_n-D_5 series demonstrated high irritant properties. Furthermore, no reproductive effectiveness was observed in any of the investigated luminogens. Considering all the parameters and properties described, the ligands D_1-A_1-D_1 and D_1-A_4-D_1 appear to be the best druggable ligands. In terms of the binding performances, it was evident that they underperformed model dye, with docking scores of -8.50 g/mol with BSA, and -10.00 and -10.10 g/mol with HSA, respectively. In addition, D_4-A_3-D_4 exhibited the most efficient docking performance with BSA by binding to its IA and IB sub-domains, which showed a -15.1 kcal/mol docking score. On the other hand, D_5-A_3-D_5 bound to IB, IIA, and IIIA sub-domains of HSA demonstrated the best binding affinity as -15.9 kcal/mol. In light of this information, we believe that our prospective NIR dye molecules used in this study will provide a valuable theoretical perspective to research in the field of fluorescence imaging (FLI) based photodynamic cancer therapy (PDT).

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AUTHOR CONTRIBUTIONS

Concept: G.K.; Design: H.N., G.K.; Control: G.K.; Sources: G.K.; Materials: H.N., G.K.; Data Collection and/or Processing: H.N., G.K., D.T.Ö., Z.Y.; Analysis and/or Interpretation: H.N., G.K.; Literature Review: H.N., G.K., D.T.Ö., Z.Y.; Manuscript Writing: H.N., G.K.; Critical Review: H.N., G.K., D.T.Ö., Z.Y.; 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

DEVELOPMENT OF ELECTROCHEMICAL SENSOR BY MOLECULARLY IMPRINTING POLYMER FOR DETECTION OF AN INFLUENZA VIRUS NEURAMINIDASE INHIBITOR PERAMIVIR

BİR İNFLUENZA VİRÜSÜ NÖRAMİNİDAZ İNHİBİTÖRÜ OLAN PERAMİVİR'İN SAPTANMASI İÇİN MOLEKÜLER BASKI POLİMER İLE ELEKTROKİMYASAL SENSÖRÜN GELİŞTİRİLMESİ

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ABSTRACT

Objective: *Influenza viruses are the most common cause of influenza. Antiviral drugs prevent the spread of the virus through the infected cells. Peramivir is one of the antiviral drugs that is an inhibitor of influenza virus neuraminidase. In our study, we aimed to develop a MIP-based electrochemical sensor to determine Peramivir. This study is the first to create an electrochemical sensor for Peramivir. MIP(PERA)/GCE was fabricated with the electropolymerization of 4-aminophenol (4-AP) and ortophenilendiamine (o-PD) in the presence of Peramivir. The developed MIP(PERA)/GCE was applied to the commercial serum sample for analysis of Peramivir.*

Material and Method: *PERA is supplied by Tobio Novelpharma pharmaceutical company (İstanbul, Türkiye). Potassium ferricyanide ($[K_3Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6].3H_2O$), and potassium chloride (KCl), 4-aminophenol (4-AP) and ortophenilendiamine (o-PD), commercial human serum sample, dopamine, ascorbic acid, uric acid, paracetamol, KNO_3, Na_2SO_4 ve $MgCl_2$ were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, acetic acid, oxalic acid, hydrochloric acid, acetonitrile, sodium hydroxide, and ethanol were purchased by Merck. The redox process was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) by the Dropsens µStat 400 Bipotantiostat/ Galvanostat (Metrohm, Herisau, Switzerland). Three-electrode systems consisted of a glassy carbon working electrode (GCE, 3mm^2, BASi, USA), a saturated Ag/AgCl reference electrode, and a Pt wire.*

Result and Discussion: *The sensor exhibits a linear range of 1-10 pM with a detection limit of 0.158 pM. The MIP(PERA)/GCE sensor could detect PERA from commercial serum samples with a high recovery of 101.81%.*

Keywords: *Commercial serum sample, determination, electrochemical sensor, molecularly imprinted polymer, peramivir*

ÖZ

Amaç: *Grip virüsleri, gribin en yaygın nedenidir. Antiviral ilaçlar virüsün enfekte hücreler yoluyla yayılmasını engeller. Peramivir, influenza virüsü nöraminidazının inhibitörü olan antiviral ilaçlardan biridir. Çalışmamızda Peramivir tayini için MIP tabanlı bir elektrokimyasal sensör geliştirilmesi amaçlanmıştır. Bu çalışma, Peramivir analizi için geliştirilen ilk elektrokimyasal*

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sensör çalışmasıdır. MIP(PERA)/GCE, Peramivir varlığında 4-aminofenol (4-AP) ve ortofenilendiaminin (o-PD) monomerlerin elektropolimerizasyonu ile geliştirilmiştir. Geliştirilen MIP(PERA)/GCE, Peramivir analizi için ticari serum örneğine uygulanmıştır.

Gereç ve Yöntem: *Peramivir, Tobio Novelpharma ilaç şirketi (İstanbul, Türkiye) tarafından sağlanmıştır. Potasyum ferrisiyanür ($[K_3Fe(CN)_6]$), potasyum ferrosiyanür ($K_4[Fe(CN)_6].3H_2O$) potasyum klorür(KCl), 4-aminofenol (4-AP) ve ortofenilendiamin (o-PD), sentetik insan serumu, dopamin, askorbik asit, ürik asit, parasetamol, KNO_3, Na_2SO_4 ve $MgCl_2$ Sigma-Aldrich'ten (St. Louis, Missouri, ABD) temin edilmiştir. Metanol, asetik asit, okzalik asit, hidroklorik asit, asetonitril sodyum hidroksit ve etanol Merck tarafından tedarik edilmiştir. Redoks işlemi, Dropsens µStat 400 Bipotantiostat/ Galvanostat (Metrohm, Herisau, İsviçre) tarafından döngüsel voltametri (CV) ve diferansiyel puls voltammetrisi (DPV) kullanılarak gerçekleşmiştir. Üç elektrotlu sistemler, bir camsı karbon çalışma elektrodu (GCE, 3mm^2, BASi, ABD), doymuş bir Ag/AgCl referans elektrodu ve bir Pt telinden oluşmuştur.*

Sonuç ve Tartışma: *Geliştirilen sensör, 0,158 pM en düşük tayin limiti ile 1-10 pM doğrusal bir aralık göstermiştir. Geliştirilen MIP sensörü ticari bir serum örneğine uygulanmıştır. MIP(PERA)/GCE sensörü ile %101.81'lik yüksek bir geri kazanımla ticari serum örneklerinden peramivir saptanmıştır.*

Anahtar Kelimeler: *Elektrokimyasal sensör, miktar tayini, molekül baskılanmış polimer, peramivir, ticari serum örneği*

INTRODUCTION

Influenza is a respiratory disease caused by Influenza A and Influenza B viruses. It usually occurs in people with weakened immune systems, children, and older people with a sudden onset of high fever. Influenza disease is treated with antiviral medication [1]. One of the antiviral drugs is Peramivir (Figure 1). Peramivir is a neuraminidase inhibitor. FDA approved the peramivir drug to prevent new influenza viruses from infecting cells in December 2014 [2].

Many analytical methods were performed for the determination of Peramivir. Especially, the chromatographic techniques such as RP-HPLC [3], HPLC-MS/MS [4-5], HILIC-SPE-LC-MS/MS [6], were used for the analysis of Peramivir. However, scientists have been looking for new analytical methods recently because of the expensive and long analysis time of chromatographic techniques. The electrochemical methods are strong alternatives to chromatographic techniques [7]. Electrochemical sensors have many advantages, such as high selectivity and sensitivity and being affordable and environmentally friendly.

A molecularly imprinted polymer (MIP) is produced by polymerization in the presence of a target molecule [8]. The polymerization comprises a monomer, initiator, cross-linker, and target molecule. The enzymes, biomarkers, viruses, bacteria, and pharmaceutical drugs can be used as target molecules. MIP aims to form artificial receptors for target molecules [9]. MIP has three main processes: 1-Polymerization, 2-creating a cavity specific to the target molecule 3-compatible target molecules into cavities. The polymerization is performed with different techniques, such as thermal polymerization [10], photopolymerization [11-13], and electropolymerization [14-15]. Polymerization is a critical step because of polymeric film stability and repeatability. The monomers such as acrylamide, o-phenylenediamine, methacrylic acid, 4-aminophenol, 4-aminobenzoic acid, aniline were generally used for polymerization of electrodes. Basically, the photopolymerization technique takes place under a UV lamp, while thermal polymerization is performed in the oven. Electropolymerization in the presence of a target molecule and monomer is carried out by applying potential with cyclic voltammetry [15]. The thickness of the polymeric film should be controlled by an indirect method with redox markers. The cavities specific to the target molecule in the polymeric matrix are formed for creating the MIP receptors [16]. For creating a cavity process, the best removal solution should be selected [17]. Therefore, the MIP receptor recognizes the target molecules. It is enhanced the sensitivity and selectivity of analytical methods. Moreover, the MIP-based electrochemical sensor is easy to prepare, cheap, and has high mechanical and chemical stability.

In our study, we aimed to develop a MIP-based electrochemical sensor to determine of PERA. This study is the first to create an electrochemical sensor for PERA. MIP(PERA)/GCE was fabricated

with the electropolymerization of 4-aminophenol (4-AP) and ortophenilendiamine (o-PD) in the presence of PERA. The developed MIP(PERA)/GCE was applied to the commercial serum sample for analysis of PERA.

MATERIAL AND METHOD

Reagents and Chemicals

PERA is obtained by Tobio Novelpharma pharmaceutical company (İstanbul, Türkiye). Potassium ferricyanide ($[K_3Fe(CN)_6]$), ferrocyanide ($K_4[Fe(CN)_6].3H_2O$), and 0.1 M KCl were used to use the 5 mM redox marker ($[Fe(CN)_6]^{3-/4-}$). The 4-aminophenol (4-AP) and ortophenilendiamine (o-PD) were supplied from Sigma-Aldrich (St. Louis, Missouri, USA). These monomers were used for composing the polymeric film. Moreover, methanol (MeOH), acetic acid (HAc), oxalic acid, hydrochloric acid (HCl), acetonitrile (ACN), sodium hydroxide (NaOH), and ethanol were purchased by Merck (Darmstadt, Germany) to select optimum removal solution. The commercial serum sample was supplied from Sigma-Aldrich (product no: H4522). The interference chemicals (dopamine, ascorbic acid, uric acid, paracetamol, KNO_3, Na_2SO_4, and $MgCl_2$) were acquired from Sigma-Aldrich.

Apparatus

The redox process was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) by the Dropsens μStat 400 Bipotantiostat/ Galvanostat (Metrohm, Herisau, Switzerland). Three-electrode systems consisted of a glassy carbon working electrode (GCE, $3mm^2$, BASi, USA), a saturated Ag/AgCl reference electrode, and a Pt wire. All electrodes were supplied from BASi, USA.

The chemicals were weighed with precision balance (Ohaus Company, China) and dissolved with distilled water or pH 5.2 acetate buffer and sonicated with an ultrasonic bath. The solutions were kept in a refrigerator until use. The pH of the solutions was arranged with a pH meter. The commercial serum sample prepared for applying MIP(PERA)/GCE in the commercial serum sample was centrifuged at 3500 rpm for 30 min by a centrifuge from Nuve Company (NF200, Nuve Company, Türkiye). The thermal shaker (TS-100, Biosan, Riga, Latvia) was used at room temperature and 650 rpm to supply the movement of the target molecule from the polymeric matrix.

The morphology of MIP(PERA)/GCE surface and the electrochemical behavior of the films were characterized. The surface of morphology was evaluated with SEM (TESCAN GAIA 3, Czech Republic). The EIS (Metrohm Autolab, Utrecht, Netherlands) was used to examine the polymeric films regarding electrochemical behavior.

Fabrication of MIP(PERA)/GCE and NIP-based Electrochemical Sensors

The polymeric film was composed of two different monomers. To prepare the PERA stock solution, PERA was dissolved in double distilled water (ddwater). To prepare the MIP(PERA)/GCE, 2 ml 10^{-3} M 4-AP, 1 ml 10^{-3} M o-PD and 1 ml 10^{-3} M PERA, and 1 ml 5.2 acetate buffer were mixed and vortexed. The polymeric film was prepared in the same protocol without 1 ml10^{-3} M PERA to prepare the NIP-based sensor. The electrode was immersed in the polymeric film solution and electropolymerized scanning between -0.2 and 0.8 V for 5 cycles (50 mV/s scan rate). Then, the electrode was immersed in a 15 M HAc solution to create the pores in the polymeric matrix. The obtained results by MIP(PERA)/GCE was compared with NIP-based electrode in terms of analytical performances using a redox marker.

Preparation of Commercial Human Serum Sample

MIP(PERA)/GCE practicability was examined with commercial serum samples. The commercial serum sample in the presence of PERA as the target molecule was prepared. 1 mM PERA (1 ml), commercial serum (3.6 ml), 5.4 ml ACN (to precipitate the protein residues) were mixed in the centrifuge tube. PERA was not added to the commercial serum sample for the blank serum solution. Two centrifuge tubes were settled in a centrifuge as an opposite and centrifuged to separate the supernatant from the precipitate [18]. The gathered supernatant was used to evaluate the accuracy of

MIP(PERA)/GCE. Moreover, the calibration plot was obtained, and recovery studies were done in commercial serum samples.

RESULT AND DISCUSSION

Surface Characterization of the Molecularly Imprinted Polymeric Film and Non-imprinting Polymeric Film

The morphological structures of molecular imprinted polymeric film and non-imprinting polymeric film were characterized using SEM and SEM-EDX measurements (Figure 1). As expected, the non-imprinting polymeric film showed smoothness (Figure 1A), whereas the molecular imprinted polymeric film showed porosity and roughness (Figure 1B). The SEM-EDX of the MIP was also analyzed. The C and O atoms in the structure of the polymeric film were proved with The EDX spectra of the polymeric film (Figure 1C).

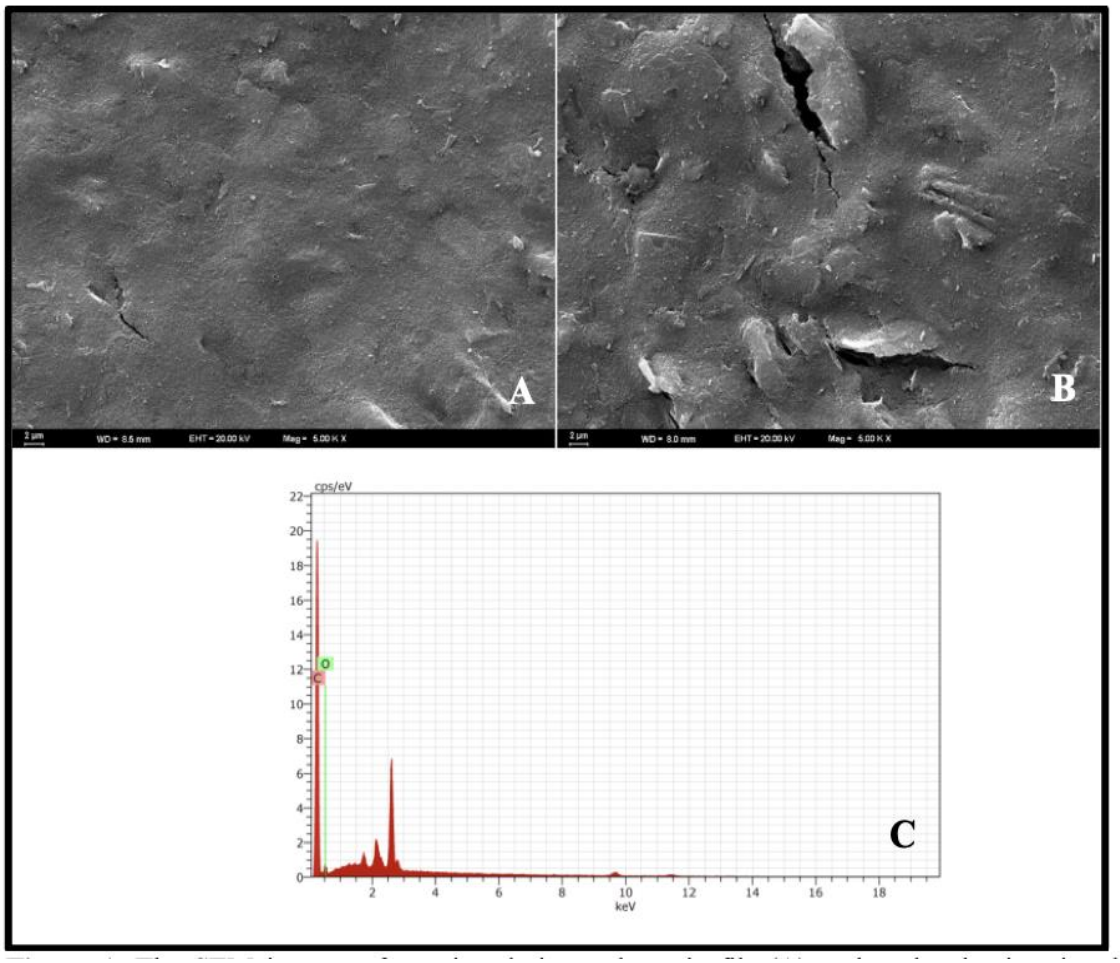

Figure 1. The SEM images of non-imprinting polymeric film(A) and molecular imprinted polymeric film (B) and EDX spectra of molecular imprinted polymeric film(C)

Electrochemical Characterization of the MIP-based Electrochemical Sensors

Electrochemical characterization of MIP-based sensors was performed with CV and EIS techniques. EIS and CV are based on charge resistance and electron transfer, respectively. After each process, the indirect measurements were performed in the solution of redox marker. The cyclic voltammograms are shown after each process in Figure 2A. Firstly, the current obtained with bare GCE was measured, and the highest current was obtained with bare GCE (black line). After

electropolymerization, the current decreased because electron transfer was slow (blue line). It was observed that the current increased (green line). Finally, the PERA was immobilized into PERA-specific cavities. The current decreased again (red line).

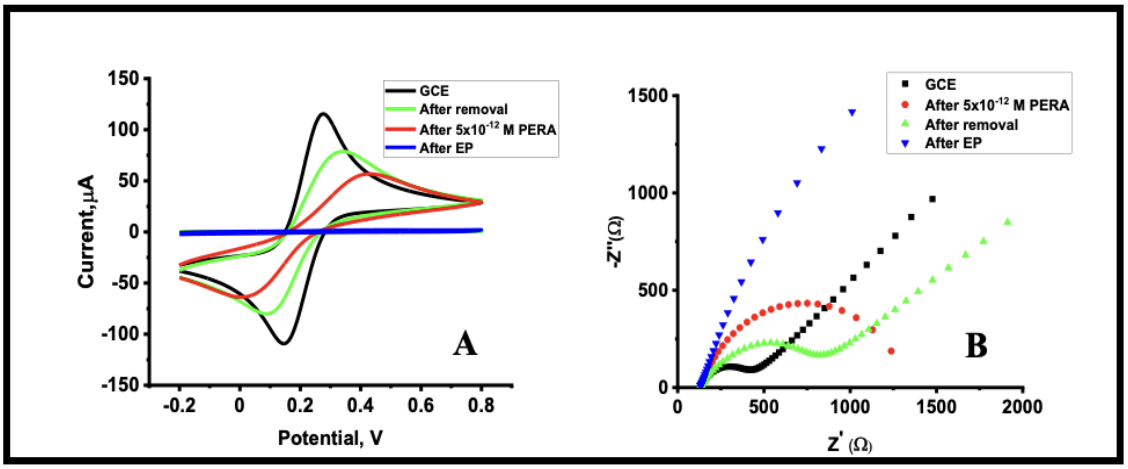

Figure 2. The cyclic voltammograms(A) and electrochemical impedance spectra(B) of MIP(PERA)/GCE in the presence of redox marker

The Nyquist plots were drawn with EIS measurement of solution of redox marker after each process in Figure 3B. The semi-circle represents the charge resistance. The lowest charge resistance was found in the bare GCE (black dots). After the EP process, it had the highest charge resistance (blue dots). After the removal process, the charge resistance decreased (green dots). Finally, the charge resistance increased again (red dots).

Optimization of Important Parameters

The crucial parameters were optimized to obtain the best MIP-based electrochemical sensor for the analysis of PERA. The o-pD:4-AP monomer ratio, monomer: template ratio, scan number, removal solution, removal time, and rebinding time were optimized. The optimization results are given in Figure 3.

The o-pD:4-AP Monomer Ratio:

Forming a polymeric matrix is an essential step for fabricating an MIP-based sensor. The o-pD and 4-AP monomers were tried separately. However, the MIP-based sensor composed of these monomers didn't respond well. For this reason, the combinations of monomers were studied. The difference (ΔI) between currents before removal and after electropolymerization was measured. Different ratios of o-pD: 4-AP (1:1; 1:2; 2:1) were tried to obtain the best polymeric film. As seen in Figure 3A., the ratio of o-pD:4-AP was found good response in 1:1.

Monomer: Template Ratio:

The monomer: template ratio was optimized after selecting the monomer and their ratio. The polymeric matrix was prepared with the combination of monomer (o-pD: 4-AP(1:1)) in the presence of PERA with an electropolymerization technique. The different ratio of the combination of monomers: template was studied between 20:1 and 2:1 while keeping the PERA constant at 1. According to Figure 3B., the best result(ΔI) was received in 10:1.

Number of Electropolymerization Scans:

The thickness of the polymeric film is related to the number of electropolymerization scans. Determining the best electropolymerization scan number is crucial for the sensitivity, repeatability, and stability of the MIIP(PERA)/GCE. The electropolymerization process on the GCE surface was carried

out by scanning the CV with a potential between -0.2 and 0.6 V. The number of EP scan were optimized from 3 to 10 cycles. The ΔI results were obtained similarly while numbers of scans 3 and 5. The results were not repeatable when scanning 3. For this reason, scan 5 was selected (Figure 3C).

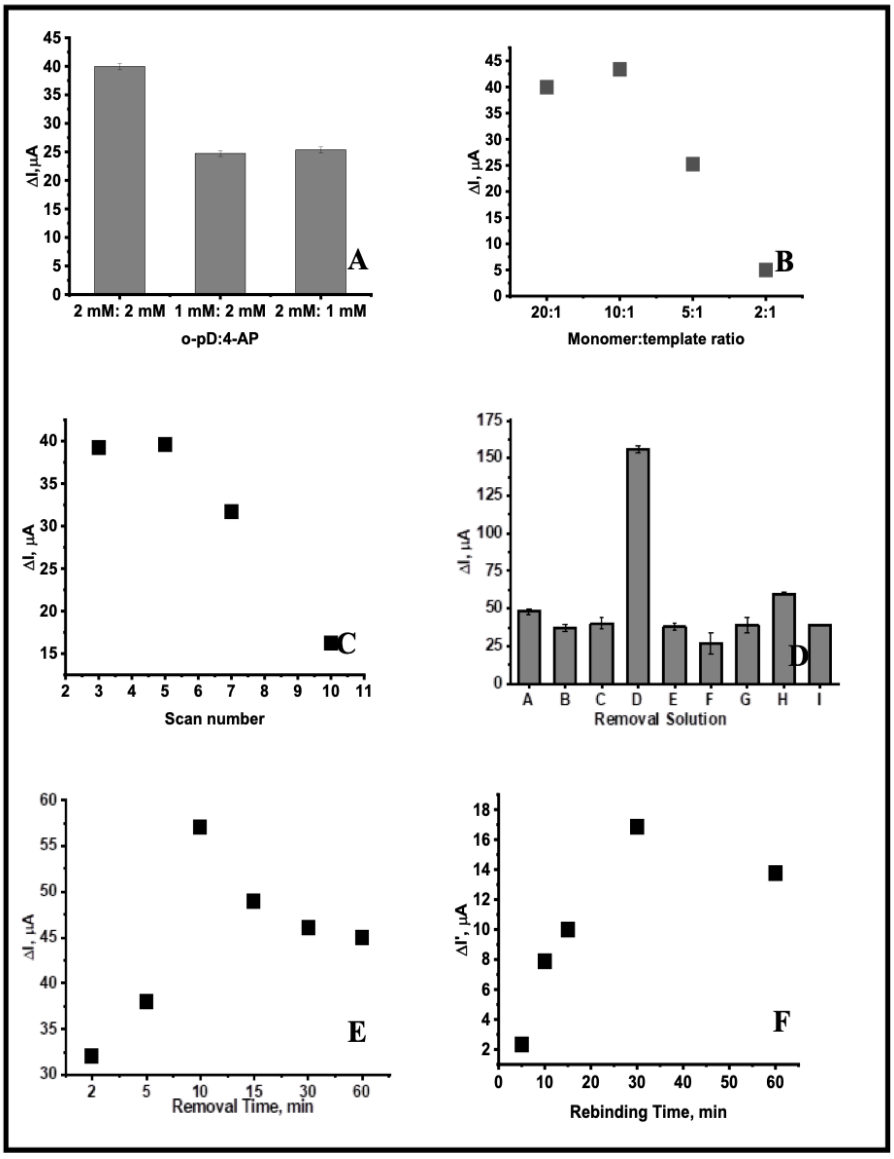

Figure 3. The optimization studies of o-pD:4-AP monomer ratio(A), monomer: template ratio(B), number of electropolymerization scan(C), removal solution(D), removal time(E) and rebinding time(F) for development of MIP(PERA)/GCE

Selection of Removal Solution and Removal Time

To remove the PERA from the cavities by broking non-covalent bonding, different removal solutions (Ethanol(A), ACN(B), NaOH. ddwater(C), 15 M HAc(D), 1M HCl(E), 1M NaOH(F), MeOH(G), 10 M HAc(H), 5 M HCl (I)) were tried. Moreover, oxalic acid and 5 M HCl were used as removal solutions; however, these solutions were not enough to remove PERA from the cavities. The obtained ΔI results were highest when 15 M HAc solution was used (Figure 3D). Therefore, the cavities were formed specific to PERA on the polymeric matrix. For determining the time of removal process

with 15 M HAc solution, the removal time from 2 to 20 min was optimized. According to Figure 3E, the removal time was chosen as 10 min.

Rebinding Time

After the removal, the PERA, the target molecule, was replaced with cavities specific to the target molecule. The 10^{-3} M PERA, the target molecule, was diluted to different concentrations. The PERA rebinding process was performed with the incubation of PERA by a thermo-shaker. The rebinding time was assessed by following the difference (ΔI') between currents after the removal process and after the rebinding process were measured. According to Figure 3F, the rebinding time was 30 min for MIP(PERA)/GCE.

Analytical Performances of MIP(PERA)/GCE and NIP-based Electrochemical Sensors

The calibration curve was drawn to examine the analytical performances of MIP(PERA)/GCE and NIP-based electrochemical sensors, and validation parameters were evaluated. Under the optimum condition, the different concentrations of PERA were incubated to MIP(PERA)/GCE. The redox solution was measured by DPV after rebinding PERA. The linear curve between 1.0 and 10 pM was obtained, plotting the concentration of PERA versus ΔI′ current. The calibration equation was found as $\Delta I' (\mu A) = 2.09x10^{12}$ (μA/M) x C (M) + 20.21 (μA) (r = 0.998) with LOD and LOQ values of 1.58 x 10^{-13} M and 5.27 x 10^{-13} M, respectively (Table 1). LOD and LOQ values are calculated as 3×sd/m and 10×sd/m, respectively [19-20] (sd: standard deviation; m: slope of calibration curve). The calibration plots for MIP(PERA)/GCE (red dots) and NIP-based sensor (black dots) and DP voltammograms for MIP(PERA)/GCE were given in Figure 4A and Figure 4B, respectively. NIP-based sensor was prepared to control the MIP(PERA)/GCE. According to the results, the MIP(PERA)/GCE showed excellent selectivity and sensitivity for the PERA analysis.

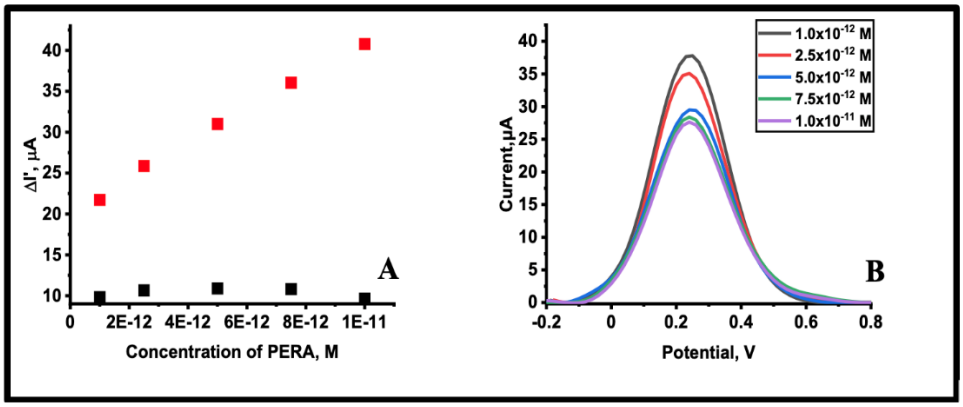

Figure 4. The calibration plots (A) for MIP(PERA)/GCE and NIP-based sensor and DP voltammograms (B) for MIP(PERA)/GCE

Analytical Application of MIP(PERA)/GCE and NIP-based Electrochemical Sensors in Commercial Human Serum Sample

MIP(PERA)/GCE practicability was investigated with commercial serum samples to demonstrate the accuracy of the sensor. The preparation of a commercial serum sample in the presence of PERA was explained in section 2.4. This sample was diluted to the required concentration of PERA and rebound to pores in the polymeric matrix. The 5 mM $[Fe(CN)_6]^{3-/4-}$ solution was measured by DPV after rebinding of PERA in the commercial serum sample. The linear curve between 1.0 and 10 pM was obtained, plotting the PERA concentration versus ΔI′ current. The calibration equation was obtained as $\Delta I' (\mu A) = 9.80x10^{11}$ (μA/M) x C (M) + 22.85 (μA) (r = 0.998) with LOD and LOQ values of 2.79 x 10^{-13} M and 9.31 x 10^{-13} M, respectively (Table 1).

Table 1. Regression data of the calibration line for PERA on MIP(PERA)/GCE

	Standard solution	**Serum sample**
Linearity range (M)	$1x10^{-12}$-$1x10^{-11}$	$1x10^{-12}$-$1x10^{-11}$
Slope (µA/M)	$2.09x10^{12}$	$9.80\ x10^{11}$
SE of slope	$6.96x10^{10}$	$3.39x10^{10}$
Intercept (µA)	20.221	22.854
SE of intercept	0.427	0.205
Correlation coefficient (*r*)	0.998	0.998
LOD (M)	$1.58x10^{-13}$	$2.79x10^{-13}$
LOQ (M)	$5.27x10^{-13}$	$9.31x10^{-13}$
Repeatability of peak current (RSD%)*	0.507	0.498
Reproducibility of peak current (RSD%)*	0.812	0.727

**Each value is the mean of three experiments*

The calibration plots for MIP(PERA)/GCE (red dots) and NIP-based sensor (black dots) and DP voltammograms for MIP(PERA)/GCE for commercial serum samples were given in Figure 5A and Figure 5B, respectively. NIP-based sensor was prepared to control the MIP(PERA)/GCE in the commercial serum sample.

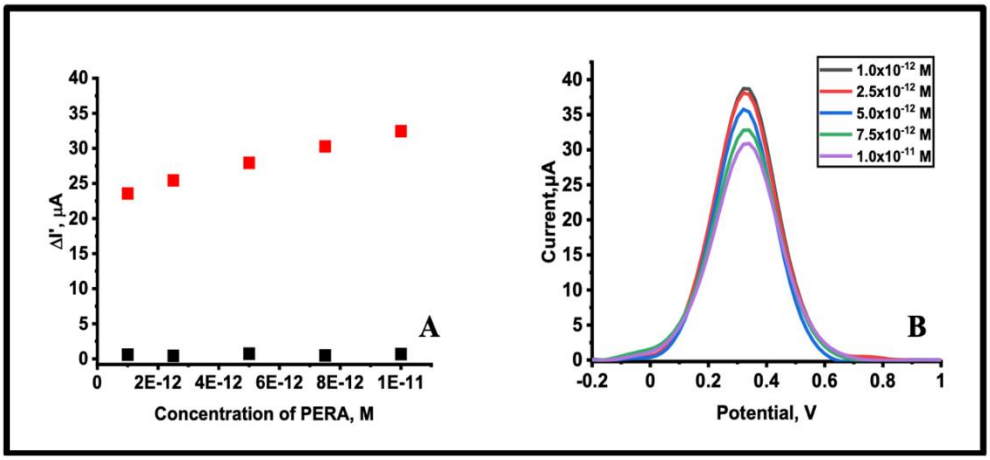

Figure 6. The calibration plots (A) for MIP(PERA)/GCE and NIP-based sensor and DP voltammograms for MIP(PERA)/GCE (B) for commercial serum samples

Furthermore, the recovery studies were performed with commercial serum samples (Table 2). Recovery and RSD % results have proven the accuracy and precision of MIP((PERA)/GCE.

Table 2. Results of the recovery experiments for commercial serum samples

	Serum Sample
Spiked amount (mg)	0.1000
Found amount (mg)*	0.1018
Average recovery (%)	101.81
RSD%	1.95
Bias%	+1.81

**Each value is the mean of three experiments*

Selectivity of MIP(PERA)/GCE and NIP-based Electrochemical Sensors

After removal, the cavities specific to PERA form in the polymeric matrix. The selectivity studies were performed by rebinding PERA and other selected compounds, such as Oseltamivir, Zanamivir, Brivudin, and Lamivudin (Figure 6). The imprinting factor (IF) was calculated by the ratio of obtained ΔI′ for MIP and NIP. The IF of PERA, Oseltamivir, Zanamivir, Brivudin, and Lamivudin were 18.69, 10, 7.5, 2.27, and 2.31, respectively. As expected, PERA shows a higher affinity to the cavities than similar compounds. The value of IF(MIP/NIP)> 1.0 confirmed that the developed sensors show the high affinity and selectivity to PERA molecule.

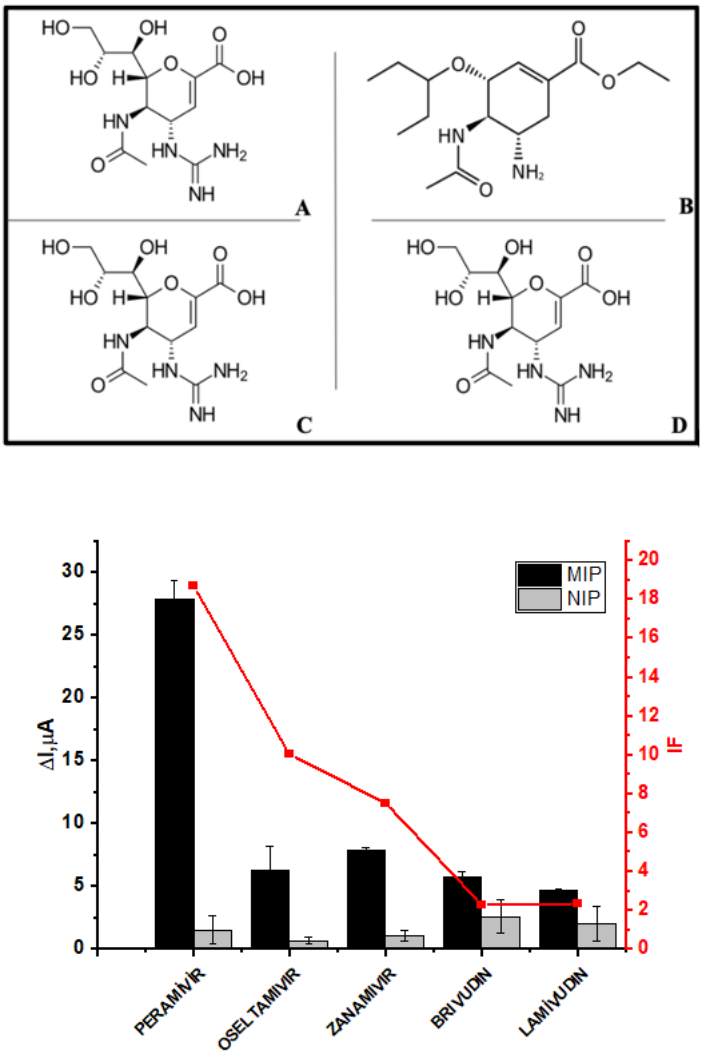

Figure 6. The structure of oseltamivir(A), zanamivir(B), brivudine(C), lamivudine(D). Selectivity studies of similar drugs and peramivir

Interference Study of MIP and NIP-based Electrochemical Sensors

The biological fluids in our body have many compounds, such as ascorbic acid, uric acid, paracetamol, KNO_3, Na_2SO_4, $MgCl_2$, and so on. In the interference study, these compounds are used as interference agents. The mixture of PERA and 10 times more interference agents were applied to the rebinding process. The 98.93-101.96% of recovery and 0.26-1.50% of RSD% showed that the interfering agents did not significantly affect the MIP(PERA)/GCE analytical performance (Table 3).

Table 3. Effect of interferents on the determination of PERA

Interferent	Recovery (%)	RSD (%)
Dopamine	100.38	1.03
Ascorbic acid	101.96	0.26
Uric acid	100.35	0.49
Paracetamol	99.41	0.82
K^+	98.93	0.89
NO_3^-	98.93	0.89
Na^+	101.07	1.50
SO_4^{2-}	101.07	1.50
Mg^{2+}	100.17	0.50
Cl^-	100.17	0.50

Stability of MIP Sensor

Stability is one of the validation parameters. The developed MIP(PERA)/GCE was tested regarding stability parameters. As a result of studies, effective results were found in daily preparation.

Conclusion

The electrochemical MIP(PERA)/GCE sensor was fabricated and used to determine PERA for the first time. The two monomers (o-pD and 4-AP) were applied to form the polymeric film consisting of the PERA. The MIP(PERA)/GCE was controlled with NIP based electrochemical sensor. The morphological and electrochemical characterization of sensors were analyzed. The required parameters were optimized to obtain the best performance of MIP(PERA)/GCE. The specificity and selectivity of the MIP(PERA)/GCE were evaluated with imprinting factors of similar structure and PERA. The MIP(PERA)/GCE analytical performance was examined, and the linear range of PERA was obtained between 1 pM and 10 pM with an LOD of 0.158 pM. The excellent recovery (101.81%) and RSD% (1.95%) results proved the feasibility and accuracy of MIP(PERA)/GCE in commercial serum samples. PERA was also analyzed in the presence of interference agents in serum samples. No interaction was observed. This study is important because it is the first MIP-based electrochemical sensor study with PERA. The developed sensor has the potential to adapt to other analytical methods.

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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

DETERMINATION OF TOXIC METALS IN SOME FOOD SUPPLEMENTS BY ICP-MS

ICP-MS İLE BAZI GIDA TAKVİYELERİNDEKİ TOKSİK METALLERİN BELİRLENMESİ

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ABSTRACT

Objective: *The presence of toxic metals in foods poses a significant risk to human health. Even low-level exposure to these metals can cause various health problems over a long period of time. In this study, we investigated the levels of toxic metals (Li, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Cd, Sb, Ba, Pb and Bi) in 34 food supplement samples purchased from pharmacies in Edirne.*
Material and Method: *The samples were digested using HNO_3 in a microwave system and the metal content was analyzed using ICP-MS.*
Result and Discussion: *Our results showed that none of the food supplements exceeded Turkish authority limits. Only Cd levels of two food supplements (0.431 µg/g and 0.316 µg/g) exceeded WHO guideline limits. We discussed the potential health effects of these metals and their compounds. Our findings suggest the need for increased regulation and monitoring of food supplements to ensure their safety and quality.*
Keywords: *Cadmium, dietary supplements, heavy metals, trace elements*

ÖZ

Amaç: *Gıdalarda toksik metallerin bulunması insan sağlığı için önemli bir risk oluşturmaktadır. Bu metallerin düşük dozda dahi uzun süreli maruziyeti çeşitli sağlık sorunlarına neden olabilir. Bu çalışmada, Edirne ili eczanelerinden satın alınan 34 gıda takviyesi örneğinde toksik metallerin (Li, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Cd, Sb, Ba, Pb ve Bi) seviyeleri araştırılmıştır.*
Gereç ve Yöntem: *Örnekler mikrodalga sistemde HNO_3 kullanılarak sindirilmiş ve metal içeriği ICP-MS kullanılarak analiz edilmiştir.*
Sonuç ve Tartışma: *Sonuçlarımız, hiçbir gıda takviyesinin Türk otorite sınırlarını aşmadığını göstermiştir. Sadece iki gıda takviyesinin Cd seviyeleri (0.431 µg/g ve 0.316 µg/g) Dünya Sağlık Örgütü (WHO) kılavuz sınırlarını aşmıştır. Bu metallerin ve bileşiklerinin potansiyel sağlık etkileri tartışılmıştır. Bulgularımız, gıda takviyelerinin güvenliği ve kalitesinin sağlanması için artan düzenleme ve izleme ihtiyacını göstermektedir.*
Anahtar Kelimeler: *Ağır metaller, eser elementler, kadmiyum, takviye edici gıdalar*

INTRODUCTION

Dietary supplements are defined in the Turkish Food Codex Regulation on Dietary Supplements as products that are prepared in capsule, tablet, lozenge, single-use powder packet, liquid ampoule, dropper bottle, and other similar liquid or powder forms, with a daily intake dose specified, of concentrated or extracted nutrients such as vitamins, minerals, proteins, carbohydrates, fiber, fatty acids,

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amino acids, or other nutrients, or plant, herbal, and animal-based substances, bioactive substances, and similar substances with nutritional or physiological effects, for the purpose of supplementing normal nutrition [1]. These food supplements are generally produced from plant-based substances or different natural sources. While it is easier for people to think that medicinal herbs, phytotherapy, and natural food supplements do not show adverse effects on health, it is important to note that the consumption of these substances can also pose potential risks and adverse effects on health [2]. Additionally, the quality and purity of herbal products and supplements can show variability, which can also affect their safety and efficacy [3]. Therefore, it is necessary to monitor the content of potentially toxic elements in these products. In addition to the different metals present in these products, plant material may be contaminated with pesticides, microbial contaminants, and other chemical toxins [4]. They can also be contaminated during chemical treatment or storage [5].

The European Commission has set maximum levels for certain contaminants in foods in Commission Regulation (EC) No 1881/2006 [6]. These regulations are also available in Turkish Food Codex Legislation [7]. In addition to other maximum levels of contaminants such as dioxins, nitrates, melamine, mycotoxins, and polycyclic aromatic hydrocarbons, certain metals such as lead, cadmium, arsenic, mercury, and tin are explained in these regulations.

The use of food supplements as a means of improving health and wellness has become increasingly popular in recent years. This trend has increased due to the health impact of the COVID-19 pandemic on the community to strengthen their immune system and protect their overall health [8]. While these products are often marketed as being safe and effective, there is growing concern about the potential presence of harmful substances, including metals. In particular, toxic metals such as lead, cadmium, arsenic and nickel pose inherent health risks even at relatively low concentrations. The toxic effects of metals such as lead, cadmium, arsenic, and nickel encompass a spectrum of health consequences. Lead exposure, especially in children, can result in plumbism, manifesting with cognitive impairments, neurological deficits, and developmental issues. Lead, inorganic lead, and organic lead compounds categorized for humans as Group 2B (Possibly carcinogenic), Group 2A (Probably carcinogenic), and Group 3 (Not classifiable as carcinogenic), respectively [9,10].

Cadmium is implicated in kidney damage, lung pathologies, and bone disorders, and it is established as a carcinogen, particularly associated with lung cancer upon extended exposure therefore classified as class 1 (Carcinogenic for humans) carcinogens by the The International Agency for Research on Cancer (IARC). Arsenic, when chronically consumed, is linked to carcinogenic effects, notably skin, lung, and bladder cancers, and classified as class 1 carcinogens by the IARC. Arsenic can also induce skin lesions, cardiovascular complications, and neurotoxicity. Nickel, upon prolonged dermal contact, can lead to skin allergies, such as dermatitis. Nickel compounds are classified as class 1 carcinogens by IARC with substantial evidence suggesting their capability to induce cancer in humans [10,11].

Some metals (such as Cr, Mn, Zn, Fe, Co, Cu, Al) are essential nutrients at trace levels and play vital roles in various physiological processes, they can become detrimental to health when ingested in excessive amounts [11].

Metals are usually determined by techniques, such as inductively coupled plasma optical emission spectrometry (ICP-OES) [12], atomic absorption spectrometry (AAS) [13], and inductively coupled plasma mass spectrometry (ICP-MS) [14]. For a convenient result, a sensitive analysis technique such as ICP-MS, more advanced than the generally used AAS, is performed in this study. This technique is widely recognized as one of the most sensitive and accurate methods for the determination of trace metals in a wide range of matrices, including food supplements. In addition to its sensitivity and accuracy, ICP-MS is also highly versatile. It can be used to analyze a wide range of sample types, including solids, liquids, and gases, and can be applied to a variety of different matrices. It provides also fast, with good reproducibility and low sample preparation requirements [15].

This study aimed to assess the metal content of food supplements in order to better understand the potential risks to human health. 34 food supplement samples randomly purchased from pharmacies in Edirne, Turkey and, analyzed them for 17 different metals content (Li, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Cd, Sb, Ba, Pb, and Bi) by using ICP-MS. Our findings provide insights into the recent status of Turkish food supplement market product safety and quality.

MATERIAL AND METHOD

Chemicals and Apparatus

All solutions were prepared with reagent grade chemicals and ultra-pure water (18 MΩ cm); nitric acid was ultrapure from Sigma Aldrich (St. Louis, MO, USA). The element standard solutions were prepared by diluting 1000 mg/l stock solution (ICP standard CertiPUR, Merck, Germany). Digestion of samples are conducted by using a closed microwave digestion system CEM Mars 5 (CEM Corporation, Mathews, North California, USA). Agilent 7700 Series ICP-MS instrument (Agilent Technologies, Waldbronn, Germany) was employed for metal analysis.

Samples

In our study, 34 dietary supplement samples were randomly selected and purchased as one package from those available in pharmacies in Edirne, Turkey. The dietary supplements had not expired, and their packages were unopened and intact. The intended uses of these supplements were diverse, including maintaining health and mental function, sexual enhancement, strengthening the immune system, and regulating the bone system, among others. The supplements were available in a variety of dosage forms, including bars, tablets, powders, lozenges, pastes, and capsules. The information about the samples is shown in Table 1.

Table 1. Sample properties

Sample No	Form of the Product	Product Specifications from Its Label	One Serving Size	Purpose of the Product
1	Bar	A Protein Bar	35 g	Sports
2	Paste	Bee Product Mixed with Herbs	7 g	Immunity
3	Tablet	Multivitamin	1.042 g	Bone System
4	Tablet	Contains Vitamin C	0.75 g	Immunity
5	Capsule	*Boswellia Serrata* Extract	0.46 g	Osteoarthritis
6	Tablet	Contains Vitamin D	0.333 g	Bone System, Immunity
7	Powder	Concentrated Herbal Tea	1.7 g	Weight Loss
8	Tablet	Contains *Panax ginseng*, Multivitamin and Minerals.	0.72 g	Maintaining Health, Immune System Enhancement
9	Tablet	Contains *Panax ginseng*, Multivitamin and Minerals	0.75g	Maintaining Health, Immune System Enhancement
10	Capsule	Contains Fish Oil	1.4 g	Heart health, Conditioning Mental Stability
11	Powder	Nutritious Meal Replacement Shake Mix	26.19 g	Weight Loss
12	Tablet	Contains Vitamin C, Green Tea, Caffeine and Mate Leaf	0.877 g	Weight Loss
13	Tablet	Contains Multivitamin and Minerals	0.9 g	Maintaining Health, Immune System Enhancement
14	Lozenge	Contains Eucalyptus, Mint and Menthol	2.8 g	Immunity, Sore Throat
15	Lozenge	Contains Vitamin C, Honey, Zinc and *Sambucus nigra*	2.5 g	Immunity, Sore Throat
16	Lozenge	Contains Linden, Lemon and Propolis	2,8 g	Immunity, Sore Throat
17	Tablet	Contains High Amounts of Calcium and Vitamin D	1.456 g	Bone System
18	Capsule	Contains Omega 3, Multivitamin and Minerals	2 g	Pregnancy Support
19	Tablet	Vitamin and Mineral Complex for Women	1.405 g	Maintaining Health, Immune System Enhancement
20	Powder	Sports Food Supplement Contains Amino Acid	26g	Sports, Muscle Building
21	Capsule	Contains Vitamin C, Zinc and *Sambucus nigra*	1 g	Maintaining Health, Immune System Enhancement
22	Powder	Contains Vitamin C, Zinc and *Sambucus nigra*	7.5 g	Maintaining Health, Immune System Enhancement

Table 1 (*continue*). Sample properties

Sample No	Form of the Product	Product Specifications from Its Label	One Serving Size	Purpose of the Product
23	Powder	Contains Multivitamin, Minerals and *Sambucus nigra*	5 g	Maintaining Health, Immune System Enhancement
24	Powder	Contains Creatine, Arginine, Beta alanine, Multivitamins, and Minerals.	7 g	Maintaining Health, Immune System Enhancement, Skin, and Hair Health
25	Paste	Bee Product Mixed with Herbs	7 g	Immunity
26	Powder	Probiotic Supplement	0.75 g	Intestinal regulator
27	Powder	Probiotic Supplement	2 g	Intestinal regulator
28	Powder	Contains Vitamin C and Zinc	1.5 g	Immunity
29	Powder	Contains Iron, Multivitamin and Minerals	1 g	Iron deficiency, Immunity
30	Tablet	Herbal Drug	0.259 g	Constipation Regulation
31	Powder	Food Supplement Containing L-Carnitine, L-Arginine, PABA, Coenzyme Q10, Multivitamin and Plant Extracts	5 g	Sexual Enhancement
32	Powder	Contains Mixed Herbs	5 g	Constipation Regulation
33	Capsule	Contains *Carthamus tinctorius* Oil	1.385 g	Weight Loss
34	Capsule	Contains *Opuntia ficus-indica* Extract, *Cinnamomum zeylanicum* Extract and Chromium	0.477 g	Weight Loss

Sample Preparation

Firstly, the homogenization of the food supplement samples is executed by using a mechanical homogenizer. This step was designed to ensure that the samples were well-mixed and representative of the entire batch. Then, a precise amount of each sample, typically in the range of 0.5 g is weighed. Nitric acid (%65) was used for the digestion of samples. Digestion of samples is executed by CEM Mars 5 closed microwave digestion system. Microwave parameters are shown in Table 2.

Table 2. Microwave device parameters

Level	Max. Power (%)	Power (W)	Time (min.)	Pressure (psi)	Temperature (°C)	Standby (min)
1	100	1800	20	800	210	15

Metal Analysis

Determination of metals in food supplement samples was performed by using the Agilent 7700 ICP-MS instrument. To proceed with the analysis, the procedure outlined in Environmental Protection Agency (EPA) Method 200.8 was followed [16]. ICP-MS Device Parameters are shown in Table 3.

Table 3. Parameters of the ICP-MS device used for element analysis

ICP-MS Device Parameters			
Power: 1550 W	Sample Acquisition Rate: 0.3 rps	OctP RF: 180 V	Deviation: 13 V
Depth: 8 mm	Max Blank Concentration: %100	Stabilization Time: 50 sec	OctP Slope: -8 V
Nebulizer Pump: 0.1 rps	Matching Voltage: 1.80 V	Energy Separator: 5 V	Calibration Curve Confidence Interval: 0.95
Omega Lens: 10 V	Carrier Gas Flow Rate: 1.05 L/min	Sample Acquisition Time: 50 sec	Relative Standard Deviation: %5
Cell Output: -50 V	S/C Temperature: 2°C	Layer Slope: -40 V	Cell Input: -30 V

ICP-MS analysis was employed to determine the metal content in food supplements by EPA Method 200.8. To evaluate the accuracy and precision of our analytical approach, recovery study was conducted with 25 ppb spike samples for each element. The results from the spike recovery study (Table 4) demonstrated the reliability and accuracy of our analytical method. The average recoveries for all elements were within acceptable ranges, indicating that our analytical method is capable of accurately measuring metal content in food supplement samples.

Table 4. Recovery values of the elements in spiked samples (n=10)

Metals	Recovery values (%)
^{7}**Li [He]**	101.51 ± 2.84
^{27}**Al [He]**	95.20 ± 2.10
^{51}**V [He]**	96.36 ± 2.00
^{52}**Cr [He]**	96.89 ± 2.14
^{55}**Mn [He]**	100.50 ± 2.25
^{56}**Fe [He]**	96.30 ± 6.20
^{59}**Co [He]**	101.35 ± 2.64
^{60}**Ni [He]**	100.09 ± 1.75
^{63}**Cu [He]**	98.87 ± 1.66
^{66}**Zn [He]**	102.38 ± 2.30
^{75}**As [He]**	100.10 ± 1.37
^{88}**Sr [He]**	96.96 ± 1.97
^{111}**Cd [No Gas]**	105.70 ± 1.98
^{121}**Sb [No Gas]**	102.40 ± 3.24
^{137}**Ba [No Gas]**	102.09 ± 1.42
^{208}**Pb [No Gas]**	100.56 ± 2.11
^{209}**Bi [No Gas]**	101.88 ± 2.06

The capabilities of the instrumentation were evaluated through the determination of the Limit of Detection (LOD) and Limit of Quantification (LOQ) using spike samples prepared at various concentrations for each individual metal. Moreover, the LOD values, which represent the minimum concentrations at which metals can be detected with a high degree of reliability, and the LOQ values, signifying the lowest concentrations at which these metals can be accurately measured with acceptable precision, were established for each specific element (Table 5).

Table 5. Method validation results of the studied elements

Metals	LOD (ppb)	LOQ (ppb)	%RSD
^{7}**Li [He]**	0.393	1.309	10.854
^{27}**Al [He]**	0.020	0.065	1.087
^{51}**V [He]**	0.007	0.023	0.284
^{52}**Cr [He]**	0.296	0.988	13.317
^{55}**Mn [He]**	0.040	0.133	1.863
^{56}**Fe [He]**	0.40	0.134	2.194
^{59}**Co [He]**	0.006	0.018	0.278

Table 5 (*continue*). Method validation results of the studied elements

Metals	LOD (ppb)	LOQ (ppb)	%RSD
^{60}Ni [He]	0.024	0.079	0.894
^{63}Cu [He]	0.042	0.140	1.522
^{66}Zn [He]	0.090	0.298	4.753
^{75}As [He]	0.009	0.031	0.383
^{88}Sr [He]	0.529	1.764	9.117
^{111}Cd [No Gas]	0.005	0.016	0.239
^{121}Sb [No Gas]	0.097	0.322	2.938
^{137}Ba [No Gas]	0.418	1.393	6.587
^{208}Pb [No Gas]	0.015	0.049	0.550
^{209}Bi [No Gas]	0.021	0.070	0.680

RESULT AND DISCUSSION

Toxic metals such as Al, Cd, Pb, As, Bi, Zn, Ba, and Ni are naturally present in the environment due to weather conditions, biological activities, and volcanic processes. However, human activities, including agricultural practices and industrial processes such as the use of chemical fertilizers and pesticides, irrigation of wastewater, intense coal combustion, and metalliferous mining, significantly amplify the contamination levels in soil, surface, and groundwater. As a result, these activities contribute to a substantial increase in the concentration of toxic elements in the environment.

The European Food Safety Agency (EFSA) and the Turkish Food Codex have established upper limits for lead and cadmium levels in toxic elements, which were evaluated in this study. The limit values for both metals are set at 3 µg/g [6,7]. Additionally, the European Pharmacopeia stipulates that the levels of cadmium in herbal drugs should be below 0.5 µg/g, while lead levels should not exceed 5.0 µg/g [17]. The World Health Organization (WHO) published an overview of the maximum limits for toxic metals set by countries in 2007, which recommends a limit of 10 µg/g for Pb and 0.3 µg/g for Cd in herbal products [18].

Heavy metals such as Cd, Pb, and As are among the most notable contaminants found in food supplements [9,15]. In the study we conducted Cd levels range from 0.002 µg/g to 0.431 µg/g, Pb levels range from 0.035 µg/g to 0.684 µg/g, and As levels range from 0.011 µg/g to 0.267 µg/g (Table 3 and 4). Our analysis of the supplements revealed that the lead and cadmium levels were below the established limits, except for samples 17 and 28. It is important to note that regulations regarding toxic metals only apply to the concentration of Pb, Cd, and Hg in plants, herbs, medicines, and food products. For other toxic metals, we have not found any authority establishments. Only, the water level of different toxic metal contaminations was established [19].

Lithium is not an essential element. Its presence in the environment can increase due to reasons such as the disposal of lithium-containing batteries. When taken through food and dietary supplements, lithium can compete to bind to carrier proteins for transport into cells due to its similarity to sodium and potassium and can cause toxic effects depending on the dose [20]. Our study found that lithium was present in very low levels ranging from 0 to 1.649 µg/g in the evaluated dietary supplements and below the recommended daily allowance level [20,21].

Natural and herbal food supplements have become increasingly popular in recent years, leading to a surge in production. As a result, the quality and safety of these supplements have become a major concern worldwide. Our research shows that most food supplements contain low levels of toxic metals (Tables 6 and 7). However, we found high concentrations of Cd (0.431 µg/g and 0.316 µg/g) in samples 17 and 28, respectively, which exceed the WHO limit for Cd in herbal products. Prolonged exposure to elevated concentrations of toxic metals can lead to a range of harmful health consequences, including skin and internal cancers, as well as cardiovascular and neurological effects [11,15]. As a result, it is

essential to ensure that plants used for producing food supplements are completely devoid of harmful and toxic components.

Table 6. Levels of Li, Al, V, Cr, Mn, Fe, Co and Ni elements (μg/g) detected (n=3) by ICP-MS

Sample	Li	Al	V	Cr	Mn	Fe	Co	Ni
1	1.649 ±0.24	1.178 ±0.06	0.019 ±0.00	0.074 ±0.01	0.441 ±0.01	4.893 ±0.05	0.025 ±0.00	0.245 ±0.02
2	1.101 ±0.15	2.666 ±0.20	0.008 ±0.00	0.032 ±0.00	0.652 ±0.01	2.692 ±0.04	0.008 ±0.00	0.216 ±0.02
3	0.935 ±0.27	20.416 ±1.05	0.858 ±0.03	1.591 ±0.02	22.801 ±0.09	106.211 ±0.96	0.473 ±0.00	0.870 ±0.02
4	0.369 ±0.17	15.655 ±0.11	0.026 ±0.00	1.195 ±0.01	0.204 ±0.00	21.494 ±1.01	0.041 ±0.00	0.569 ±0.01
5	0.386 ±0.08	7.490 ±0.08	0.008 ±0.00	0.048 ±0.00	0.104 ±0.01	2.921 ±0.03	0.002 ±0.00	0.056 ±0.01
6	0.447 ±0.14	1.221 ±0.06	0.015 ±0.00	0.060 ±0.01	0.213 ±0.01	2.299 ±0.04	0.009 ±0.00	0.128 ±0.01
7	0.308 ±0.21	7.050 ±0.19	0.027 ±0.00	0.759 ±0.01	19.625 ±0.31	58.117 ±0.17	0.085 ±0.00	0.516 ±0.02
8	1.005 ±0.22	6.289 ±0.31	0.234 ±0.00	16.969 ±0.13	321.852 ±3.51	4634.714 ±46.41	1.811 ±0.03	1.393 ±0.02
9	0.621 ±0.21	13.419 ±0.40	0.303 ±0.01	0.714 ±0.01	269.875 ±1.09	2527.304 ±15.33	0.178 ±0.01	0.517 ±0.01
10	0.360 ±0.12	0.648 ±0.12	0.003 ±0.00	0.027 ±0.00	0.315 ±0.01	4.355 ±0.13	0.001 ±0.00	0.029 ±0.01
11	0.647 ±0.13	4.603 ±0.23	0.036 ±0.00	0.266 ±0.06	14.790 ±0.10	48.343 ±0.61	0.157 ±0.01	1.142 ±0.01
12	0.395 ±0.11	433.181 ±2.84	0.167 ±0.01	0.798 ±0.01	95.212 ±0.70	26.297 ±0.42	0.341 ±0.01	3.539 ±0.08
13	0.360 ±0.16	7.158 ±0.22	0.628 ±0.02	1.633 ±0.02	805.887 ±13.61	4781.454 ±72.38	0.737 ±0.03	1.148 ±0.03
14	0.264 ±0.05	0.359 ±0.12	0.003 ±0.00	0.097 ±0.00	0.11 ±0.01	1.997 ±0.08	0.001 ±0.00	0.035 ±0.01
15	0.194 ±0.13	1.37 ±0.38	0.013 ±0.00	0.087 ±0.00	2.112 ±0.01	4.672 ±0.02	0.022 ±0.00	0.057 ±0.00
16	0.220 ±0.06	0.476 ±0.12	0.004 ±0.00	0.053 ±0.00	0.072 ±0.00	1.061 ±0.07	0.002 ±0.00	ND
17	0.177 ±0.07	11.22 ±0.16	2.036 ±0.06	2.910 ±0.04	127.078 ±1.56	22.131 ±0.50	0.565 ±0.00	1.4 ±0.02
18	0.029 ±0.14	1.304 ±0.16	0.095 ±0.01	0.038 ±0.00	8.630 ±0.05	2325.056 ±7.60	0.365 ±0.00	0.262 ±0.01
19	0.029 ±0.09	16.597 ±0.29	0.206 ±0.01	8.044 ±0.07	107.294 ±0.86	2356.312 ±20.88	0.504 ±0.01	0.233 ±0.01
20	0.055 ±0.09	1.574 ±0.10	0.028 ±0.00	0.069 ±0.00	9.692 ±0.07	153.181 ±0.64	0.034 ±0.00	ND
21	ND	18.146 ±0.26	0.107 ±0.01	0.350 ±0.01	0.774 ±0.01	10.623 ±0.05	0.010 ±0.00	0.141 ±0.02
22	ND	0.548 ±0.04	0.005 ±0.00	0.122 ±0.00	4.047 ±0.05	10.312 ±0.09	0.024 ±0.00	0.157 ±0.02
23	0.133 ±0.11	0.337 ±0.03	0.013 ±0.00	0.246 ±0.01	4.655 ±0.09	158.878 ±2.20	0.048 ±0.00	0.203 ±0.00
24	0.020 ±0.05	1.392 ±0.49	0.021 ±0.00	0.420 ±0.01	0.543 ±0.00	661.933 ±2.01	0.005 ±0.00	0.007 ±0.01
25	0.046 ±0.15	13.399 ±0.60	0.015 ±0.00	0.049 ±0.00	5.095 ±0.03	8.912 ±0.18	0.030 ±0.00	0.381 ±0.02
26	0.090 ±0.10	0.150 ±0.03	0.005 ±0.00	0.828 ±0.02	0.153 ±0.00	2.405 ±0.02	0.009 ±0.00	0.111 ±0.01
27	0.125 ±0.02	0.059 ±0.05	0.004 ±0.00	0.018 ±0.00	4.476 ±0.05	0.582 ±0.03	0.009 ±0.00	ND
28	0.255 ±0.13	21.986 ±0.54	0.056 ±0.00	0.172 ±0.01	5.408 ±0.05	5.431 ±0.05	0.087 ±0.01	0.221 ±0.01
29	0.891 ±0.35	3.970 ±0.04	0.198 ±0.00	0.286 ±0.01	196.882 ±0.84	11930.25±128.86	1.693 ±0.04	0.234 ±0.01
30	0.133 ±0.07	0.941 ±0.14	0.006 ±0.00	0.918 ±0.02	0.074 ±0.01	3.271 ±0.00	0.003 ±0.00	0.086 ±0.01
31	0.090 ±0.08	0.565 ±0.05	0.012 ±0.00	0.325 ±0.01	0.323 ±0.01	3.609 ±0.01	0.211 ±0.00	0.134 ±0.02
32	1.318 ±0.08	103.515 ±1.50	0.353 ±0.01	0.288 ±0.01	25.966 ±0.29	95.965 ±2.05	0.250 ±0.00	0.790 ±0.03
33	0.186 ±0.06	0.157 ±0.05	0.002 ±0.00	0.029 ±0.00	0.008 ±0.00	1.097 ±0.02	0.001 ±0.00	ND
34	0.613 ±0.07	48.180 ±0.49	0.080 ±0.01	149.011 ±2.28	21.747 ±0.48	56.236 ±0.81	0.119 ±0.00	0.561 ±0.03

Abbreviations: Not Detected (ND)

Table 7. Levels of Zn, As, Sr, Cd, Sb, Ba, Pb, Bi and Cu elements (μg/g) detected (n=3) by ICP-MS

Sample	Zn	As	Sr	Cd	Sb	Ba	Pb	Bi	Cu
1	5.691 ±0.10	0.023 ±0.00	1.485 ±0.01	0.008 ±0.00	0.105 ±0.02	0.549 ±0.00	0.047 ±0.00	2.321 ±0.00	0.916 ±0.01
2	2.199 ±0.03	0.022 ±0.01	0.581 ±0.01	0.008 ±0.00	0.100 ±0.07	0.294 ±0.00	0.055 ±0.00	2.328 ±0.03	0.289 ±0.00
3	1.111 ±0.04	0.210 ±0.02	156.516 ±1.02	0.250 ±0.00	0.044 ±0.01	1.754 ±0.03	0.176 ±0.00	1.269 ±0.13	0.387 ±0.00
4	0.135 ±0.02	0.054 ±0.02	0.264 ±0.01	0.006 ±0.00	0.072 ±0.02	0.162 ±0.00	0.101 ±0.00	2.146 ±0.02	0.356 ±0.00
5	0.279 ±0.05	0.022 ±0.00	0.254 ±0.01	0.004 ±0.00	0.038 ±0.01	0.240 ±0.00	0.052 ±0.00	2.222 ±0.01	0.046 ±0.00
6	12.51 ±0.03	0.048 ±0.01	0.532 ±0.01	0.011 ±0.00	0.177 ±0.08	0.678 ±0.00	0.041 ±0.00	2.059 ±0.02	0.292 ±0.01
7	139.14 ±1.2	0.026 ±0.00	2.832 ±0.04	0.025 ±0.00	0.012 ±0.00	3.350 ±0.04	0.061 ±0.00	1.939 ±0.05	18.45 ±0.00
8	3500 ±19.3	0.099 ±0.02	61.733 ±0.62	0.204 ±0.00	0.120 ±0.03	0.759 ±0.00	0.167 ±0.00	1.596 ±0.02	242.85 ±1.2
9	331.6 ±2.32	0.033 ±0.01	13.142 ±0.06	0.027 ±0.00	0.037 ±0.00	1.375 ±0.01	0.074 ±0.00	1.839 ±0.01	209 ±0.49
10	0.446 ±0.03	0.025 ±0.01	0.124 ±0.01	0.010 ±0.00	0.030 ±0.02	0.275 ±0.00	0.045 ±0.00	2.444 ±0.45	0.298 ±0.01
11	99.67 ±1.73	0.017 ±0.00	6.280 ±0.08	0.051 ±0.00	0.011 ±0.00	2.359 ±0.00	0.065 ±0.00	1.906 ±0.02	18.08 ±0.02
12	11.71 ±0.14	0.044 ±0.01	25.29 ±0.52	0.224 ±0.01	0.060 ±0.07	15.32 ±0.04	0.206 ±0.01	1.738 ±0.02	7.065 ±0.07
13	5597 ±122	0.041 ±0.00	32.544 ±0.97	0.192 ±0.01	0.044 ±0.02	1.334 ±0.03	0.142 ±0.00	1.600 ±0.04	626.03 ±12
14	0.846 ±0.05	0.024 ±0.01	0.188 ±0.00	0.005 ±0.00	0.013 ±0.01	0.052 ±0.01	0.052 ±0.01	2.127 ±0.03	0.395 ±0.01
15	1303 ±7.30	0.019 ±0.00	19.122 ±0.15	0.012 ±0.00	0.008 ±0.00	18.39 ±0.22	0.062 ±0.00	2.019 ±0.03	0.770 ±0.02
16	0.547 ±0.04	0.012 ±0.01	0.266 ±0.01	0.002 ±0.00	0.007 ±0.00	0.128 ±0.00	0.043 ±0.00	2.114 ±0.03	0.252 ±0.00
17	1184 ±12.5	0.085 ±0.00	54.179 ±0.50	0.431 ±0.01	0.024 ±0.00	7.108 ±0.02	0.095 ±0.00	1.381 ±0.00	205.35 ±2.5
18	1547.2 ±4.9	0.012 ±0.00	0.101 ±0.00	0.033 ±0.00	0.009 ±0.01	0.054 ±0.00	0.199 ±0.00	2.164 ±0.00	0.194 ±0.00
19	1527.1 ±11	0.036 ±0.01	16.507 ±0.08	0.083 ±0.01	0.315 ±0.00	0.628 ±0.01	0.145 ±0.00	1.563 ±0.06	91.27 ±1.09
20	94.94 ±1.26	0.022 ±0.01	2.567 ±0.03	0.007 ±0.00	0.019 ±0.00	0.967 ±0.01	0.036 ±0.00	1.90 ±0.03	10.47 ±0.06
21	8135 ±76.5	0.018 ±0.01	1.5 ±0.02	0.058 ±0.01	0.017 ±0.01	3.067 ±0.03	0.206 ±0.00	1.846 ±0.06	1.124 ±0.01
22	587.15 ±5.5	0.018 ±0.01	0.230 ±0.00	0.009 ±0.00	0.009 ±0.01	0.148 ±0.00	0.049 ±0.00	2.008 ±0.02	0.045 ±0.01
23	934.3 ±19.5	0.014 ±0.00	1.691 ±0.04	0.281 ±0.01	0.002 ±0.00	0.137 ±0.00	0.094 ±0.00	1.859 ±0.02	0.284 ±0.00
24	505.14 ±1.6	0.013 ±0.00	0.441 ±0.01	0.005 ±0.00	0.007 ±0.00	0.125 ±0.01	0.045 ±0.00	1.712 ±0.00	0.049 ±0.01
25	2.554 ±0.04	0.023 ±0.01	1.678 ±0.03	0.021 ±0.00	0.002 ±0.00	1.625 ±0.01	0.086 ±0.00	1.897 ±0.00	1.294 ±0.01
26	10.78 ±0.17	0.018 ±0.01	0.140 ±0.01	0.005 ±0.00	0.001 ±0.00	0.059 ±0.00	0.047 ±0.00	2.01 ±0.01	0.124 ±0.00
27	0.942 ±0.05	0.039 ±0.01	0.098 ±0.00	0.005 ±0.00	0.001 ±0.00	0.015 ±0.00	0.035 ±0.00	1.987 ±0.02	0.046 ±0.00
28	8884 ±33.4	0.021 ±0.00	0.681 ±0.01	0.316 ±0.01	0.005 ±0.00	0.837 ±0.00	0.084 ±0.00	2.048 ±0.1	0.048 ±0.00
29	11303 ±115	0.034 ±0.01	0.095 ±0.01	0.197 ±0.01	0.02 ±0.00	0.086 ±0.00	0.151 ±0.00	2 ±0.00	0.127 ±0.04
30	1.002 ±0.08	0.011 ±0.01	0.224 ±0.01	0.004 ±0.00	0.001 ±0.00	0.090 ±0.00	0.043 ±0.00	2.257 ±0.01	0.04 ±0.01
31	2298 ±9.73	0.038 ±0.01	0.397 ±0.02	0.020 ±0.01	0.004 ±0.00	0.135 ±0.01	0.169 ±0.00	2.099 ±0.02	0.408 ±0.01
32	20.07 ±0.3	0.267 ±0.03	298.412 ±1.96	0.042 ±0.00	0.027 ±0.01	53.84 ±0.43	0.694 ±0.01	1.874 ±0.02	6.419 ±0.03
33	0.632 ±0.04	0.005 ±0.00	0.090 ±0.00	0.003 ±0.00	ND	0.016 ±0.00	0.040 ±0.00	2.213 ±0.03	0.030 ±0.00
34	8.476 ±0.27	0.262 ±0.01	15.217 ±0.2	0.041 ±0.00	0.015 ±0.00	6.704 ±0.05	0.315 ±0.01	2.109 ±0.02	0.382 ±0.01

Abbreviations: Not Detected (ND)

The multiple use of dietary food supplements (Samples 11, 17, 23, 28, and 29) which are used for different health effects, may increase the risk of high cadmium intake. When taken together in a day as one serving, these supplements can provide a total of 4.04 μg of cadmium. This is 28.9% of the minimum recommended daily intake of cadmium, which is 14 μg. If these supplements are taken twice daily or if other supplements are also taken, the cadmium intake could be even higher. In addition, if the individual

is exposed to other sources of cadmium, such as food, smoke, or fumes, it would be even easier to exceed the minimum recommended daily intake.

Prolonged exposure to elevated levels of aluminum has been associated with neurological and bone disorders, such as Alzheimer's disease and osteomalacia. The exact mechanisms of aluminum toxicity are still the subject of ongoing research, but minimizing exposure to high levels of aluminum, especially through daily sources, remains an important public health concern [22]. In our study the aluminum levels were ranging from 0.150 ±0.03 to 433.181 ±2.84 (Table 6).

According to Table 8, none of the food supplements have exceeded the daily intake limits with their single-size serving use. However, it is important to note that the hazardous effects of metals may occur due to the multiple uses of these supplements. Accumulation of toxic metals may lead to intoxication in individuals. The limits in the table are based on literature and daily intakes may vary depending on an individual's conditions, physiological factors, sex, and other factors.

Table 8. Minimum Risk Levels/Recommended Dietary Allowances/No Observed Adverse Effect Levels of the Elements/Day

Elements	AI/RDA/NOAEL/MRL/DI/PTDI	References
Li	RDA: 1 mg/day for adults	[20,21]
Al	DI: 0.10-0.12 mg of Al/kg/day for adults	[14]
V	MRL: 210 µg for adults	[14]
Cr	RDA: 35 µg for males and 25 µg for females	[23]
Mn	RDA: 2.3 mg for males and 1.8 mg for females	[23]
Fe	PTDI: 48 mg	[24]
Co	DI: 0.005-1.8 mg	[14]
Ni	DI: 100-300 µg	[24]
Cu	RDA: 900 µg for adults	[23]
Zn	AI: 11 mg for males and 8 mg for females	[23]
As	MRL: 21 µg	[23]
Sr	DI: 2-4 mg	[25]
Cd	MRL: 14 µg	[14]
Sb	MRL: 0.4 and 6 µg/kg/d	[26]
Ba	MRL: 0.2 mg/kg/d	[26]
Pb	DI: 490 µg	[27]
Bi	RDA: 0.6-0.8 g	[28]

Abbreviations: AI, adequate intake; DI, daily intake; RDA, recommended dietary allowance; PTDI, provisional tolerable daily intake; NOAEL, no observed adverse effect level; MRL, minimum risk level. Non-specified intakes are for 70kg healthy adults

In conclusion, the use of ICP-MS to measure trace elements in food supplements enables rapid analysis with high precision and accuracy. Our results demonstrate that the food supplements examined contained elements in the microgram per gram range, and the concentrations of these elements varied significantly. Notably, the levels of toxic elements differed significantly among the samples analyzed, which can be attributed to a range of factors such as environmental and agronomic conditions, varying exposure to pollutants, inconsistent storage conditions, and unreliable supply sources. Additionally, differences in geographic location, composition, and production processes may also contribute to variations in the levels of toxic metals. Therefore, our findings suggest that it is crucial to monitor the concentration of toxic metals in food supplements, particularly those used for human consumption.

AUTHOR CONTRIBUTIONS

Concept: Ç.O., M.D.; Design: Ç.O., M.D.; Control: Ç.O., M.D.; Sources: Ç.O., M.D.; Materials: Ç.O., M.D.; Data Collection and/or Processing: Ç.O., M.D.; Analysis and/or Interpretation: Ç.O., M.D.;

Literature Review: Ç.O., M.D.; Manuscript Writing: Ç.O., M.D.; Critical Review: Ç.O., M.D.; Other: Ç.O., M.D.

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

UNVEILING THE THERAPEUTIC POTENTIAL OF *GINKGO BILOBA*: A NETWORK PHARMACOLOGY APPROACH FOR PARKINSON'S DISEASE

GİNKGO BİLOBA'NIN TEDAVİ POTANSİYELİNİN ORTAYA ÇIKARILMASI: PARKİNSON HASTALIĞINA YÖNELİK AĞ FARMAKOLOJİSİ YAKLAŞIMI

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ABSTRACT

Objective: *The aim of the current study is to identify the major phytoconstituents in Ginkgo biloba that could modulate the role of major therapeutic targets involved in the pathogenesis of Parkinson's disease using approaches in network pharmacology.*
Material and Method: *The phytoconstituents in Ginkgo biloba and their therapeutic targets and the targets of Parkinson's disease were identified using various online databases and software. The identified phytoconstituents were subjected to evaluation of several pharmacokinetic properties and druglikeness study. The phytoconstituents with favourable pharmacokinetic and druglikeness properties and targets with better topological parameters were subjected to molecular docking study and MMGBSA analysis.*
Result and Discussion: *This study identified the presence of 125 major phytoconstituents in Ginkgo biloba and out of 125 phytoconstituents, 30 phytoconstituents passed the pharmacokinetics and druglikeness property. The therapeutic targets for these selected phytoconstituents were found to be 468 and the disease targets in PD were found to be 2033. The common targets between phyto-targets and disease targets were found to be 44 targets. Out of 44 common targets, 5 top proteins CNR1, HPGDS, AR, RXRA and HDAC1 were identified on the basis of the topological parameters such as degree centrality and betweenness centrality in the Cytoscape 3.9.1 software. The docking studies and MMGBSA analysis revealed that beta-eudesmol has better interaction with the top 5 therapeutic targets.*
Keywords: *Ginkgo biloba, network pharmacology, parkinson's disease, schrödinger, sitoscape*

ÖZ

Amaç: *Mevcut çalışmanın amacı, ağ farmakolojisindeki yaklaşımları kullanarak Parkinson hastalığının patogenezinde yer alan ana terapötik hedeflerin rolünü modüle edebilen Ginkgo biloba'daki ana bitki kaynaklı bileşenleri belirlemektir.*
Gereç ve Yöntem: *Ginkgo biloba'daki bitki kaynaklı bileşenler ile bunların terapötik hedefleri ve Parkinson hastalığının hedefleri, çeşitli çevrimiçi veritabanları ve yazılımlar kullanılarak belirlendi. Tanımlanan bitki kaynaklı bileşenlerin, çeşitli farmakokinetik ve ilaç benzeri özellikleri değerlendirildi Uygun farmakokinetik ve ilaç benzeri özelliklere sahip bitki kaynaklı bileşenler ve daha iyi topolojik parametrelere sahip hedefler, moleküler yerleştirme çalışmasına ve MMGBSA*

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analizine tabi tutuldu.
Sonuç ve Tartışma: *Bu çalışma ile Ginkgo biloba'da 125 ana bitki kaynaklı bileşenin varlığı saptandı ve 125 bitki kaynaklı bileşenden 30'u uygun farmakokinetik ve ilaç benzeri özellik gösterdi. Seçilen bu bitki kaynaklı bileşenler için 468 terapötik hedef ve Parkinson için 2033 hastalık hedefi bulundu. Fito-hedefler ile hastalık hedefleri arasındaki ortak hedefler 44 hedef olarak bulundu. 44 ortak hedeften, Cytoscape 3.9.1 yazılımındaki derece merkeziliği ve arasındalık merkeziliği gibi topolojik parametrelere dayanılarak 5 üst protein CNR1, HPGDS, AR, RXRA ve HDAC1 tanımlandı. Yerleştirme çalışmaları ve MMGBSA analizi, beta-eudesmol'ün ilk 5 terapötik hedefle daha iyi etkileşime sahip olduğunu ortaya çıkardı.*
Anahtar Kelimeler: *Ağ farmakolojisi, cytoscape, Ginkgo biloba, parkinson hastalığı, schrödinger*

INTRODUCTION

The current approach in drug discovery is to design highly selective ligands to either activate or inhibit a specific target that is proven to play a crucial role in the pathogenesis of a specific disease. This approach is based on the assumption that a ligand that exhibits a high degree of specificity towards a single target would be safe and effective with minimal adverse effects [1]. This 'one gene, one drug, one disease' approach towards discovery of novel drugs is challenged by the recent developments in the field of systems biology and network pharmacology. Network pharmacology aims to discover drug leads and understand mechanism of action of potential drugs through interaction with multiple therapeutic targets [2]. Network pharmacology is considered to be an essential method for development of phytochemicals as potential therapeutic agents because of the potential of natural phytoconstituents to act on multiple targets in various signalling pathways [3]. Network pharmacology elucidates the interaction between drug and targets; drug and drug and the impact of potential drugs on biological pathways and networks. Through comprehensive analysis of biological pathways and networks, network pharmacology can reveal the underlying mechanisms of drug action, identify potential therapeutic targets, predict adverse effects and help in drug discovery and development. *Ginkgo biloba*, native to China, is a large ancient tree reaching a height of 20-35 m that belongs to the family, Ginkgoaceae. Phytochemical analysis of *Ginkgo biloba* has revealed the presence of terpene trilactones such as ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J and bilobalide, flavonol glycosides biflavones, proanthocyanidins, alkylphenols, phenolic acids, 6-hydroxykynurenic acid, 4-*O*-methylpyridoxine and polyprenol [4,5]. *Ginkgo biloba* has been used medicinally for around 2000 years mostly in China but also in other parts of the world. Several studies have revealed the potency and efficacy of *Ginkgo biloba* in various ailments like Alzheimer's disease, epilepsy, peripheral vascular disease, ischemia induced oxidation, liver injury, hyperlipidaemia, depression, tardive dyskinesia, generalized anxiety, diabetes, inflammation and cancer [6]. *Ginkgo biloba* has also been reported in literature to exerts its beneficial effects in various in vitro and in vivo animal models of Parkinson's disease [7]. Parkinson's disease is a complex neurological condition that involves the loss of dopaminergic neurons in the substantia nigra as well as the emergence of Lewy bodies and Lewy neurites. The prominent hallmarks of the disease include resting tremors, stiffness, bradykinesia, and postural instability, along with a number of non-motor indications, including autonomic dysfunction, sleep issues, exhaustion, constipation, depression, cognitive decline, and loss of smell or taste, among others [8]. The present study aims to identify the main phytochemicals of the *Ginkgo biloba* that modulate the considerable therapeutic targets of Parkinson's disease using network pharmacology and computational approaches such as molecular docking, MMGBSA, and molecular dynamics.

MATERIAL AND METHOD

Identification and Retrieval of Phytoconstituents of *Ginkgo biloba*

The phytoconstituents of the plant, *Ginkgo biloba* were identified and retrieved from Indian Medicinal Plants Phytochemistry and Therapeutics (IMPPAT) Dr. Duke's Phytochemical and Ethnobotanical databases and through comprehensive literature survey. The chemical structure of the phytochemicals present in *Ginkgo biloba* was obtained in SDF format from PubChem [9].

Evaluation of Drug-likeliness and BBB Permeation

The phytochemicals present in *Ginkgo biloba* were analysed for their drug likeness and permeation through blood brain barrier (BBB) using SwissADME. For prediction of drug-likeness and BBB permeability, SwissADME received input in the Canonical SMILES format of phytochemicals. The Canonical SMILES format of phytochemical compounds was given as the input in SwissADME for prediction of drug-likeness and permeation through BBB. The potential toxicity of the phytochemicals was predicted using AdmetSAR. The compounds that pass the BBB, have one or less than one violation of Lipinski rule of five and exhibited no toxicity were considered for further analysis [10].

Prediction of Phytochemical Targets and Disease Targets

The therapeutic targets of the retrieved phytochemicals present in *Ginkgo biloba* were predicted using SwissTargetPrediction. A list of potential targets was obtained by giving the Canonical SMILES format of phytochemicals compounds as input in SwissTargetPrediction.[11]. The disease targets were obtained using DisGeNet database. The list of therapeutic targets involved in the pathogenesis of Parkinson's disease was obtained by typing the name of the disease (Parkinson's disease) in the DisGeNet database followed by retrieval of the disease-gene associations [12].

Identification of Common Targets

The common targets between the phytochemical targets and Parkinson's disease targets were identified using Venny2.1.0 tool. A Venn diagram was constructed to identify the common targets between the phytochemicals and the Parkinson's disease targets. The list of phytochemical targets and Parkinson's disease targets were given as input to Venny tool in two separate lists. Venny tool analyses the list of phytochemical targets and Parkinson's disease targets and provides a list of the common targets in the results box.

Target Network Construction and Topological Analysis

A network of common targets based on their functional relationship were constructed using GeneMania (Multiple Association Network Integration Algorithm), a Cytoscape plugin [13]. The topological parameters, betweenness centrality (BC) and degree centrality (DC) were determined using CytoNCA to identify the top 5 essential therapeutic targets involved in Parkinson's disease [14].

Molecular Docking

The structure of the top 5 proteins CNR1 (6N4B), HPGDS (1IYH), AR (2YHD), BCHE (6ESJ), HDAC1 (4BKX) were downloaded in the PDB format from the RCSB Protein data bank (PDB). Further, the 2D structures of the selected ligands were obtained from the PubChem online database and downloaded in sdf format. The 3D structure of the protein was prepared using Schrodinger Maestro protein preparation wizard [15,16]. The 2D structures of all the selected phytoconstituents were transformed into a minimal 3D structure using the LigPrep wizard of Schrodinger maestro. For precise tautomer enumeration and to determine the protonation state in biological status, the potential ionisation state was created using Epik at the target pH of 7.0 ±2.0. By maintaining certain chiralities, stereoisomers might be created with a maximum of 32 per ligand. OPLS3 force field settings were employed and the processes that followed used the lowest penalty state. The receptor grid files were generated by using the default options of the Receptor Grid generation function of Schrodinger maestro. The ligand required for docking was determined to create the grid around it. The centroid of the ligand served as the centre of the grid box, and "Dock ligands similar in size to the Workspace ligand" was selected as the grid size. The top 5 selected target proteins were docked with the chosen phytoconstituents using the extra precision (XP) glide of Schrodinger maestro. It takes into account the penalties applied to non-cis/trans amide bonds. The docking procedure was validated through measurement of root mean square deviation (RMSD) [15,16]

Molecular Mechanics with Generalised Born and Surface Area Solvation (MMGBSA)

MMGBSA was used to calculate the binding free energy of the top 5 target-phytoconstituent docked complexes. The binding free energy was calculated using the OPLS 2005 force field, VSGB solvent model, and rotamer search methods [17]. The binding free (ΔGbind) energy calculated using the below formula:

$$\Delta Gbind = Gcomplex - (Gprotein + Gligand)$$

where Gcomplex, Gprotein, and Gligand are the free energies of the complex, protein, and ligand respectively.

RESULT AND DISCUSSION

Identification and Retrieval of Phytoconstituents of the Plant *Ginkgo biloba*

Network pharmacology is a distinctive *in-silico* approach that allows targeting multiple therapeutic targets involved in the pathogenesis of a disease instead of a single target. It has enabled a paradigm shift from the classical existing 'one disease–one target–one drug' dogma to the current 'multicomponent, multi-target' approach [18]. In the current study, network pharmacology analysis was adopted to identify the potential phytochemicals present in *Ginkgo biloba* that can modulate the therapeutic targets involved in the pathogenesis of PD.

The current principle of 'one disease–one target–one drug' in drug design and discovery is challenged by large scale functional genomic studies. These findings ascertain that modulation of single proteins in a network do not affect the disease networks to a significant degree whereas modulation of multiple proteins might offer significant therapeutic benefits. Single gene knock-out studies have revealed that knockout of single genes have little or no effect at all on the phenotype of an organism[19-21]. For instance, a systematic single gene deletion study in yeast has discovered that only 15 % of the single gene deletions result in defects in the fitness whereas the remaining deletions have no significant effect on yeast[22]. These findings reveal that a biological pathway is highly robust, redundant, and has alternative signalling routes. These properties of a disease network emphasize that instead of modulating the function of a single protein in a pathway, network pharmacology recommends that modulating the functions of multiple proteins in a disease network would offer significant therapeutic benefits.

A total of 125 phytoconstituents reported to be present in *Ginkgo biloba* were identified and retrieved from Indian Medicinal Plants Phytochemistry and Therapeutics (IMPPAT) and Dr. Duke's Phytochemical and Ethnobotanical database. The list of phytoconstituents in *Ginkgo biloba* is presented in the supplementary file.

Drug-likeness of the Retrieved Phytochemicals Compounds

Physicochemical, drug-likeness, various pharmacokinetic, and toxicity properties of 125 phytochemicals found in Ginkgo biloba were estimated using Swiss ADME and AdmetSAR. Out of 125 selected phytochemicals, 30 phytochemicals passed the drug- likeness and blood-brain barrier BBB permeation. The complete list of the 30 phytochemicals that passed pharmacokinetic prediction is provided in the supplementary file and represented in Figure 1. The results of the pharmacokinetic properties of the top five phytoconstituents are represented in Tables 1, 2, and 3.

Table 1. Physicochemical properties and drug-likeness of selected phytoconstituents

Phyto-constituent	Physicochemical properties					Drug-likeness	
	Mol.Wt.	Number of rotatable bonds (ROTB)	Number of Hydrogen Bond acceptors (HBA)	Number of Hydrogen Bond Donors (HBD)	Aqueous Solubility (LogS)	Bioavailability score	Lipinski's violations
Beta-Eudesmol	222.37	1	1	1	-3.51	0.55	0

Table 1 (*continue*). Physicochemical properties and drug-likeness of selected phytoconstituents

Phyto-constituent	Physicochemical properties					Drug-likeness	
	Mol.Wt.	Number of rotatable bonds (ROTB)	Number of Hydrogen Bond acceptors (HBA)	Number of Hydrogen Bond Donors (HBD)	Aqueous Solubility (LogS)	Bioavailability score	Lipinski's violations
Linoleic acid	280.45	14	2	1	-5.05	0.85	1
Elemol	222.37	3	1	1	-3.8	0.55	0
Linolenic acid	278.43	13	2	1	-4.78	0.85	1
Myristic acid	228.37	12	2	1	-4.31	0.85	0

Table 2. Lipophilicity and medicinal chemistry of selected phytoconstituents

Phytoconstituent	Lipophilicity		Medicinal chemistry		
	Topological Polar Surface Area (TPSA)	Partition Coefficient (CLogP)	PAINS alert	Brenk alert	Lead-likeliness
Beta-Eudesmol	20.23	3.61	0	1	2
Linoleic acid	37.3	5.45	0	1	2
Elemol	20.23	3.77	0	1	2
Linolenic acid	37.3	5.09	0	1	2
Myristic acid	37.3	4.45	0	0	3

Table 3. Pharmacokinetic and toxicity profile of selected phytoconstituents

Phytoconstituent	Pharmacokinetic parameters							Toxicity	
	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Ame's mutagenicity	Carcinogenicity
Beta-Eudesmol	High	Yes	No	No	Yes	No	No	No	No
Linoleic acid	High	Yes	Yes	No	Yes	No	No	No	No
Elemol	High	Yes	No	No	Yes	No	No	No	No
Linolenic acid	High	Yes	Yes	No	Yes	No	No	No	No
Myristic acid	High	Yes	Yes	No	No	No	No	No	No

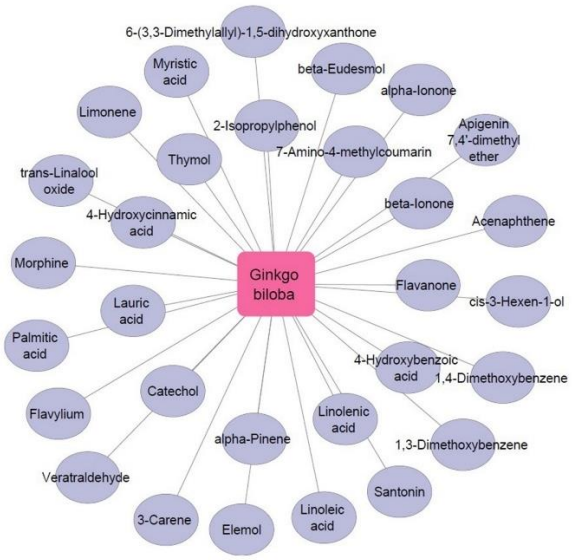

Figure 1. Pictorial representation of selected phytoconstituents of *Ginkgo biloba*

The figure represents the major phytoconstituents present in *Ginkgo biloba.*

Prediction of Phytochemical Targets and Disease Targets

The therapeutic targets associated with the phytoconstituents present in *Ginkgo biloba* and Parkinson's disease were retrieved from SwissTargetPrediction and DisGeNET databases respectively. A total of 468 targets was associated with phytoconstituents present in *Ginkgo biloba* and 2033 targets were associated with Parkinson's disease. A Venn diagram analysis of the targets revealed that 44 targets are common between phytoconstituents targets and Parkinson's disease targets. The list of phytoconstituent targets, disease targets and common targets are provided in the supplementary file. The results are represented in Figure 2.

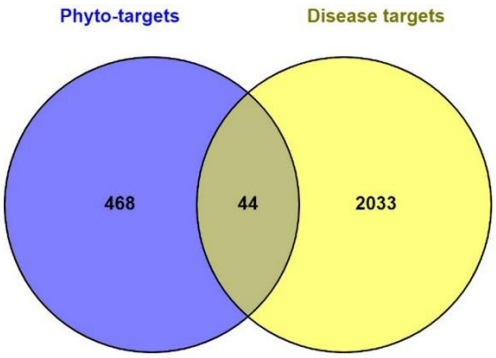

Figure 2. Common Targets between *Ginkgo biloba* and Parkinson's disease (The figure represents the total number of phyto-targets, disease targets and common targets)

Target Network Construction and Topological Analysis

A protein-protein interaction network of selected common targets was constructed using GeneMania, a Cytoscape plugin. The topological parameters like Degree Centrality (DC), Betweenness Centrality (BC) and Closeness Centrality (CC) of the network were analyzed to determine the most important targets in the network. The top five proteins in the network were selected based on Degree Centrality (DC), Betweenness Centrality (BC) and Closeness Centrality (CC). Degree centrality measures the number of connections a node (protein/target) makes with other nodes in a network. Betweenness centrality determines the influence of a node (protein/target) in controlling the interaction between a pair of nodes (protein/target) passing through this node in the network. Closeness centrality measures the inverse of average distance of a node from all other nodes in a network. It is widely accepted that nodes with higher degree, betweenness and closeness centrality values may represent important targets and play a crucial role in a biological network. The targets selected based on topological analysis were: CNR1 (Cannabinoid receptor 1), HPGDS (Prostaglandin-D synthase), AR (Androgen receptor), BCHE (Butyrylcholinesterase) and HDAC1 (Histone deacetylase 1). The network is represented in Figure 3 and the results are given in Table 4.

Table 4. Topological analysis of the target proteins

Protein	**Topological Parameters**		
	Degree Centrality (DC)	**Betweenness Centrality (BC)**	**Closeness Centrality (CC)**
BCHE (6ESJ)	**7**	457.0333	0.172691
CNRI (6N4B)	**7**	450.4714	0.167315
HPGDS (1IYH)	**7**	407.8857	0.17623
AR (2YHD)	**7**	165.4762	0.173387
HDAC1 (4BKX)	**7**	96.66429	0.172

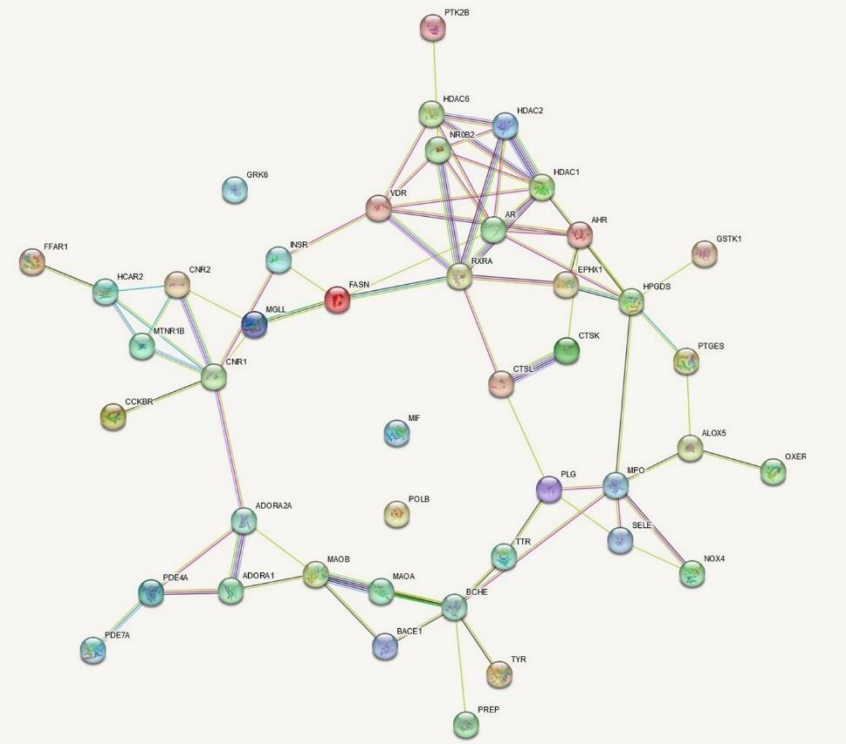

Figure 3. Protein Interaction Diagram of the Common targets

Proteins are represented by circular nodes in the PPI diagram and their interactions are represented by lines called edges. The purple edges represent interactions determined by using curated databases and the pink edges represent experimentally determined protein-protein interactions. The green, red and blue edges represent predicted determined protein-protein interactions based on gene neighbourhood, gene fusions and gene co-occurrence respectively. The yellow and black edges represent protein-protein interactions based on text mining and co-expression respectively.

Molecular Docking

The binding interactions between the top 5 phytoconstituents selected based on pharmacokinetic profile and the top 5 proteins selected based on topological parameters were studied using molecular docking studies. Molecular docking studies revealed the binding interactions and binding mode of the selected phytoconstituents with the target proteins. Among the 5 selected phytoconstituents, beta-eudesmol exhibited favourable docking scores with the five selected target proteins. Beta-eudesmol exhibited maximum docking scores with four out of five targets compared to other phytoconstituents. The docking scores of beta-eudesmol are CNR1 (-4.54 kcal/mol), HPGDS (-5.894 kcal/mol), AR (-4.73 kcal/mol) and BCHE (-6.30 kcal/mol). Beta-eudesmol exhibited the second maximum docking score with HDAC1 (-1.613 kcal/mol). Beta eudesmol forms eight hydrophobic interactions with PHE 155, LEU 209, ILE 212, ILE 216, ILE 227, VAL 228, ALA 233 and ALA 236 and two polar interactions with THR 210 and THR 229 with 6N4B. It forms seven hydrophobic interactions with TYR 8, PHE 9, MET 11, MET 99, TRP 104, CYS 156, LEU 199 and one polar interaction with THR 159 with 1IYH. Beta-eudesmol with 2YHD forms seven hydrophobic interactions with LEU 712, VAL 713, VAL 716, VAL 730, MET 734, ILE 737, AND MET 894 and two polar interactions with GLN 733 and GLN 738. With 6ESJ, beta-eudesmol forms two hydrogen bonds with TRP 82 and TYR 440 and six hydrophobic interactions with ALA 328, PHE 329, TYR 332, TRP 430, MET 434 and MET 437 and three polar interactions with SER 79, THR 120 and HID 438. With 4BKX, beta-eudesmol forms two hydrophobic interactions with LEU161 and LEU 164. The dock score of beta-eudesmol with 6N4B, 1IYH, 2YHD and 6ESJ was -4.545, -5.894, -4.731 and -6.308 kcal/mol and the dock score of respective reference compounds were -4.569, -4.464, -2.307 and -10.806 kcal/mol respectively. Root mean square deviation

(RMSD) was measured to validate the accuracy of docking results. The value of RMSD calculated between the reference and docked pose was found to be 1.21 Å, 0.98 Å, 1.32 Å and 0.90 Å for 6N4B, 1IYH, 2YHD and 6ESJ respectively. The results of the molecular docking studies are given in Table 5 and 2D interaction diagrams are represented in Figure 4.

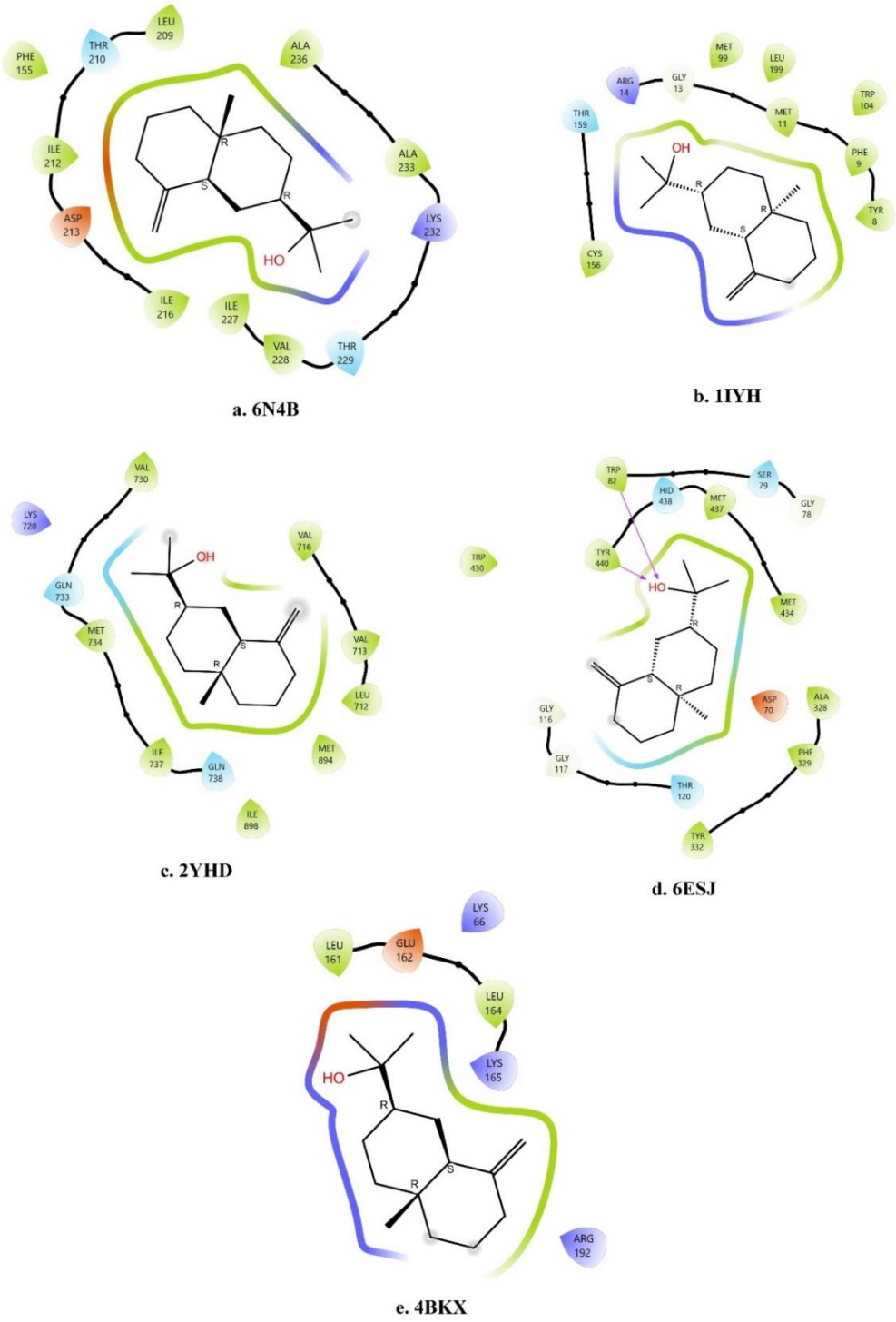

Figure 4. 2D Interactions diagrams of the top 5 proteins with beta-Eudesmol (The figure represents the 2D interaction diagrams of beta eudesmol with the top five therapeutic targets selected based on topological parameters. Figures a-e represent the 2D interaction diagrams of 6N4B, 1IYH, 2YHD, 6ESJ and 4BKX respectively)

Table 5. Dock Score of the selected Phytoconstituents

Phytoconstituents	Dock Score (kcal/mol)				
	6N4B	**1IYH**	**2YHD**	**6ESJ**	**4BKX**
Beta-Eudesmol	-4.545	-5.894	-4.731	-6.308	-1.613
Linoleic acid	-3.889	-5.827	-4.154	-4.524	-2.304
Elemol	-2.418	-5.646	-3.499	-6.273	-2.2
Linolenic acid	-4.552	-4.052	-2.261	-4.43	-2.078
Myristic acid	-1.625	-3.09	-2.05	-3.656	-1.888
Co-crystal	-4.569	-4.464	-2.307	-10.806	

Molecular Mechanics with Generalised Born and Surface Area Solvation (MMGBSA)

The binding free energy of top 5 phytoconstituents based on pharmacokinetic profile and top 5 proteins based on topological parameters were studied using determined MMGBSA technique. Beta-eudesmol showed better binding free energy with all the proteins especially it showed maximum binding free energy with CNR1 and HDAC1 and second best with HPGDS and BCHE. Linoleic acid also exhibited favourable binding free energy with CNR1, BCHE and HDAC1. The results of the MMGBSA studies are given in Table 6.

Table 6. Binding Free Energy of the Target Proteins

Phytoconstituent	Binding Free Energy (ΔGbind)				
	6N4B	**1IYH**	**2YHD**	**6ESJ**	**4BKX**
Beta-eudesmol	-73.71	10.22	-21.97	-73.71	21.01
Linoleic acid	-52.39	-4.16	-5.84	54.85	7.13
Elemol	-30.03	4.49	-41.1	-129.3	47.2
Linolenic acid	-59.75	-6.79	-36.19	29.61	-0.42
Myristic acid	-38.27	-17.68	-6.45	48.6	9.69
Co-crystal	-50.12	25.42	-38.17	-192.14	

Ginkgo biloba extract (EGb761) has been proven in the past to improve cell viability, reduce apoptosis and protect mitochondrial membrane potential, increased tyrosine hydroxylase positive cells, bcl-2 positive cells and decreased the caspase-3 positive cells on PC12 cells injured by paraquat [23]. A similar *in vitro* study carried out by Yang et al., on PC12 cells using MPTP as a neurotoxic agent also proved that leaf extract of *Ginkgo biloba* prevents apoptosis in Parkinson's disease [24]. *Ginkgo biloba* dropping pill is a leaf extract preparation of *Ginkgo biloba* which has been reported in the literature for its antioxidant and neuroprotective properties. Yu et al., studied the neuroprotective effect of *Ginkgo biloba* dropping pill in PD using 6-OH model in zebra fish and MPTP model in mice. The results of this study showed that *Ginkgo biloba* dropping pill prevented the loss of dopaminergic neurons in zebra fish and improved cognitive abilities and decreased damage to the dopaminergic neurons in the mice probably through Akt/GSK3β pathway [25]. Patricia Rojas et al., have proven that *Ginkgo biloba* extract prevents the mice form MPTP induced PD probably through regulation of copper levels in the corpus striatum, midbrain and hippocampus [26]. In another study, Patricia Rojas et al., have proven that *Ginkgo biloba* extract prevents the mice form MPTP induced PD through up regulation of tyrosine hydroxylase (Th), vesicular monoamine transporter 2 (Vmat2), dopamine transporter (Dat), dopamine D2 receptor (Da-d2r), and transcription factors (Pitx3 and Nurr1) related to dopamine neurotransmission [27]. Kuang et al., have proven that A53T transgenic mice fed and treated with *Ginkgo biloba* extract improves locomotor activity, levels of superoxide dismutase and glutathione peroxidase, expression of tyrosine hydroxylase and dopamine transporters and inhibits the expression of methane dicarboxylic aldehyde [28]. All the above finding ascertains the neuroprotective effect of *Ginkgo biloba* extract in various *in vitro, in vivo* and transgenic models of PD. In the current, an attempt is made to identify the phytoconstituents of *Ginkgo biloba* that are vital for its neuroprotective action and its potential therapeutic targets using network pharmacology and molecular docking studies.

The network pharmacology study revealed the presence of 125 major phytoconstituents in *Ginkgo biloba*. All 125 phytoconstituents were subjected to prediction of pharmacokinetic and druglikeness studies. Out of 125 phytoconstituents in *Ginkgo biloba*, 30 phytoconstituents showed favourable pharmacokinetic like the ability to penetrate the blood brain barrier and druglikeness property. The therapeutic targets for these selected phytoconstituents were found to be 468 and the disease targets in PD were found to be 2033. The common targets between phyto-targets and disease targets were found to be 44 targets. Out of 44 common targets, 5 top proteins CNR1, HPGDS, AR, RXRA and HDAC1 were identified on the basis of the topological parameters such as degree centrality and betweenness centrality in the Cytoscape 3.9.1 software. Further, the top 5 proteins were docked with 5 potential phytochemicals such as beta-eudesmol, linoleic acid, elemol, linolenic acid and myristic acid. Molecular docking was performed using the XP ligand docking in Maestro Schrodinger. The docking studies reveals that beta-eudesmol has the highest docking scores in most of the proteins. The complexes of protein-ligand were subjected to MM-GBSA studies to determine the stability of the protein-ligand complex. MM-GBSA assay revealed that beta-eudesmol forms stable complexes with maximum number of proteins.

Consistent with our findings, *Eucalyptus citriodora* L. leaf extract that contains beta-eudesmol as one of its major phytoconstituent delayed the loss of climbing ability and attenuated the oxidative stress in transgenic Drosophila melanogaster[29]. In a related study, beta eudesmol has been reported to control the hallucinations associated with PD on 1-(2,5-Dimethoxy-4- odophenyl)-2-aminopropane induced Head Twitch Response in Mice[30]. α-synuclein, mitochondrial dysfunction, oxidative stress, neuroinflammation and deficiency of trophic factors have been reported to impair neurite outgrowth leading to consequences like impaired neuroplasticity and a compromise in synaptic function[31,32]. Beta-eudesmol has been reported by Yutaro et al., to induce neurite outgrowth at concentrations of 100 and 150μM in rat pheochromocytoma (PC-12) cells mediated by MAPK pathway[33].

In conclusion, the current study reveals that beta-eudesmol present in *Gingko biloba* could act as a potential lead molecule in the management and prevention of Parkinson's disease through activation of multiple therapeutic targets.

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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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ÖZGÜN MAKALE / ORIGINAL ARTICLE

GEBE KADINLARIN GEBELİK SÜRECİNDE İLAÇ KULLANIMI HAKKINDA BİLGİ TUTUM VE DAVRANIŞLARININ DEĞERLENDİRİLMESİ

EVALUATION OF KNOWLEDGE, ATTITUDES AND BEHAVIORS OF PREGNANT WOMEN ON MEDICINE USE DURING PREGNANCY

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ÖZ

Amaç: *Bu çalışma, Türkiye'de bir üniversite hastanesinde kadın doğum polikliniklerine başvuran gebe kadınların gebelikte ilaç kullanımlarına ilişkin bilgi, tutum ve davranışlarını değerlendirmeyi amaçlamıştır.*
Gereç ve Yöntem: *Çalışma kesitsel bir anket çalışmasıdır. 13 Mart-14 Nisan 2023 tarihleri arasında bir üniversite hastanesi kadın doğum polikliniklerinde 18 yaş üstü gebe kadınlarda yüz yüze olacak şekilde yürütülmüştür.*
Sonuç ve Tartışma: *Ankete katılan gebe kadınların %64.4'ü 22-29 yaşları arasındaydı ve %42.3'ü üniversite mezunuydu. Gebelerin kadınların çoğunluğu (%83.4) vitamin-mineral takviyesi almaktaydı. Gebe kadınların bilgi, tutum ve davranış puanlarının ortalama±standart sapması sırasıyla 3.38±0.91, 12.8±1.72 ve 4.73±0.578'di. Gebe kadınların genel olarak bilgi, tutum ve davranışları iyi bulunmuştu ancak yine de bazı bilgi eksiklikleri bulunmaktaydı. Gebe kadınların gebelik sırasında güvenli ilaç kullanımı ve kaçınılması gereken ilaçlar konusunda eğitilmesine ihtiyaç bulunmaktadır.*
Anahtar Kelimeler: *Bilgi, davranış, gebeler, ilaç kullanımı, tutum*

ABSTRACT

Objective: *This study aimed to evaluate the knowledge, attitudes and behaviors of pregnant women who applied to the obstetrics and gynecology outpatient clinics of a university hospital in Türkiye regarding medicine use during pregnancy.*
Material and Method: *The study was a cross-sectional survey study. It was conducted face-to-face with pregnant women over the age of 18 in a university hospital obstetrics outpatient clinics between March 13 and April 14, 2023.*
Result and Discussion: *Most of the pregnant women surveyed (64.4%) were between the ages of 22-29 and 42.3% were university graduates. Most of the pregnant women (83.4%) were taking vitamin-mineral supplements. The mean±standard deviation of the knowledge, attitude and behavior scores of the pregnant women were 3.38±0.91, 12.8±1.72 and 4.73±0.578, respectively. The knowledge, attitudes and behaviors of the pregnant women were found to be good in general, but there were still some lack of knowledge. There is a need to educate pregnant women on rational drug use and drugs to be avoided during pregnancy.*
Keywords: *Attitude, behavior, medicine use, knowledge, pregnant women*

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GİRİŞ

Gebelik, plasenta ve fetüsün büyümesiyle birlikte bireyde fizyolojik değişikliklerin meydana geldiği karmaşık bir süreçtir [1]. Oluşan bu farklılarla beraber tedavide kullanılacak ilacın dozu, etkisi, farmakodinamiği ve farmakokinetiği değişmektedir [2]. Gebelik sürecinde hem anne hem de fetüs için ilaç kullanımı önemli ve üzerinde durulması gereken bir konudur. Etik nedenlerden dolayı gebe kadınlar pazarlama öncesi klinik araştırmaların büyük çoğunluğuna dahil edilememektedir [3]. Bunun sonucu olarak çoğu ilaç, gebelikte doğrudan belirlenmiş bir güvenlik profili olmaksızın piyasaya sürülmektedir [4]. Şimdiye kadar çok az ilacın majör teratojen olduğu gösterilmesine rağmen, minor teratojenite riski veya fetal gelişim üzerindeki daha hafif etkilerin hala belirlenmesi gerekmektedir [5].

Kronik hastalığı olan gebelerin gebelik esnasında ilaç kullanımını tamamen bırakması bazen mümkün olmamaktadır. Diyabet, epilepsi, astım, hipertansiyon gibi hastalık durumları dahil olmakla beraber belirli durumlarda gebelikte ilaç kullanımı zorunlu hale gelmektedir [6,7]. Hastalık durumları dışında ayrıca folik asit, vitamin ve mineral desteği gibi gebelikte tavsiye edilen ilaçların kullanımı da mümkün olabilmektedir [8].

Avrupa, Kuzey-Güney Amerika ve Avusturalya'da yapılan çok uluslu bir çalışmada, gebe kadınların %81.2'si hamilelik sırasında en az bir ilaç (reçeteli ve reçetesiz) kullandığı tespit edilmiştir. %66.9'u ise reçetesiz ilaç kullanmıştır [5]. İtalya'da 33.343 doğum arasında yapılan bir çalışma sonucuna göre kadınların %70'i hamilelik sırasında en az bir reçeteli ilaca maruz kalmıştır. Vitamin ve mineral ürünleri hariç tutulduğunda ise %48'i en az bir reçeteli ilaç kullanmıştır. Amoksisilin, fosfomisin ve ampisilin gibi antibiyotikler en fazla kullanılan ilaçlardır [9]. Oslo Üniversitesi'nde yapılan bir çalışmada ise gebe kadınların %80'inden fazlası parasetamol, penisilinler ve reflü ilaçları kullandıkları tespit edilmiştir. Gebeler bu süreçte ilaç kullanımı hakkında daha fazla bilgiye ihtiyaç duyduklarını bildirmişlerdir. Ayrıca gebe kadınların bitkisel ilaçları geleneksel ilaçlara göre daha güvenli alternatifler olarak algıladıkları yönünde sıklıkla dile getirilen iddianın aksine, çalışmada bitkisel ilaçlara ilişkin düşük bir risk algısı bulunmuştu [10].

Gebelik sırasında ilaç kullanımı, potansiyel teratojenik etkilerden dolayı özel bir dikkat gerektirmektedir. Bununla birlikte gebeler ilaçların teratojenitesinden korkarak yeterli tedaviyi alamayabilmektedirler. Bu durum anne bebek sağlığını olumsuz etkilemektedir [1]. Bu nedenle, anne ve bebeğin sağlık sonuçlarının iyileştirilmesi için gebe kadınların ilaç kullanımlarına ilişkin bilgi, tutum ve davranışlarının araştırılmasına ihtiyaç vardır. Bu çalışma, Türkiye'de bir üniversite hastanesinde kadın doğum polikliniklerine başvuran gebe kadınların gebelikte ilaç kullanımlarına ilişkin bilgi, tutum ve davranışlarını değerlendirmeyi ve sosyodemografik faktörler ile bilgi, tutum ve davranış arasındaki ilişkiyi belirlemeyi amaçlamıştır.

GEREÇ VE YÖNTEM

Çalışma kesitsel bir anket çalışmasıdır. 13 Mart 2023 ve 14 Nisan 2023 tarihleri arasında Süleyman Demirel Üniversitesi Araştırma ve Uygulama Hastanesi Kadın Hastalıkları ve Doğum polikliniklerine başvuran, bilgilendirilmiş onam alınan 18 yaş üstü gebe kadınlara uygulanmıştır. Çalışmanın etik onayı Süleyman Demirel Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu'ndan alınmıştır (Onay No: 289 /Tarih:10.10.2022).

Gebeler çalışmanın amacı hakkında bilgilendirildikten ve onamı alındıktan sonra anket yüz yüze yaklaşık 15 dakika sürecek şekilde uygulanmıştır. Örneklem büyüklüğü %5 hata payı, %95 güven aralığı ve %50 dağılım oranıyla 95 bulunmuştur [11].

Bu çalışmada daha önce yayınlanmış bir çalışmadaki anket soruları araştırmacılar tarafından revize edilerek kullanılmıştır [12]. Çalışmanın ilk yazarından e-mail yoluyla anket kullanım izni alınmıştır. Anketin orjinal dili İngilizce olup Türkçe'ye çevrilmiştir. 30 gebe kadında pilot çalışma yapılıp anketin anlaşılabilirliğine bakılmıştır. Anket anlaşılır bulunmuştur ve herhangi bir değişiklik yapılmamıştır. Anketin iç tutarlılığı Cronbach alfa skoruyla hesaplanmıştır ve 0.72 bulunmuştur.

Ankette toplam 23 soru bulunmaktadır. İlk 6 soru sosyodemografik özelliklerle ilgilidir. Bilgi ve davranış soruları ''Evet'' ve ''Hayır'' olacak şekilde tutum soruları ise 3'lü Likert ölçeği ''Katılıyorum'', ''Kararsızım'' ve ''Katılmıyorum'' şeklindedir. Bilgi ve davranış bölümlerinde 'Evet', 1 ve 'Hayır' 0 olarak puanlanmıştır. Bilgi sorularında katılımcıların hep ''Evet'' demesini engellemek için eğer evet ise belirtiniz diye cevapları açıklamaları istenmiştir. Açıklama yanlışsa da 0 olarak puanlanmıştır. Tutum bölümünde ''Katılıyoruma'' 1, ''Karasızım'' 2 ve ''Katılmıyorum'a'' 3 puan

verilmiştir. Bazı sorular ters skorlanmıştır. Bilgi, tutum ve davranışları etkileyen faktörleri belirlemek amacıyla, gebeler kümülatif puana göre düşük ve yüksek puanı kategorilerine ayrılmıştır (bilgi ve davranış için 0-2 ve 3-5 ve tutum için 6-11 ve 12-18).

Veriler Statistical Package for the Social Sciences (SPSS) 20.0 ile analiz edilmiştir. Değişkenler % ve sayı ile ifade edilmiştir. Kategorik değişkenleri karşılaştırmak için Ki-Kare testi kullanılmıştır. Anlamlılık seviyesi (α), tüm istatistiksel testler için 0.05'tir

SONUÇ VE TARTIŞMA

Anket 104 gebe tarafından yanıtlanmıştır. Gebelerin %64.4'ü 22-29 yaşları arasındaydı ve %42.3'ü üniversite mezunuydu. Gebelerin %43.3'ünün daha önce çocuğu olmamıştı ve gebelerin çoğu (%76.9) daha önce düşük tecrübesi yaşamamıştı. Gebelerin %41.3'ü 2.trimesterindeydi. Tablo 1'de gebelerin sosyodemografik özellikleri gösterilmiştir.

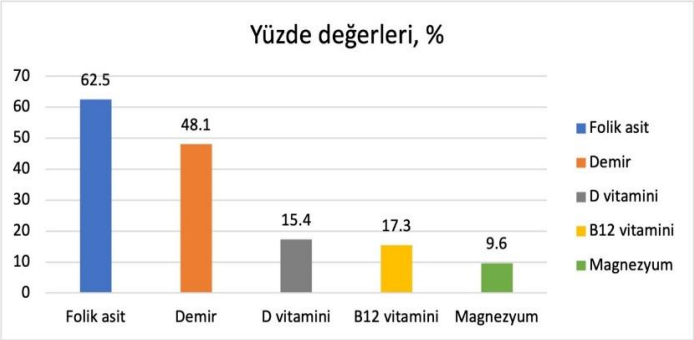
Tablo 1. Çalışmaya katılan gebe kadınların sosyodemografik özellikleri

Özellikler	**Gebe sayısı (%)**
Yaş	
22-29	67 (64.4)
30-44	37 (35.6)
Eğitim Düzeyi	
İlkokul	1 (1)
Ortaokul	19(18.3)
Lise	38 (36.5)
Üniversite	44 (42.3)
Yüksek Lisans/Doktora	2 (1.9)
Meslek	
Ev hanımı	64 (61.5)
Çalışan	40 (38.5)
Çocuk sayısı	
0	45 (43.3)
1	40 (38.5)
2	18 (17.3)
≥3	1 (1)
Daha önce düşük tecrübesi	
Hayır	80 (76.9)
Evet	24 (23.1)
Gebelik trimesteri	
1.trimester	26 (25)
2.trimester	43 (41.3)
3.trimester	35 (33.7)

Bilgi Düzeyi

Bilgi düzey ortalama±standart sapması 3.38±0.91'di. Gebelerin büyük çoğunluğu (%91.2) en az bir ilaç (reçeteli-reçetesiz) kullanmaktaydı ve bunların çoğu (%83.7) vitamin-mineral takviyesiydi. Gebelerin %62.5'u folik asit, %48.1'i demir, %17.3'ü D vitamini kullanmaktaydı (Şekil 1). Bunun dışında %4.8'i antikoagülan, %4.8'i progesteron hormonu, %3.8'i antitiroid, %3.8'i antitrombotik ve %2.9'u antihistaminik kullanmaktaydı.

Gebe kadınların tamamı kullandıkları ilaçların adını biliyordu ve %86.81 kullandıkları ilaçların kullanımlarını biliyordu. Gebelerin çoğu (%80.8) gebelik sırasında bazı ilaçların güvenli olmayabileceğini bilmiyordu ve herhangi bir örnek veremedi. Gebe kadınların %66.3'ü ilaçların fetüs üzerinde etkili olma olasılığının yüksek olduğu kritik dönemi biliyordu. Tablo 2'de gebe kadınların ilaç kullanımı konusundaki bilgi düzeyleri gösterilmiştir.



Şekil 1. Gebe kadınların kullandığı vitamin ve mineral takviyelerinin dağılım yüzdesi

Tablo 2. Gebe kadınların ilaç kullanımı konusundaki bilgi düzeyleri

Sorular	Yanıt	Gebe sayısı (%)
Halihazırda kullanmakta olduğunuz ilaç(lar)ın adını biliyor musunuz?[a]	Evet	91 (100)
Şu anda almakta olduğunuz tüm ilaç(lar)ın kullanım(lar)ını biliyor musunuz?	Evet	79 (86.81)
Gebelik sırasında bazı ilaçların güvenli olmayabileceğini biliyor musunuz?	Evet	20 (19.2)
Bazı ilaçların gebelik sırasında alınmasının önemli olduğunu biliyor musunuz?	Evet	67 (64.4)
Gebelik sırasında ilaçların fetüs üzerinde etkili olma olasılığının yüksek olduğu kritik dönemi biliyor musunuz?	Evet	69 (66.3)

[a]Toplam 91 kişi yanıtlamış ve 13 kişi herhangi bir ilaç kullanmadığını belirtmiştir. İlk soru hariç diğer sorular ilaç kullanan 91 kişiye sorulmuştur.

Tutum Düzeyi

Tutum düzey ortalama ± standart sapması 12.8±1.72'di. Çalışmaya katılan gebe kadınların %10.6'sı tüm ilaçların fetüse zararlı olduğu fikrine katılıyordu. Gebe kadınların %33.7'si gebe kadınların gebelik sırasında ilaç almayı bırakmasının fetüs için daha iyi olduğu fikrindeydi. Gebe kadınların %39.4'ü gebe kadınların hastalığı tedavi etmek için ilaç kullanması, tedavi edilmemesine göre fetüs için daha iyi olduğunu düşünüyordu. Gebe kadınların yalnızca %7.7'si doktorların gebe kadınlara çok fazla ilaç reçete ettiğine katılıyordu. Ayrıca gebe kadınların yaklaşık yarısı (%49) gebelik sırasında doğal tedavilerin tercih edilmesi gerektiğini fikrindeydi. Tablo 3'te gebe kadınların ilaç kullanımı konusunda tutumları gösterilmiştir.

Davranış Düzeyi

Davranış düzeyi ortalama ± standart sapması 4.73±0.578'di. Gebe kadınların %9.6'sı gebeliği sırasında reçetesiz ilaç aldığını belirtti. Bunların yaklaşık %8.7'si parasetamol, %1'i ibuprofendi. Gebe kadınların %91.3'ü prospektüsü kontrol ediyordu ve %99'u doktoruyla görüşüyordu. %94.2'si doktor reçete yazarken ilaçların amacını soruyordu. Gebe kadınlar ilaçla ilgili bilgileri %56.7 oranında kadın doğum uzmanından ve %42.3 oranında eczacıdan öğrenmekteydi. Tablo 4 gebelerin ilaç kullanımı hakkında davranışlarını göstermektedir.

Gebe kadınların bilgi, tutum ve davranışlarıyla sosyodemografik özellikleri arasında istatistiksel olarak ilişkili bulunmamıştır ($p>0.05$). Tablo 5'te gebe kadınların sosyodemografik özellikleriyle bilgi, tutum ve davranışları arasındaki ilişki gösterilmiştir.

Bu çalışmada gebe kadınların gebelik sürecinde ilaç kullanımı hakkında bilgi, tutum ve davranışları değerlendirilmiştir. Çalışmamızın sonuçlarına göre gebe kadınların %91'i en az bir ilaç (reçeteli-reçetesiz) kullanmaktaydı ve bunların büyük çoğunluğu vitamin- mineral takviyesiydi. İtalya'da yapılan bir çalışmada %59.6 [13], Etiyopya'da %88.4 [14], Suudi Arabistan'da %40 [15], İskoçya'da %85.2[16], Amerika Birleşik Devletlerinde (ABD) [17] %82.5, Türkiye'de ise sırasıyla %45.6, %76.1 ve %90.2 oranında [18-20] gebeler en az bir ilaç kullanmaktaydı. Çalışmamızın sonuçları

daha önce yapılmış çalışmaların çoğuna göre biraz daha yüksek olduğu görülmektedir.

Tablo 3. Gebe kadınların ilaç kullanımı hakkında tutumları

Sorular	Yanıt	Gebe sayısı (%)
Tüm ilaçlar fetüse zararlıdır.	Katılıyorum	11(10.6)
	Kararsızım	32 (30.8)
	Katılmıyorum	61 (58.7)
Gebe kadınların gebe olmayan kadınlara göre ilaç kullanma eğilimleri daha yüksektir.	Katılıyorum	18 (17.3)
	Kararsızım	20 (19.2)
	Katılmıyorum	66 (63.5)
Gebe kadınların hastalığı tedavi etmek için ilaç kullanması, tedavi edilmemesine göre fetüs için daha iyidir.	Katılıyorum	41 (39.4)
	Kararsızım	37 (35.6)
	Katılmıyorum	26 (25)
Gebelik sırasında alınan ilaçlar her yıl birçok doğmamış çocuğun hayatını kurtarabilir.	Katılıyorum	33 (31.7)
	Kararsızım	52 (50)
	Katılmıyorum	19 (18.3)
Doktorlar gebe kadınlara çok fazla ilaç reçete etmektedir.	Katılıyorum	8 (7.7)
	Kararsızım	20 (19.2)
	Katılmıyorum	76 (73.1)
Gebe kadınlar gebelik sırasında doğal tedavileri tercih etmelidir.	Katılıyorum	51 (49)
	Kararsızım	31 (29.8)
	Katılmıyorum	22 (21.2)

Tablo 4. Gebelerin ilaç kullanımı hakkında davranışları

Sorular	Yanıt	Gebe sayısı
Mevcut gebeliğiniz sırasında reçetesiz ilaç(lar) alıyor musunuz?	Evet	10 (9.6)
	Hayır	94 (90.4)
İlaçla birlikte verilen prospektüs içeriğini normal olarak kontrol ediyor musunuz?	Evet	95 (91.3)
	Hayır	9 (8.7)
Gebelik sırasında doktorunuzla düzenli olarak görüşüyor musunuz?	Evet	103 (99)
	Hayır	1 (1)
Doktor reçete yazarken reçete edilen ilaç(lar)ın amacını soruyor musunuz?	Evet	98 (94.2)
	Hayır	6 (5.8)
Eczacıya ilaç(lar)ın nasıl kullanılacağını soruyor musunuz?	Evet	102 (98.1)
	Hayır	2 (1.9)

Tablo 5. Gebelerin demografik özellikleriyle bilgi, tutum ve davranışlarının karşılaştırılması

Değişkenler	Bilgi Düzeyi			Tutum Düzeyi			Davranış Düzeyi		
	Düşük (%) n	Yüksek (%) n	p	Negatif (%) n	Pozitif (%) n	p	Negatif (%) n	Pozitif (%) n	p
Yaş (yıl)									
22-29	12(17.9)	55(82.1)	0.761	12(17.9)	55(82.1)	1	1 (100)	66(64.7)	1
30-44	5 (13.5)	32(86.5)		7(18.9)	30(81.1)		-	37(35.9)	
Eğitim düzeyi									
<Lise	5 (27.8)	13(72.2)	0.167	6(33.3)	12(66.7)	0.092	1 (100)	17(16.5)	0.173
≥Lise	12(14)	74(86)		13(15.1)	73(84.9)		-	86(83.5)	
Meslek									
Ev hanımı	13(20.3)	51(79.7)	0.267	10(15.6)	54(84.4)	0.534	1 (100)	63(61.2)	1
Çalışan	4(10)	36(90)		9(22.5)	31(77.5)		0	40(38.8)	

Tablo 5 (*devamı*). Gebelerin demografik özellikleriyle bilgi, tutum ve davranışlarının karşılaştırılması

Değişkenler	Bilgi Düzeyi			Tutum Düzeyi			Davranış Düzeyi		
	Düşük (%) n	Yüksek (%) n	p	Negatif (%) n	Pozitif (%) n	p	Negatif (%) n	Pozitif (%) n	p
Çocuk sayısı									
<1	8 (18.2)	36(81.8)	0.869	9(20.5)	35(79.5)	0.813	1 (100)	43(41.7)	0.423
≥1	9 (15)	51(85)		10(16.7)	50(83.3)		-	60(58.3)	
Düşük tecrübesi									
Hayır	15(18.2)	65(81.2)	0.348	14(17.5)	66(82.5)	0.765	1 (100)	79(76.7)	1
Evet	2 (8.3)	22(91.7)		5(20.8)	19(79.2)		0	24(23.3)	
Gebelik trimesteri									
1.trimester	2(7.7)	24(92.3)	0.146	3(11.5)	23(88.5)	0.588	-	26(25.2)	0.37
2.trimester	6(14)	37(86)		9(20.9)	34(79.1)		-	43(41.7)	
3.trimester	9(25.7)	26(74.3)		7(20)	28(80)		1	34 (33)	

Çalışmamızda gebe kadınların bilgi düzeyi genel olarak yüksek bulunmuştur. Türkiye'de [19] ve ABD'de yapılan çalışmalarda [21] gebe kadınların bilgi düzeyi bizim çalışmamıza benzer şekilde yüksekti; ancak Nijerya [22], Malezya [23], Hindistan'da [12] yapılan çalışmalarda ise gebe kadınlar düşük bilgi düzeyine sahipti. Çalışmamızda olumlu bir şekilde gebe kadınların çoğu kullandıkları ilaçların kullanımlarını biliyordu. Ancak gebelik sırasında güvenli olmayabilecek ilaçları katılımcıların çok azı (%19.2) bilmekteydi. Hindistan'da yakın zamanda yapılan bir araştırmada da benzer bir bulgu gözlemlendi; hamile bireylerin %90'ından fazlasının hamilelikte kaçınılması gereken ilaçlar hakkında hiçbir bilgisi yoktu [12]. Nijerya'da yapılan bir çalışmada gebe kadınların %81.6'sı gebelik sırasında kaçınılması gereken ilaçlar konusunda emin değildi [22]. Tanzanya'da yapılan bir araştırmada hamile kadınların yalnızca %31.5'i gebelikte kısıtlanan bazı ilaçları bildiği ortaya konmuştur [24]. Çalışmamızda gebe kadınların %66.3'ü gebelik sırasında ilaçların fetüs üzerinde etkili olma olasılığının yüksek olduğu kritik dönemi biliyordu. Bu oran diğer çalışmalara göre yüksektir. Hindistan'da yapılan çalışmada bu oran %29.38'di [12] ve Etiyopya'da ise %24.5'ti [25]. Suudi Arabistan'da yapılan bir çalışmada ise gebelikte kullanılan tüm ilaçlar trimesterden bağımsız olarak zararlıdır ifadesine katılımcıların sadece %28.6'sı katılmadı [26].

Çalışmamızda gebe kadınların ilaç kullanım konusunda tutumları genel olarak pozitifti. Katılımcıların çok azı (%10.6) tüm ilaçların fetüse zararlı olduğunu düşünüyordu ve %39.4'ü gebe kadınların hastalığı tedavi etmek için ilaç kullanması, tedavi edilmemesine göre fetüs için daha iyi olduğu fikrindeydi. Etiyopya'da yapılan çalışmada [25] gebelerin %62.5'i, Suudi Arabistan'da yapılan çalışmada [26] ise %27'si gebelikte tüm ilaçların fetüse zararlı olduğu fikrindeydi. Çalışmamızda olumlu bir şekilde, katılımcıların çoğu doktorların gebe kadınlara çok fazla ilaç reçete ettiği fikrine katılmadı ve bu da diğer çalışmalarla tutarlıydı [12,22]. Ancak gebe kadınların yaklaşık yarısı gebelik sırasında doğal tedavileri tercih edilmesi gerektiğini düşünüyordu**.** Etiyopya, Hindistan, ABD, Nijerya'da yapılan çalışmalarda gebe kadınlar bu fikre katılmamıştı [12,21,22,25]. İtalya, Avusturalya ve Norveç'te yapılan çalışmalarda ise gebelik döneminde doğal tedavilerin sık kullanıldığı sonucuna varılmıştır [27-29]. Ayrıca Türkiye'de yapılan bir çalışmada gebe kadınların %47.3'ünün gebelik süresince en az bir bitkisel ürün kullandığını ortaya koymuştur [30]. Gebe kadınların bu tutumu göz önüne alındığında, sağlık profesyonelleri tarafından gebe kadınların bu tür tedavilerin yararları ve riskleri konusunda bilgilendirilmesi gerektiği açıktır. [28].

Araştırmamızda gebe kadınların ilaç kullanım konusunda davranışları olumluydu. Ayrıca gebe kadınların reçetesiz ilaç kullanımı düşüktü (%9.6). Türkiye'de yapılan bir çalışmada [19] gebelerin kendi kendine ilaç kullanımı %23.6'ydı ve yine Türkiye'de yapılan bir çalışmada gebe kadınların %42.8'i eczaneden reçetesiz ilaç aldığını belirtmişti [31]. Teksas'da İspanyol gebe kadınlarda yapılan çalışmada reçetesiz ilaç kullanımı %23 [32], Hollanda'da yapılan çalışmada [33] %12.5 ve ABD'de yapılan bir çalışmada [34] ise %92.6'dı. Çalışma sonucumuzun diğer çalışmalara göre düşük olduğu görülmektedir. Ayrıca çalışmamızda gebe kadınların büyük çoğunluğu prospektüs içeriğini kontrol ediyordu, doktoruyla düzenli görüşüyordu, eczacıya ilaçların nasıl olarak kullanılacağını soruyordu. Alptekin ve ark. [19] yaptıkları çalışmada gebe kadınların %64.1'i prospektüs okuduğunu, Kahraman

ve ark. [31] çalışmalarında ise gebe kadınların %39.4'ü prospektüsü okuduğunu belirtti. Çalışmamızda gebe kadınlar ilaçla bilgileri kadın doğum uzmanından (%56.7) ve eczacıdan (%42.3) öğrendiğini belirtti. Olumlu olarak, bilgi kaynağı olarak eczacıların oranı yapılan diğer çalışmalara göre yüksek bulunmuştur [13,15,21,23]. Eczacıların gebelikte akılcı ilaç kullanımında ve hastalara ilaç danışmanlığında önemli rolleri bulunmaktadır [35]. Bazı gebelikler özellikle erken evrelerde belirgin olamayabileceğinden, eczacıların ilaç vermeden önce kadınlardan gebelik durumları hakkında bilgi alması ve ilaç danışmanlığı sunması önemlidir [22]. Bu çalışmada, sosyodemografik değişkenlerle gebe kadınların bilgi, tutum ve davranışları arasında istatistiksel olarak anlamlı ilişki bulunmamakla birlikte; bazı çalışmalarda yaş ve eğitim seviyesi anlamlı değişkenler olarak bulunmuştur ($p<0.05$) [12,23].

Çalışmamızın bazı sınırlamaları bulunmaktadır. Çalışma tek bir hastanede kısıtlı bir sürede ve sınırlı sayıda gebe kadında gerçekleştirilmiştir. Bu nedenle genel popülasyonu temsil etmeyebilir. Bulgularımız, daha geniş bir örneklemle gebe kadınlarda ilaç kullanımını değerlendirmeyi amaçlayan gelecekteki çalışmalara bilgi sağlamak için kullanılabileceğini düşünüyoruz.

Sonuç olarak, gebelerin gebelik süresince ilaç kullanımı konusunda bilgi, tutum ve davranışları genel olarak iyi bulunmuştur. Çalışmamızda gebelerin çoğu yaygın olarak vitamin ve mineral ilaçları kullanmaktadır ve reçetesiz ilaç kullanımı düşüktür. Ayrıca gebe kadınların bazı konularda bilgi eksikleri bulunmaktadır. Gebe kadınların gebelik sırasında güvenli ilaç kullanımı ve kaçınılması gereken ilaçlar konusunda eğitilmesine ihtiyaç vardır.

TEŞEKKÜR

Çalışmaya katılan gebe kadınlara teşekkür ederiz.

YAZAR KATKILARI

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Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler.

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

KNOWLEDGE, ATTITUDES AND PRACTICES OF COMMUNITY PHARMACISTS ABOUT PROTON PUMP INHIBITORS

SERBEST ECZACILARIN PROTON POMPASI İNHİBİTÖRLERİ HAKKINDAKİ BİLGİ, TUTUM VE UYGULAMALARI

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ABSTRACT

Objective: *This study aimed to evaluate the knowledge, attitudes, and practices of community pharmacists about proton pump inhibitors (PPIs).*
Material and Method: *The study was a cross-sectional online survey study It was conducted with community pharmacists in Türkiye between 1 November 2022 and 15 April 2023. The survey questions were created by revising the previous study and consisted of 32 questions. Of these, 4 were about demographic information, 16 were about knowledge, 6 were about attitude, and 6 were about practices.*
Result and Discussion: *The vast majority of pharmacists who participated into the study (97.6%) knew that drugs such as pantoprazole, omeprazole, etc. were PPIs, and a majority of pharmacists (84.5%) knew that PPIs were inactive pro-drugs. 63.1% of the pharmacists answered correctly that omeprazole had the most individual variability. The vast majority (93.5%) of pharmacists believed that PPIs were overused in Türkiye. 86.3% of pharmacists believed that excessive consumption of PPIs resulted in increased costs and adverse drug reactions. 46 pharmacists (27.38%) declared that they have used PPIs in the last 1 year. Pantoprazole (15.5%) and lansoprazole (7.7%) were the most commonly used PPIs, respectively. According to our study, although pharmacists' knowledge, attitudes, and practices about PPIs were generally good, there were also some deficiencies in their knowledge. Therefore, pharmacists need to update themselves and be supported by interdisciplinary continuous educations.*
Keywords: *Attitudes, community pharmacists, knowledge, practices, proton pump inhibitors*

ÖZ

Amaç: *Bu çalışma, serbest eczacıların proton pompası inhibitörleri (PPİ'ler) hakkındaki bilgi, tutum ve uygulamalarını değerlendirmeyi amaçlamıştır.*
Gereç ve Yöntem: *Çalışma, kesitsel bir çevrimiçi anket çalışmasıydı. 1 Kasım 2022-15 Nisan 2023 tarihleri arasında Türkiye'deki serbest eczacılarla yapılmıştı. Anket soruları bir önceki çalışmanın revize edilmesiyle oluşturulmuş olup 32 sorudan oluşmaktadır. Bunlardan 4'ü demografik bilgiler, 16'sı bilgi, 6'sı tutum ve 6'sı uygulamalara ilişkindir.*
Sonuç ve Tartışma: *Eczacıların büyük çoğunluğu (%97.6) pantoprazol, omeprazol vb. ilaçların PPİ olduğunu, eczacıların büyük çoğunluğu (%84.5) PPİ'lerin inaktif ön ilaç olduğunu biliyordu. Eczacıların %63.1'i omeprazolün bireysel değişkenliğe sahip olduğunu doğru yanıtlamıştı. Eczacıların büyük çoğunluğu (%93.5) PPİ'lerin Türkiye'de gereğinden fazla kullanıldığına*

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inanmaktaydı. Eczacıların %86.3'ü aşırı PPİ tüketiminin maliyet artışına ve advers ilaç reaksiyonlarına yol açtığına inanmaktaydı. 46 eczacı (%27.38) son 1 yılda PPİ kullandığını beyan etti. Pantoprazol (%15.5) ve lansoprazol (%7.7) sırasıyla en sık kullanılan PPİ idi. Çalışmamıza göre eczacıların PPİ'lerle ilgili bilgi, tutum ve uygulamaları genel olarak iyi olmakla birlikte bazı bilgi eksiklikleri de vardı. Bu nedenle eczacıların kendilerini güncellemeleri ve meslek içi sürekli eğitimlerle desteklenmeleri gerekmektedir.
Anahtar Kelimeler: *Bilgi, proton pompa inhibitörleri, serbest eczacılar, tutum, uygulamalar*

INTRODUCTION

Proton pump inhibitors (PPIs) are acid secretion-inhibiting prodrugs that are widely used to treat gastric acid-related pathologies, including gastroesophageal reflux disease, duodenal ulcer, and gastric ulcer [1]. PPIs are the most widely prescribed and most effective class of gastrointestinal drugs. Omeprazole, esomeprazole, lansoprazole, dexlansoprazole, pantoprazole, and rabeprazole are among the PPIs approved by the Food and Drug Administration (FDA) [2].

One of the reasons why they are widely prescribed is that they have very few side effects, but with the research, it has been understood that there are many adverse effects of inappropriate and/or long-term use. These adverse effects include an increased risk of both acute and chronic kidney diseases, hypomagnesemia, Clostridium difficile infection, pneumonia, and osteoporotic fractures. Before using PPIs for patients with the potential for these adverse effects, the benefit-harm relationship and whether it is necessary for the patient should be considered [3-5].

Many studies show positive results in pharmacist-led proton pump inhibitor (PPI) management [6-10]. In a study conducted by Tandun et al. in a long-term care facility, pharmacists recommended deprescribing PPI and 80% of residents successfully completed deprescribing PPI [6]. Wahking et al. reduced the use of inappropriate PPIs in both inpatients and outpatients as part of a pharmacist-led PPI stewardship program [7]. In a study conducted in a family medicine clinic, deprescribing long-term PPI therapy was successful by implementing a clinical pharmacist-led program that included detailed dose reduction instructions, patient education, and follow-up [8]. A clinical pharmacist-led guidance team at a tertiary hospital in China implemented the Plan-Do-Check-Act (PDCA) method to increase rational PPI use. As a result, the irrational PPI rate and cost, including duration, route of administration, indication, and dosing frequency, were significantly reduced [9]. In a study of hospitalized older adults in Türkiye, appropriate PPI use increased with a pharmacist-led stewardship program [10]. Community pharmacists also have an important role in providing drug counseling to patients [11]. They need to inform patients about when to take PPIs, how long they should use them, and their long-term side effects [12]. Therefore, the knowledge, attitudes, and practices of community pharmacists towards PPIs are important to prevent side effects, drug-drug interactions, and inappropriate PPI use [13].

This study aimed to evaluate community pharmacists' knowledge, attitudes and practices regarding PPIs. It was also designed to identify community pharmacists' knowledge gaps in properly counseling patients about PPIs.

MATERIAL AND METHOD

Study Design and Participants

The study was an observational cross-sectional online survey study. A convenience sampling technique was used. It was conducted with community pharmacists in Türkiye between 1 November 2022 and 15 April 2023. Ethical approval of the study was obtained from Suleyman Demirel University Clinical Research Ethics Committee (Approval No: 194 / Date: 07.07.2022).

The survey was created with Google Form. Before their participation, pharmacists were informed about the purpose and definition of the research on the first page of the questionnaire. Those who read and approved the Informed Consent Form electronically participated in the study.

There are approximately 26,759 community pharmacists in Türkiye [14] and the sample size was determined as 96 with a sampling error of 0.10 and a probability of occurrence of 0.5 [15-17].

Data Collection

The questionnaire was created by 2 pharmacists by revising the previous study [13]. In addition, expert opinion was obtained from 2 pharmacists. A pilot study was conducted among 30 participants to check the intelligibility and readability of the questionnaire. As a result, minor changes were made. For internal consistency, the Cronbach alpha score was calculated and found to be 0.64. (%95 confidence interval (0.510-0.791), F=2.995, p<0.001).

The questionnaire consisted of 32 questions. Of these, 4 were about demographic information, 16 were about knowledge, 6 were about attitude, and 6 were about practices. The answers to all of the knowledge questions were ‘‘Yes’’ or ‘‘No’’. The correct answer was scored as ‘‘1’’ and the incorrect answer as ‘‘0’’. A 5-point Likert scale was used in questions about attitude. It was scored as 5 for Strongly Agree, 4 for Agree, 3 for Uncertain, 2 for Disagree and 1 for Strongly Disagree. In the practical questions, the first question was about whether the PPI was used, and if it was, the next 5 questions were answered by the participants.

It was always scored as 1 point, frequently 2 points, occasionally 3 points, rarely 4 points, and never 5 points. More than ≥80% of the total score was associated with a higher level of knowledge, attitude, and practice, and <80% was associated with a lesser level of knowledge, attitude, and practice.

Statistical Analysis

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) 20.0. Descriptive statistics were calculated as numbers, percentage, mean ± standard deviation (SD). Normality of the data were evaluated by the Kolmogorov–Smirnov test. The Chi-square test was used to compare categorical variables and the Student’s t-test was used to compare non-categorical variables. P values less than 0.05 were considered statistically significant.

RESULT AND DISCUSSION

The questionnaire was answered by 168 (23.24%) community pharmacists. About half of the pharmacists (51.2%) were women and the mean ± SD age was 46.26±14.053. 93.5% of the pharmacists had a bachelor's degree and 85.1% had more than 5 years of experience. Table 1 shows the sociodemographic characteristics of pharmacists.

Table 1. Sociodemographical characteristics of pharmacists

Characteristics	Values
Gender (%)	
Female	86 (51.2)
Male	82 (48.8)
Age, years (mean±SD)	46.26±14.053
Education (%)	
Bachelor's degree	157 (93.5)
Postgraduate	11 (6.5)
Work experience, years (%)*	
< 5	25 (14.9)
≥ 5	143 (85.1)

SD: standard deviation, * ≥ 5 years was considered more experienced

Knowledge

The vast majority of pharmacists (97.6%) knew that drugs such as pantoprazole, omeprazole, etc. were PPIs, and a majority of pharmacists (84.5%) knew that PPIs were inactive pro-drugs. 63.1% and 69% of pharmacists answered correctly that omeprazole had the most individual variability and could be used in pediatric patients, respectively. Most of the pharmacists (98.2%) answered correctly to the question of whether PPIs are taken after meals. Only 33.9% of pharmacists answered correctly to the

duration of treatment of PPIs in gastric ulcers. Table 2 shows the level of knowledge of pharmacists about the use of PPIs.

The knowledge level of 64.3% of the pharmacists was found to be good. Education level at bachelor level (bachelor level etc. postgraduate, p=0.018) and more work experience (<5 years etc. ≥5 years, p=0.003) were associated with better knowledge level (Table 5).

Table 2. Pharmacists' knowledge of proton pump inhibitors (PPIs)

Questions	Correct answers frequency (%)
Is a PPI an inactive pro-drug?	142 (84.5)
Are omeprazole, pantoprazole, lansoprazole, rabeprazole, esomeprazole PPIs?	164 (97.6)
Do PPIs treat acid-related diseases by suppressing hydrochloric acid secretion?	158 (94)
Can PPIs be used to prevent stress ulcers?	156 (92.9)
Can PPIs be used in the treatment of acute pancreatitis?	105 (62.5)
Does omeprazole have the most individual variability compared to other PPIs?	106 (63.1)
Should omeprazole be selected for pediatric patients?	116 (69)
Is rabeprazole first choice in pregnant patients?	131 (78)
Do you think that more PPI consumption will create a better and safer effect?	155 (92.3)
Are PPIs usually available as enteric-coated capsules or tablets?	137 (81.5)
Should the PPI usually be taken before breakfast?	160 (95.2)
Should a PPI be taken after a meal?	165 (98.2)
Is it advisable to increase the dose frequency rather than a single dose to improve effect?	108 (64.3)
Should patients take PPI for only 7 days in Helicobacter pylori eradication treatment?	143 (85.1)
Does PPI treatment of gastric ulcer take 2 weeks to 4 weeks?	57 (33.9)
Do you think long-term use of PPI may cause adverse reactions such as osteoporosis, etc.?	150 (89.3)

Attitude

The vast majority (93.5%) of pharmacists believed that PPIs were overused in Türkiye. 68.4% of pharmacists stated that the reason for the high use of PPIs was abuse by the patient or physician. 86.3% of pharmacists believed that excessive consumption of PPIs resulted in increased costs and adverse drug reactions. 86.9% of pharmacists believed that health workers should receive extensive training on this subject, and 80.3% believed that community pharmacy management should be strengthened. Table 3 shows the attitudes of pharmacists towards PPIs.

The attitude level of 61% of the pharmacists was found to be good. There was no significant relationship between any socio-demographic variable and the level of attitudes of the pharmacists (p>0.05) (Table 5).

Practices

46 pharmacists (27.38%) declared that they have used PPIs in the last 1 year. Figure 1 shows the PPIs used by pharmacists in the last 1 year. Pharmacists declared that they never used PPIs for abdominal pain, ventosity, nausea, and vomiting in 19%, 16.7%, 19.6% and 17.3%, respectively. Table 4 shows pharmacists' practices regarding the use of proton pump inhibitors.

There was no significant relationship between any socio-demographic variable and pharmacists' use of PPIs (p>0.05) (Table 5).

Table 3. Pharmacists' attitudes to proton pump inhibitors (PPIs) use

Questions	Agreement	Frequency (%)
Currently, PPIs are overused in Türkiye.	Strongly agree	89 (53)
	Agree	68 (40.5)
	Uncertain	6 (3.6)
	Disagree	4 (2.4)
	Strongly disagree	1 (0.6)
The main cause of PPI overuse is physician or patient abuse of the PPI.	Strongly agree	56 (33.3)
	Agree	59 (35.1)
	Uncertain	25 (14.9)
	Disagree	27 (16.1)
	Strongly disagree	1 (0.6)
Stress ulcer prophylaxis is the main reason for the overuse of PPIs.	Strongly agree	48 (28.6)
	Agree	72 (42.9)
	Uncertain	25 (14.9)
	Disagree	20 (11.9)
	Strongly disagree	3 (1.8)
Overuse of PPIs will result in increased adverse drug reactions and medical cost.	Strongly agree	64 (38.1)
	Agree	81 (48.2)
	Uncertain	15 (8.9)
	Disagree	5 (3)
	Strongly disagree	3 (1.8)
Large scale education on the rational use of PPIs is needed for healthcare professionals and the public.	Strongly agree	67 (39.9)
	Agree	79 (47)
	Uncertain	9 (5.4)
	Disagree	11 (6.5)
	Strongly disagree	2 (1.2)
In this regard, community pharmacy management should be strengthened.	Strongly agree	57 (33.9)
	Agree	78 (46.4)
	Uncertain	14 (8.3)
	Disagree	18 (10.7)
	Strongly disagree	1 (0.6)

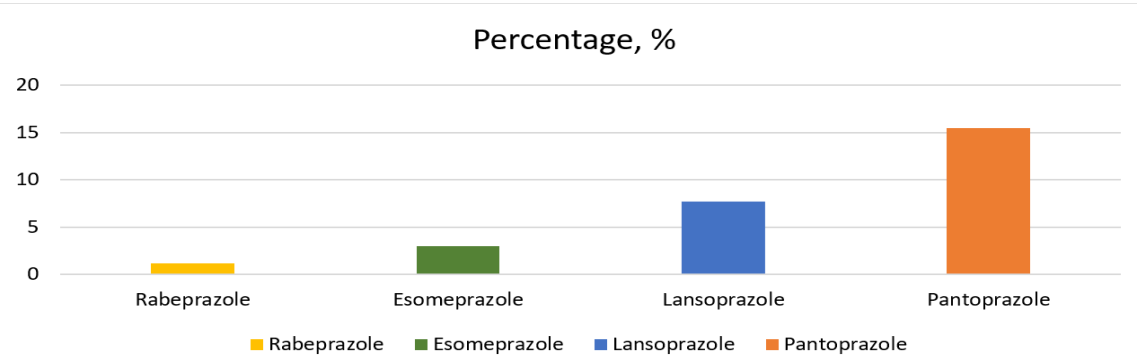

Figure 1. Types of proton pump inhibitors used by pharmacists in the last 1 year

Table 4. Pharmacists' practices on the use of proton pump inhibitors (PPIs)

Questions	Agreement	Frequency (%)*
Use PPI when abdominal pain	Always	1 (0.6)
	Often	-
	Sometimes	4 (2.4)
	Rarely	9 (5.4)
	Never	32 (19)
Use PPI when ventosity	Always	1 (0.6)
	Often	1 (0.6)
	Sometimes	7 (4.2)
	Rarely	9 (5.4)
	Never	28 (16.7)
Use PPI when nausea	Always	-
	Often	-
	Sometimes	4 (2.4)
	Rarely	9 (5.4)
	Never	33 (19.6)
Use PPI when vomiting	Always	-
	Often	-
	Sometimes	6 (3.6)
	Rarely	11 (6.5)
	Never	29 (17.3)

* Only 46 pharmacists using PPIs responded

Table 5. Responses to questions related to knowledge, attitudes and usages in relation to PPIs use

Variables	Knowledge Level			Attitude Level			PPI Usage		
	Poor (%) n	Good (%) n	p	Poor (%) n	Good (%) n	p	Unused (%) n	Used (%) n	p
Gender									
Male	30 (50)	52 (48.1)	0.818	36 (56.2)	46 (44.2)	0.13	58 (47.5)	24 (52.2)	0,717
Female	30 (50)	56 (51.9)		28 (43.8)	58 (55.8)		64 (52.5)	22 (47.8)	
Age years (mean±SD)	45.17 ±16.26	46.86 ±12.7	0.487	45.55 ±14.107	46.59 ±14.069	0.609	45.29± 13.95	48.8± 14.15	0.146
Education									
Bachelor's degree	52 (86.7)	105 (97.2)	0.018	58 (90.6)	99 (95.2)	0.336	113 (92.6)	44 (95.7)	0.729
Postgraduate	8 (13.3)	3 (2.8)		6 (9,4)	5 (4.8)		9 (7.4)	2 (4.3)	
Work experience, years (%)									
< 5	16 (26.7)	9 (8.3)	0.003	10 (15.6)	15 (14.4)	1	20 (16.4)	5 (10.9)	0,47
≥ 5	44 (73.3)	99 (91.7)		54 (84.4)	89 (85.6)		102 (83.6)	41 (89.1)	

PPIs: proton pump inhibitors, SD: standard deviation

In our study, although pharmacists' knowledge, attitudes, and practices regarding PPIs were generally good, there were also some deficiencies in their knowledge. In the study conducted in China, which drug is a PPI was answered correctly 76.61% by doctors, 66.42% by nurses and 77.78% by pharmacists. The mechanism of action was answered correctly 93.2% by doctors, 89.93% by nurses, and 92.36% by pharmacists. 68.48% of physicians, 52.61% of nurses, and 74.65% of pharmacists knew that PPIs were prodrugs [13]. These rates were 100%, 81.4% and 71.2%, respectively, in the study conducted with community pharmacists in Cyprus [18]. The results of our study are in line with the literature. In our study, only 33.9% of the participants correctly answered the duration of PPI treatment in gastric ulcers. These results were low in line with other studies. In other studies, this rate was 30%, 54.51%, and 20.3% in pharmacists, respectively [13,18,19]. This may be because the dose and duration of the PPI are the responsibility of the prescribing physician. However, pharmacists must also collaborate with the prescriber to reduce healthcare costs and improve outcomes [10,20].

In our study, pharmacists thought that PPIs were overused (93.5%) and believed that this would cause an increase in adverse drug reactions and medical costs (86.3%). PPIs are one of the most commonly prescribed drugs worldwide. There were many studies on the unnecessary and misuse of PPIs [21,22]. There were also studies in Türkiye about the unnecessary and widespread use of PPIs [10,23]. Inappropriate long-term use of PPIs, especially in elderly patients, causes serious adverse effects [24]. These adverse effects are; pneumonia, vitamin B12, calcium deficiency, increased risk of fracture, Clostridium difficile infection and gastric carcinoid tumor [25].

In our study, 86.9% of pharmacists believed that the public and health workers should be trained on the rational use of PPI, and 80.3% of pharmacists believed that community pharmacy management should be strengthened. Both community pharmacists and clinical pharmacists play an important role in reducing the inappropriate use of PPIs, reducing healthcare costs and preventing adverse reactions. Many studies have shown that pharmacists reduce the use of inappropriate PPIs [10,26,27]. Due to the low knowledge of the patients and easy access to PPIs from the pharmacy, it causes excessive use of PPIs, so the public and community pharmacists should be made aware of this issue [13].

In our study, 27.4% of pharmacists used PPIs in the last 1 year. The most commonly used PPIs were pantoprazole (56.5%) and lansoprazole (28.26%). In a study conducted in China [13], 40% of pharmacists used PPIs in the last 1 year, and omeprazole was the most commonly used PPI. In a study conducted in Cyprus [18], about half of the pharmacists used PPIs in the last 1 year and omeprazole was used most frequently. The reason why pantoprazole was frequently used by pharmacists in our study may be that pantoprazole was frequently prescribed by doctors and the number of generic drugs and their availability in the market were high. A study investigating the impact of PPI consumption on the budget in Türkiye revealed that physicians preferred omeprazole less over the years and preferred more expensive molecules instead. Pantoprazole, esomeprazole, and rabeprazole, which were preferred with low rates in 2006, dominated more than 50% of the market in 2011 [28]. Additionally, omeprazole may have been less preferred because it has a greater potential for drug-drug interactions than other PPIs [29].

In our study, there was no pharmacist who always and frequently used PPIs in the presence of vomiting and nausea. Only 1 person (0.6%) declared that they always used PPIs when they had abdominal pain and ventosity. This rate was quite low compared to other studies [13,19].

This study has some limitations. The number of pharmacists participating in the study was low, so the generalizability of the study was limited. In addition, since the survey was an online survey, the participants administered it on their own. Therefore, the participants could not be observed by the researchers, and we do not know whether they looked at any material while answering the questions.

According to our study, although pharmacists' knowledge, attitudes, and practices about PPIs were generally good, there were also some deficiencies in their knowledge. Therefore, pharmacists need to update themselves and be supported continuously with various trainings.

AUTHOR CONTRIBUTIONS

Concept: A.A.; Design: A.A., İ.Y.; Control: A.A., İ.Y.; Sources: A.A., İ.Y.; Materials: A.A., İ.Y.; Data Collection and/or Processing: İ.Y.; Analysis and/or Interpretation: A.A.; Literature Review: A.A., İ.Y.; Manuscript Writing: A.A., İ.Y.; Critical Review: A.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

Ethical approval of the study was obtained from Suleyman Demirel University Clinical Research Ethics Committee (Approval No: 194 / Date: 07.07.2022).

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

IN VITRO AND *IN SILICO* STUDIES ON LIGNAN SECOISOLARICIRESINOL DIGLUCOSIDE

LİGNAN SEKOİZOLARİSİRESİNOL DİGLUKOSİT ÜZERİNE İN VİTRO VE İN SİLİKO ÇALIŞMALAR

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ABSTRACT

Objective: *Lignans are important biologically active compounds in diphenolic structure. Secoisolariciresinol diglucoside (SDG) is a significant type of lignan known to have anti-cancer properties. This study aimed to investigate the antiproliferative activity properties of SDG on hepatocellular carcinoma cells (HepG2), colorectal cancer cells (DLD-1), lung carcinoma (A549), and prostate cancer (PC3) cell lines.*
Material and Method: *Cell viability of cancer cells was determined by the MTT method after treatment with various concentrations of SDG at 48 or 72 hours. The DFT (Density Functional Theory) analysis of the SDG was performed using Spartan'10 and visualized. Drug-likeness and absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) properties of this compound were examined. Molecular docking was carried out to research the biological activity of SDG.*
Result and Discussion: *Our results showed that SDG exhibited significant cytotoxicity only against DLD-1 cells with IC_{50} value of 37.45 µM, but inactive against other cancer cell lines as in vitro. 4UYA, which biomarker for colon cancer, is the crystal structure of the MLK4 kinase domain. The binding energy value for the SDG-MLK4 kinase domain was calculated as -6.1 kcal/mol. Anticancer potential was verified by in vitro assay and in silico molecular docking study. In conclusion, this study revealed the protective aspect of SDG against colon cancer and showed that it has promising anticancer activity.*
Keywords: *Cancer, cytotoxicity, DFT, molecular docking, SDG*

ÖZ

Amaç: *Lignanlar, difenolik yapıda biyolojik olarak aktif önemli bileşiklerdir. Sekoizolarisiresinol diglukosit. (SDG), kanser önleyici özelliklere sahip olduğu bilinen önemli bir lignan türüdür. Bu çalışmada SDG'nin hepatoselüler karsinom hücreleri (HepG2), kolorektal kanser hücreleri (DLD-1), akciğer karsinomu (A549) ve prostat kanseri (PC3) hücre hatları üzerindeki antiproliferatif*

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aktivite özelliklerinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: *Kanser hücrelerinin hücre canlılığı, 48 veya 72 saatte çeşitli SDG konsantrasyonları ile muamele edildikten sonra MTT yöntemiyle belirlendi. SDG'nin DFT (Yoğunluk Fonksiyonel Teorisi) analizi Spartan'10 kullanılarak yapıldı ve görselleştirildi. Bu bileşiğin ilaca benzerliği ve emilim, dağılım, metabolizma, atılım ve toksisite (ADME-Tox) özellikleri incelendi. SDG'nin biyolojik aktivitesini araştırmak için moleküler yerleştirme gerçekleştirildi.*
Sonuç ve Tartışma: *Sonuçlarımız SDG'nin yalnızca IC_{50} değeri 37,45 µM olan DLD-1 hücrelerine karşı anlamlı sitotoksisite sergilediğini, diğer kanser hücre hatlarına karşı ise in vitro olarak inaktif olduğunu gösterdi. Kolon kanseri biyobelirteci olan 4UYA, MLK4 kinaz bölgesinin kristal yapısıdır. SDG-MLK4 kinaz alanına ait bağlanma enerjisi değeri -6,1 kcal/mol olarak hesaplandı. Antikanser potansiyeli in vitro analiz ve in silico moleküler yerleştirme çalışmasıyla doğrulandı. Sonuç olarak bu çalışma SDG'nin kolon kanserine karşı koruyucu yönünü ortaya koyarak umut verici antikanser etkinliğe sahip olduğunu göstermiştir.*
Anahtar Kelimeler: *DFT, kanser, moleküler yerleştirme, SDG, sitotoksisite*

INTRODUCTION

One of the main ways to deal with degenerative diseases such as cancer has long been seen as a focus on diet, and as a result of increasing awareness in people, the demand and orientation for functional foods is increasing. In this context, particular emphasis is placed on foods rich in lignans. Lignans, belonging to the phytoestrogen class, are natural compounds in diphenolic structure and have different biological activities [1,2]. Secoisolariciresinol diglucoside (SDG) is an essential bioactive lignan species that is present in small amounts in a variety of foods and plants but is particularly high ratio in flaxseed (Linum usitatissimum) [3,4]. After ingestion of SDG, it is metabolized by colon bacteria to the mammalian lignans enterodiol and enterolactone [5,6]. It has been observed that studies involving lignans especially focus on the estrogenic activities of these structures and their potential for effect on cancer types such as hormone-related breast cancer [7-9]. The chemical structure of SDG is shown in Figure 1.

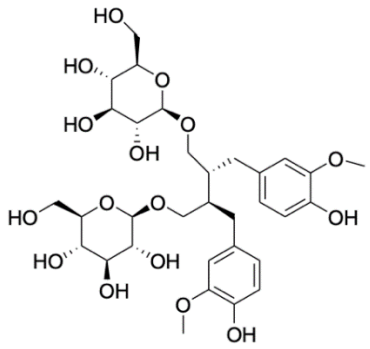


Figure 1. Chemical structure of SDG

After consumption of plant lignan SDG, it is metabolized to mammalian lignans enterodiol and enterolactone by demethylation and dehydroxylation processes by some bacteria in the human colon, such as *Peptostreptococcus Eubacterium* and *Eggerthella* [5,10]. The biological activity of SDG is generally attributed to this metabolic conversion [2]. It has been reported that lignans from flaxseed have the ability to interfere with the cellular properties of malignant tumors, affect molecular signaling junctions, and regulate related signaling pathways [11]. It has been demonstrated by various studies that SDG exhibits protective effects against various cancers such as colon, prostate, and breast, with its antiproliferative, antioxidant, and anti-estrogenic properties and/or by inhibiting metabolic-related enzymes [2,10,12-14]. There are more studies showing the anti-cancer treatment potential of the SDG metabolites enterodiol and enterolactone alone or in combination [7,8,15,16]. There are relatively few studies in the literature on the effects of SDG alone on cancer cells.

In this study, the cytotoxic activity of SDG on DLD-1 human colon cancer, A549 lung cancer, PC3 prostate cancer, and HepG2 liver cancer cell lines was evaluated. In addition, docking study was performed to analyze the binding conformation of the SDG molecule at the 4UYA active site, and *in silico* ADME-Tox profile studies were carried out. This study aimed to observe the inhibitory effectiveness of SDG, which is considered a potential anticancer candidate, in preventing cancer cell development, through molecular structure compatibility and cytotoxicity.

MATERIAL AND METHOD

Reagents, Solvents, and Materials

Human liver carcinoma (HepG2) (ATCC® HB-8065™) cell line, human lung cancer (A549) (ATCC® CCL-185™) cell line, human colon cancer (DLD-1) (ATCC® CCL-221™) cell line, and human prostate cancer (PC3) (ATCC® CRL-1435™) cell line was purchased from American Type Culture Collection. SDG was purchased commercially from Cayman chemical company and used without further purification. SDG was dissolved in 0.5% DMSO for cell culture studies.

In vitro Cytotoxic Activity Studies

The cells were seeded into 96-well plates at 5 x 10^3 cells/well densities for cytotoxic activity studies [17,18]. Cells were exposed to the SDG for different concentrations, varying from 300 to 0.5 µM (for A549), 300 to 37.5 µM (for HepG2), 300 to 9.375 µM (for DLD-1 and PC3), after 24 h. MTT stock solution was prepared at 5 mg/ml and 50 µl was added to each well after 48 h (for HepG2 and DLD-1) and 72 h (for A549 and PC3) and incubated for a further 2 h. The absorbance values were measured with an Epoch 2 Elisa plate reader at 590 nm.

Computational Methods

Frontier molecular orbitals (FMOs) and molecular electrostatic potential (MEP) map calculations were performed utilizing the Spartan software program (Spartan'10, version 1.1.0. Wavefunction) [19] with DFT: B3LYP/6–31G* method [20]. Drug-likeness and ADMET analysis of the SDG compound was performed using SwissADME [21], Pro Tox-II [22], and SwissTargetPrediction [23] prediction tools. The docking studies were performed utilizing UCSF Chimera and AutoDock Vina software [24]. From the PubChem database (https://pubchem.ncbi.nlm.nih.gov), the available structures of SDG were retrieved.

RESULT AND DISCUSSION

Cytotoxic Activity Studies

The cytotoxic activities of SDG were tested in the hepatocellular carcinoma cells (HepG2), colorectal cancer cells (DLD-1), lung carcinoma (A549), and prostate cancer (PC3) cell lines at concentrations of 300, 150, 75, 37.5, 18.75, and 9.375 µM as *in vitro* for 48 or 72 h. The IC_{50} values calculated with the GraphPad Prism 5 program are given in Table 1.

Table 1. The calculated IC_{50} results for SDG

Compound	**IC_{50} (µM)**			
	HepG2	**A549***	**DLD-1**	**PC3**
SDG	>300	>300	37.45	>300

Considering that SDG is metabolized in the colon, it is possible to say that lignan may exert its inhibitory effect on colon tumor cells through mechanisms other than estrogenic activity [2]. Özgöçmen *et al*. determined the IC_{50} values as 100 µM for 24 h and 150 µM for 48 and 72 h in SW480 cells in which they applied 40-200 µM SDG and found that cell proliferation was inhibited by almost half at all-

time intervals [25]. In another study, lower doses (0-40 µM) of SDG application showed a dose- and time-dependent decrease in cell numbers in SW480 human colon cancer cells, but cell viability was recorded above 80%. It has been claimed that SDG may be mediated by a cytotoxic mechanism associated with cyclin A expression in colon cancer cells [12]. Chen *et al*. found that SDG treatment was able to significantly inhibit cell viability over time (0-24 h) in a different colon cancer cell line HCT116. The IC_{50} value for HCT116 cells was determined as 24.5 µmol/l [26]. The results we obtained support the literature, and it was found that among the cancer cell lines studied, colon cancer cells (DLD-1) were the cells most affected by SDG cytotoxicity with an IC_{50} value of 37.45 µM. It was observed that SDG did not have an antiproliferative effect (IC_{50}>300 µM) against A549 and HepG2 cancer cells in the studied conditions. Only at a high concentration of SDG (300 µM), HepG2 cell viability was determined as 37%. PC3 cells were also cytotoxictly affected by 75 µM and above SDG concentration, and cell viability decreased depending on the concentration (87.1%, 67.2%, and 47.8% for 75, 150, and 300 µM, respectively). Considering the estrogenic properties of lignan, inhibition of the proliferation of hormone-dependent cancer types may be possible through a mechanism based on this.

It has been reported that SDG can show higher stability than its metabolite enterolactone, and this high stability is due to the ability of bulky glucose groups in its chemical structure to resist possible electrophile attacks [12]. However, in a study examining the antiproliferative activity of SDG, END, and ENL on acute myeloid leukemia cell lines (KG-1 and Monomac-1), ENL showed promising cytotoxicity activity in both cell lines, whereas SDG was found to have a minimal anti-proliferative effect in KG-1 cells after 24 hours of application. However, it was found that SDG had a proliferative effect for 48 hours against KG-1 cells. Contrary to expectations, it was determined that cell proliferation increased in both time periods (24 and 48 hours) due to the increase in SDG and END concentrations in Monomac-1 cells [16]. From these results and the data we obtained from the study, it is possible to assume that the relevant lignans may exhibit different metabolic behaviors depending on the cell type.

Computational Structural Analysis

The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are referred FMOs [27]. The reactivity and stability of compounds are estimated using molecular orbital energies. The energy difference (HOMO-LUMO gap) is a critical factor in the chemical kinetic stability and reactivity of the compound. The small energy difference between the HOMO-LUMO molecular orbitals indicates that the molecule is soft, on the contrary, it is hard when it is large [28-30]. The optimization of the SDG compound was carried out using DFT with a functional B3LYP/6–31G* basis set in the gaseous phase. In the gaseous environment, the ΔE value was found to be 3.8531 eV for SDG. The HOMO, LUMO energies, ΔE energy ranges, and the visuals of them were given in Table 2 and Figure 2.

The MEP method is a very useful and practical method for determining the electrophilic and nucleophilic fields of molecules. The MEP map is a coded by colours map of the electron density surfaces of molecules. In this map, the red colour symbolizes electron-rich regions (partially negative charge) and the blue colour symbolizes electron-poor regions (partially positive charge). The yellow colour indicates regions with fewer electrons than the other regions and the green colour represents neutral regions with zero potential [31,32]. The molecular potential surfaces of the SDG were easily obtained using the Spartan program. As shown in Figure 3, analysis of the MEP map reveals that negative regions marked in red are located on O atoms in the rings. As can be understood from the MEP map, it is predicted that the compound will exhibit nucleophilic behaviour from the red region where the oxygens are present.

Table 2. The HOMO, LUMO energies, and ΔE energy ranges of SDG

SDG				
Medium	**$E_{HOMO(a.u.)}$**	**$E_{LUMO(a.u.)}$**	**$\Delta E_{(a.u.)}$**	**$\Delta E_{(eV)}$**
Gaseous	-0.2912	-0.1496	0.1416	3.8531

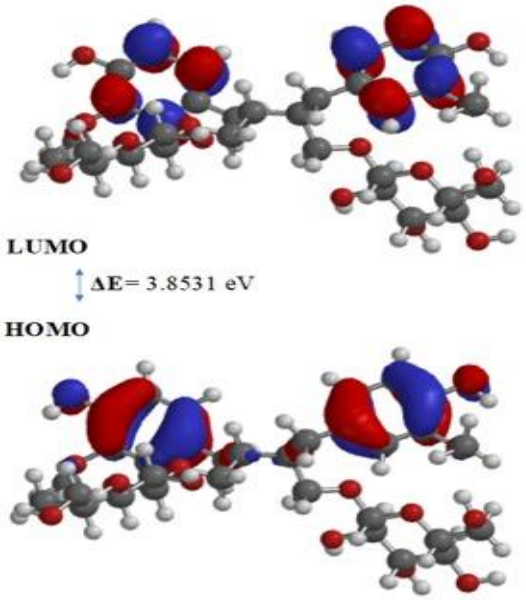


Figure 2. HOMO and LUMO energy plots of SDG in the gaseous media

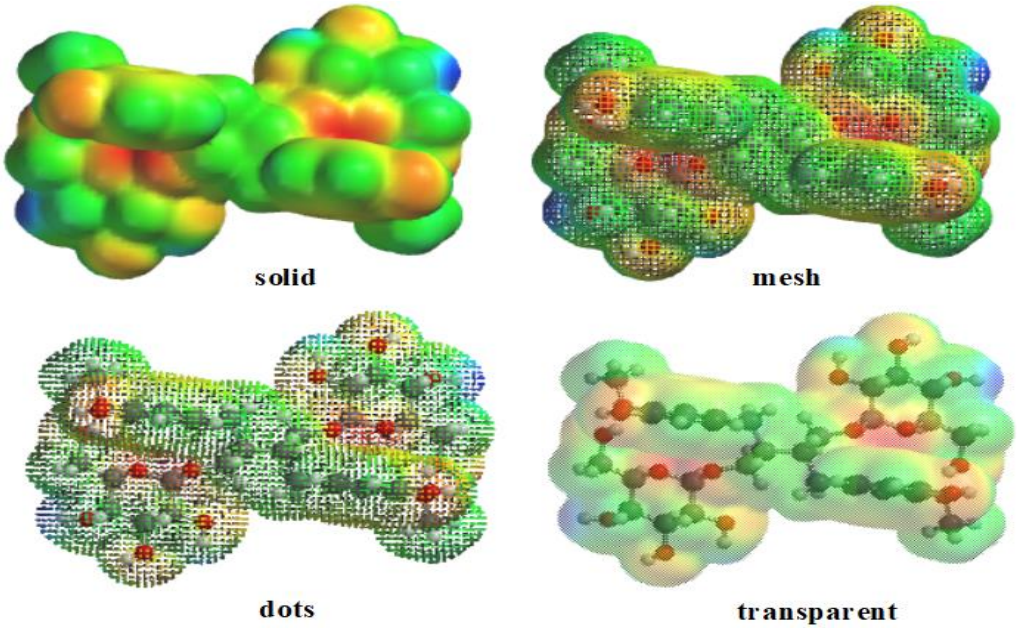
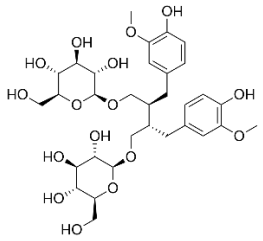


Figure 3. Showing the molecular electrostatic potential maps of the SDG

Molecular Docking

Molecular docking analyses aim at predicting the possible molecular interactions between proteins and ligands. The interaction between the SDG molecule and the MLK4 kinase was determined using docking analysis. The good energy value of the docking result (-6.1 kcal/mol) for the SDG molecule is shown in Table 3. Ligand-receptor 3D interactions were visualized owing to the Biovia Discovery Studio Visualizer program [33]. The 3D structure of the MLK4 kinase domain (PDB ID: 4UYA) at 2.80 Å resolution was acquired via the RSCB PDB website (https://www.rcsb.org/). SDG molecule formed secondary interactions with the MLK4 kinase. These interactions are shown in Figure 4.

Table 3. Docking analysis results of SDG compound

Compound Name	Chemical Structure Depiction	Docking Score (kcal/mol)	Amino Acid Residues
SDG		-6.1	ILE16, VAL21, LYS34, LEU71, LEU73, THR145, ASP146

The best interaction of the SDG compound was determined as conventional hydrogen bond, π-donor hydrogen bond, van der Waals interaction, alkyl interaction, and π-alkyl interaction including ILE16, VAL21, LYS34, LEU71, LEU73, THR145, ASP146 residues.

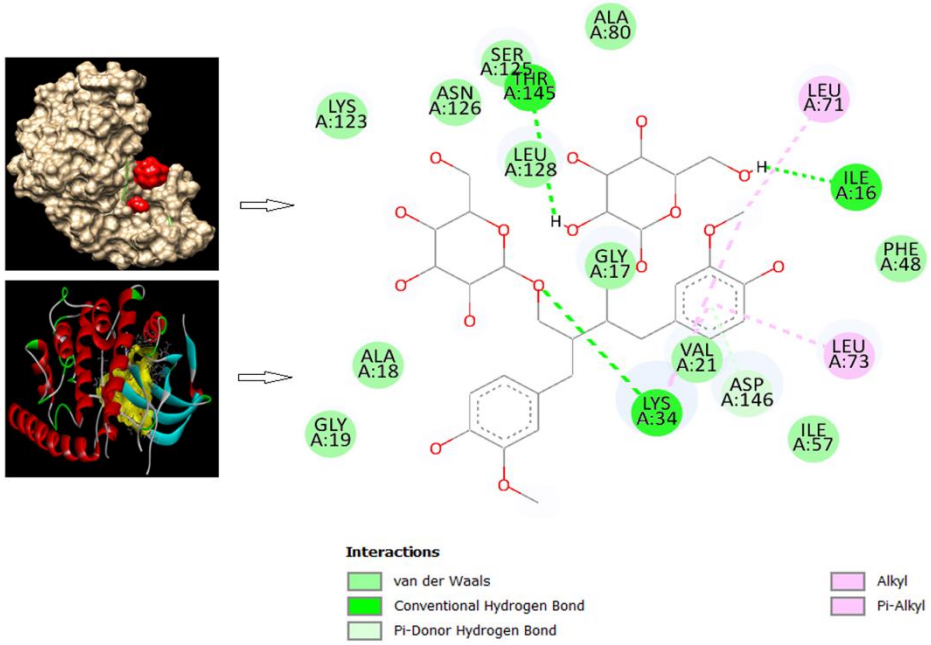


Figure 4. Images of the protein-ligand interaction by Discovery Studio Visualizer

Druggability and ADMET Properties

Druggability features refer to the physicochemical parameters and ADME-Tox properties of the compound. *In silico* methods have been widely used to estimate the ADME properties of molecules because *in vivo* and *in vitro* analyses are costly and time-consuming [34]. To determine the ADME-Tox properties of the SDG, several web-based *in silico* tools were used, including SwissADME, SwissTargetPrediction, and ProTox-II.

Prediction of pharmacokinetic and toxicokinetic properties greatly increases the success of reaching the target in the discovery of potential drug candidate compounds. Lipinski's rule of five has become standard for the prediction of the drug-likeness of the compounds [35]. The SDG does not comply with Lipinski's four rules. It complies with a rule only because its lipophilicity coefficient is LogP≤5. Due to the bulky nature of SDG, their gastrointestinal absorption is low, it cannot cross the blood-brain barrier (BBB) and cannot be used as substrates of P-glycoprotein (P-gp). The solubility of SDG in octanol/water is also low. SDG molecule does not interact with or inhibit CYP2C19, CYP1A2,

CYP2D6, CYP2C9 and CYP3A4. The results obtained by the SwissADME web tool are shown in Table 4.

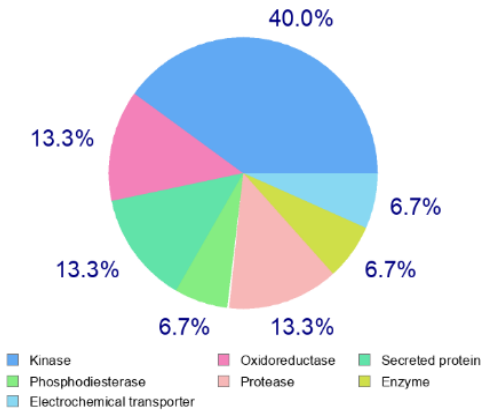
Table 4. ADME features estimated by SwissADME of the SDG

	SwissADME
	Physicochemical Properties
Formula	$C_{32}H_{46}O16$
Molecular weight	686.70 g/mol
Number heavy atoms	48
Number aromatic heavy atoms	12
Fraction Csp^3	0.62
Number rotatable bonds	15
Number hydrogen bond acceptors	16
Number hydrogen bond donors	10
Molar refractivity	164.05
TPSA	257.68 $Å^2$
Lipophilicity	
$LogP_{o/w}$	1.07
Water Solubility	
LogS	-2.87
Solubility	9.25e-01 mg/ml
Absorption	
GI absorption	Low
Distribution	
BBB permeation	No
P-gp substrate	No
Metabolism	
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
$LogK_p$ (skin permeation)	-10.96 cm/s
Drug-likeness	
Lipinski	No
Ghose	No
Veber	No
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 2 violation: MW >350
Synthetic Accessibility	6.71
Bioavailability Score	0.17

With regard to Table 5 of the SDG could be an inhibitor of 40% probability for kinase, 13.3% for oxidoreductase, 13.3% for secreted protein, 13.3% for protease, 6.7% for phosphodiesterase, 6.7% for enzyme and 6.7% for electrochemical transporter. In line with this data obtained using SwissTargetPrediction, kinase proteins were preferred for molecular docking analysis.

The toxicity prediction was performed owing to the Pro-Tox II web tool. The predicted data are given in Table 6. In terms of toxicity results, the SDG molecule was not hepatotoxic, carcinogenic, mutagenic, or cytotoxic but had immunotoxic effects. The predicted toxicity class of SDG was categorized as 5.

Table 5. The predicted biological target list and percentage distribution



Target	Common name	Uniprot ID	ChEMBL ID	Target Class
Protein kinase C delta	PRKCD	Q05655	CHEMBL2996	Kinase
Tyrosinase	TYR	P14679	CHEMBL1973	Oxidoreductase
Testis-specific androgen-binding protein	SHBG	P04278	CHEMBL3305	Secreted protein
Phosphodiesterase 5A	PDE5A	O76074	CHEMBL1827	Phosphodiesterase
Protein kinase C gamma	PRKCG	P05129	CHEMBL2938	Kinase
Protein kinase C beta	PRKCB	P05771	CHEMBL3045	Kinase
Protein kinase C epsilon	PRKCE	Q02156	CHEMBL3582	Kinase
Protein kinase C eta	PRKCH	P24723	CHEMBL3616	Kinase
Protein kinase C theta	PRKCQ	Q04759	CHEMBL3920	Kinase
Thrombin	F2	P00734	CHEMBL204	Protease
Coagulation factor VII	F7	P08709	CHEMBL3991	Protease
Tyrosyl-DNA phosphodiesterase 1	TDP1	Q9NUW8	CHEMBL1075138	Enzyme
Interleukin-2	IL2	P60568	CHEMBL5880	Secreted protein
Steroid 5-alpha-reductase 1	SRD5A1	P18405	CHEMBL1787	Oxidoreductase
Sodium/glucose cotransporter 2	SLC5A2	P31639	CHEMBL3884	Electrochemical transporter

Table 6. The toxicity computation of SDG molecule by Pro-Tox II web tool

Toxicity Model Report (Predicted Toxicity Class:5)			
Classification	**Target**	**Shorthand**	**Prediction**
Organ toxicity	Hepatotoxicity	dili	Inactive
Toxicity end points	Carcinogenicity	carcino	Inactive
Toxicity end points	Immunotoxicity	immuno	Active
Toxicity end points	Mutagenicity	mutagen	Inactive
Toxicity end points	Cytotoxicity	cyto	Inactive

In this study, the SDG molecule, which is commercially purchased, was tested *in vitro* against different cancer cell lines. The results showed that SDG, which is an important lignan species, had antiproliferative activity against the colon cancer cell line (DLD-1) while it was found to be inactive against other cell lines such as prostate, lung, and liver. The molecular docking behaviour of commercially purchased SDG against MLK4 kinase was further investigated. The predicted binding energy was found to be -6.1 kcal/mol. The docking results showed that SDG was inhibited through secondary interactions.

In general, it can be said that SDG may exhibit a greater therapeutic potential for the prevention of colon cancer based on the cytotoxic analyses obtained. As seen in the literature, the therapeutic efficacy of SDG for oncological cases is related to the type of malignant tumor and cancer cell

characteristics. However, further studies are needed to clearly demonstrate the validity of SDG supplementation/treatment.

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AUTHOR CONTRIBUTIONS

Concept: İ.B., S.Ç.Y., S.A.; Design: İ.B., S.Ç.Y.; Control: S.A.; Kaynaklar: İ.B., S.Ç.Y.; Sources: İ.B., S.Ç.Y., S.A.; Data Collection and/or Processing: İ.B., S.Ç.Y., S.A.; Analysis and/or Interpretation: İ.B., S.Ç.Y., S.A.; Literature Review: İ.B., S.Ç.Y., S.A.; Manuscript Writing: İ.B., S.Ç.Y., S.A.; Critical Review: İ.B., S.Ç.Y., S.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

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

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

TABLETING AND EVALUATION OF MULTIPLE-UNIT COMPOSITION OF ISOSORBIDE DINITRATE WITH MODIFIED RELEASE

İZOSORBİD DİNİTRATIN MODİFİYE SALIM YAPAN ÇOK BİRİMLİ BİLEŞİMİNİN TABLET ŞEKLİNDE BASILMASI VE DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *This study was aimed to create a multiple-unit dosage form of isosorbide dinitrate by pressing spherical matrix granules into tablets, selecting the optimal technology parameters and evaluating the obtained tablets.*
Material and Method: *The tablet cores were prepared from mixtures of active matrix granules and shock-absorbing auxiliary granules in various ratios using diverse tabletting forces. The dissolution profiles of the obtained tablet compositions were evaluated in comparison with the reference preparation.*
Result and Discussion: *An optimal ratio of spheroids with the active component with auxiliary granules along with optimal compression parameters were determined. The resulting as multiple-unit tablets exhibited a release profile similar to that of Cardicket Retard. Used technological approach makes it possible to regulate the dissolution profile of tablets by changing the ratio of active granules with different kinetics of the active substance release.*
Keywords: *Active substance release profile, as multiple-unit tablets, isosorbide dinitrate, spherical matrix granules*

ÖZ

Amaç: *Bu çalışma, küresel matris granüllerinin tabletler şeklinde basılmasıyla izosorbid dinitratın çok birimli dozaj formunu oluşturmayı, optimum teknoloji parametrelerini seçmeyi ve elde edilen tabletleri değerlendirmeyi amaçlamıştır.*
Gereç ve Yöntem: *Tablet çekirdekleri, çeşitli oranlarda ve çeşitli tabletleme kuvvetleri kullanılarak aktif matris granülleri ve yardımcı granüllerin karışımlarından hazırlandı. Elde edilen tablet bileşimlerinin çözünme hızı profilleri, referans ürün ile karşılaştırılarak değerlendirildi.*
Sonuç ve Tartışma: *Used technological approach makes it possible to regulate the dissolution profile of tablets by changing the ratio of active granules with different kinetics of the active substance release. Aktif sferoidler ve yardımcı granüllerin optimum oranı, optimum basınç parametresi ile belirlendi. Elde edilen çok birimli tabletler Cardicket Retard'ınkine benzer etkin madde salım profili göstermişti. Kullanılan teknolojik yaklaşım, etkin maddenin farklı kinetiklerle*

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salımı ile aktif granüllerin oranı kullanılarak tabletlerin çözünme profilini düzenlemeyi mümkün kılmaktadır.
Anahtar Kelimeler: *Aktif maddenin salım profili, izosorbid dinitrat, küresel matris granülleri, multidozlu tabletler*

INTRODUCTION

Isosorbide dinitrate (Figure 1) belongs to the group of organic nitrates. It is a drug for the treatment and prevention of angina pectoris attacks. When taking traditional forms of isosorbide dinitrate (tablets, sprays), the effect comes immediately and the total duration of action does not exceed 6 hours [1,2]. Existing forms contribute to rapid relief of angina attacks, but are not suitable for their prevention, when a gradual release of active ingredient for an extended period (about 10-12 hours) is needed. In turn, prolonged forms of isosorbide dinitrate have a high prophylactic efficacy. Modified release preparations combine high pharmacological activity, a long period of therapeutic action and the absence of serious side effects characteristic of all nitrate group preparations [2,3].

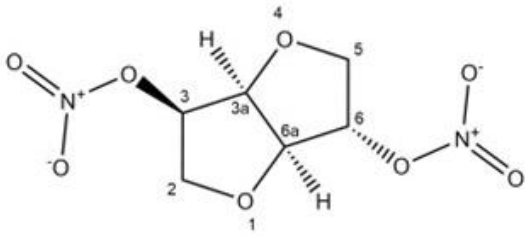

Figure 1. The chemical formula of isosorbide dinitrate

The most common dosage forms with modified release are matrix tablets. Such forms are produced by incorporating active pharmaceutical ingredients into polymer matrices to achieve controlled drug release [4-6]. In turn, today multicomponent dosage forms are increasingly used in comparison with monolithic medicinal forms. They have a number of potential benefits, such as predicted gastrointestinal movement, overdose risk absence, ability to manage the release profile, increased bioavailability, and also less intra-subject and inter-subject variability in pharmacokinetic characteristics [7-9]. The drugs requiring long-term therapeutic effect for the treatment of such cardiovascular diseases, in particular such as arterial hypertension and angina pectoris, are suitable for the development of sustained release multi-dose formulations [10,11].

The key characteristics of pellets to be compressed into tablets are the size, shape and density, type and amount of the polymer coating. The core of the granule must be strong, but with some plasticity [12]. Given that the granules contain a large proportion of a substance with a narrow therapeutic range, maintaining the integrity of the granules and their coating after tableting is a primarily responsible for the dosage form safety.

The extrusion-spheronization technology was chosen to produce isosorbide dinitrate pellets. This approach provided a high concentration of the active substance, high density and smooth surface of the granules [13-17]. The tabletability and tear resistance of the functional coating of the granules are highly dependent on their deformability. Harder particles are less deformed during pressing, which contributes to the preservation of the outer layer. As expected, the strength of pellets depends on the composition chosen for them.

Microcrystalline cellulose was used as the main auxiliary substance for the manufacture of spheroids. It has excellent ductility and cohesiveness when wetted, as well as the ability to absorb, retain and release water. The predominant mechanism of residual deformation of microcrystalline cellulose granules is plastic deformation. In addition to the above properties, microcrystalline cellulose particles are small in size and allow to obtain granules with a smoother surface than when using other fillers [18-24].

To provide complete leaching from the matrix of the active component lactose was included into

the pellets. Due to high solubility the use of lactose as a filler is unacceptable when moistened with water. However, binary mixtures of microcrystalline cellulose and lactose retain the absorption and adsorption properties of microcrystalline cellulose. And they are absent or minimal in the compositions with lactose [14,25].

Polyacrylate copolymer in the form of an aqueous 30% dispersion of Eudragit NE 30D was selected as the polymer, that modifies the release of the active component. In addition, the polymer used is the key in pellets binder to maintain release characteristics. This polymer is insoluble in water, highly ductile and it doesn't require plasticizer. Along with microcrystalline cellulose the polyacrylate dispersion forms a polymer matrix and provides plastic deformation of the pellets during pressing. Film of Eudragit® NE 30 D is very flexible and the drug release from pellets is not affected by compression [26,27].

Another important aspect of maintaining the release from multi-unit tablets is the use of auxiliary cushioning components. Such tablet components may be either primary powder particles or in the form of secondary agglomerates, such as granules. Before tableting process, excipients are mixed with pellets as cushioning agents to prevent the direct contacts between pellets. And thereby to avoid or to reduce the damage to coating films. The protective effect of the excipient depends on the particle size and characteristics of the material. The amounts of excipients in the tablets are also important for the cushioning effect [10].

The general goal of this work was to develop multi-unit tablets of isosorbide dinitrate with a dissolution profile similar to those of the original product "Cardiket Retard". Given the potential risk of uncontrolled release of the active substance, secondary goals were to develop a technology for the manufacture of isosorbide dinitrate and auxiliary shock-absorbing granules, find the optimal ratio of these granules, and adjust the characteristics of the compressing process.

MATERIAL AND METHOD

Reference Drug

Prolonged-release tablets "Cardiket Retard" 40 mg (Aesica Pharmaceuticals GmbH, Germany) were used. That was done to determine the reference release profile of isosorbide dinitrate for multiple-unit compositions.

Excipients, which were used: Isosorbide dinitrate (RPF "MICROKHIM", Ukraine);Polyacrylate dispersion Eudragit NE 30D (Evonik, Germany);Microcrystalline Cellulose HEWETEN 101 (JRS Pharma, USA); Lactose monohydrate Pharmatose 200M and Lactochem Super Fine Powder (DFE Pharma, Germany); Hydroxypropylmethylcellulose Mantrocel E-6 (Mantrose-Haeuser, USA); Talc (Imifabi, USA); Maize starch (AVEBE, Germany); Colloidal silicon dioxide Aerosil 200 (Evonik, Germany); Povidone Kollidon 30 (BASF, Germany); Magnesium stearate (FACI, Italy).

Determination of the Required Amount of a Mixture of Matrix Granules in a Given Ratio

The content of the isosorbide dinitrate in the obtained matrix pellets was determined by means of their own validated analytical HPLC technique. The analysis was performed on a Shimadzu LC-20AD XR liquid chromatograph with a diode-array detector under the following conditions: Supelco Discovery C18 chromatographic column (150 mm x 4.6 mm, 5 μm); mobile phase - water R - buffer solution (pH 4.7) - methanol R2 (35:10:55); elution mode - isocratic; mobile phase velocity – 1.0 ml / min; the detection wavelength is 220 nm [28].

To prepare buffer solution (pH 4.7) 15.4 g of ammonium acetate R was poured into a volumetric flask with a capacity of 1000.0 ml. After 300.0 ml of water R and 11.5 ml of glacial acetic acid R were added. Content was mixed and volume of solution was adjusted the with water to the mark. The pH of the solution was adjusted as needed with glacial acetic acid R.

To prepare a comparison solution of 0.120 g (precise portion) CRS isosorbide dinitrate was added to a volumetric flask with a capacity of 500.0 ml. Then 300.0 ml of methanol R2 was added and kept in an ultrasonic bath for 10 minutes. The volume of the solution as adjusted to the mark by methanol R2 and mixed thoroughly.

To prepare the test solution, about 0.24 g (exact portion) powder of ground matrix granules was

placed into a volumetric flask with a capacity of 200.0 ml. Then 100.0 ml of methanol R2 was added and kept on ultrasonic bath for 30 minutes at 40-50°C. The solution volume was adjusted to the mark with methanol R2 and mixed thoroughly. The obtained solution (25.00 ml) was transferred to a volumetric flask with a capacity of 50.0 ml and the volume of the solution was adjusted with the mobile phase to the mark was adjusted. Afterwards mixed thoroughly and filtered through the PES syringe filter (d = 25 mm; 0.45 μm) or similar, discarding the first portions of the filter installment.

The required weight of the mixture's sample of uncoated and coated granules which is needed to match the dosage of the isosorbide dinitrate in the experimental compositions was calculated by means of the formulas:

$$\boldsymbol{M_0 = \frac{D_{ISDN}}{W_0}};$$

$$\boldsymbol{W_0 = \frac{(W_{uncoat} + k \times W_{coat})}{1 + k}},$$

where $\boldsymbol{M_o}$ is the calculated mass of the mixture's sample of matrix granules;

$\boldsymbol{D_{ISDN}}$ is the isosorbide dinitrate dosage (amount) in the experimental composition;

$\boldsymbol{W_o}$ is the isosorbide dinitrate mass fraction in the mixture of matrix granules;

$\boldsymbol{W_{uncoat}}$ is the isosorbide dinitrate mass fraction in uncoated spherical granules;

$\boldsymbol{W_{coat}}$ is the isosorbide dinitrate mass fraction in coated spherical granules;

$\boldsymbol{k}$ is the ratio coefficient between the obtained amounts of coated and uncoated granules, respectively.

Obtaining of the Auxiliary Protective Granules

Auxiliary protective granules were obtained by means of the extrusion-spheronization with an aqueous solution of povidone as a moisturizer and binder liquid.

Weighted amounts of dry components were mixed in a drum mixer HSD5-100 (SaintyCo, China). The obtained dry mixture was moistened with 10% of the mass by an aqueous solution of povidone and was mixed in a planetary granulator-mixer XF DH-5L (Nantong KMM, China). The resulting mass was extruded on a screw radial extruder YC-910 (Pilotech, China) with a sieve diameter of 1,0 mm.

The obtained extrudate was spheronized in a laboratory spheronization installation YC-910 (Pilotech, China), equipped with a corrugated disk with a diameter of 250 mm with a corrugation step of 2 mm (600 rpm).

After spheronization, granules were dried in an oven at 60°C for 8 hours.

Screening of the Auxiliary Granules

A set of stainless steel laboratory sieves with a mesh size of 0.25 mm; 0.5 mm; 0.8 mm and 1.0 mm was used [29] to determine the particle size composition of the obtained granules and separation them into fractions.

Obtaining of the Tablet Cores of Multiple-Unit Composition

Compression of the tablet mass obtained from an active spheroids'mixture and auxiliary granules was performed on a rotary tablet press U& M-1000 (Shanghai Unique Machinery Technology, China) using biconvex punches with a diameter of 8 mm.

Determination of Tablet Cores' Friability

To test the friability, a sample of whole tablet cores weighing as close as possible to 6.5 g was taken. Before testing, the tablets were thoroughly dedusted, the sample was accurately weighed, and the tablets were placed in a drum. After 100 revolutions of the drum, the tablets were removed, the dust was removed and weighed accurately again [29].

Friability was calculated by the formula:

$$F = \frac{m_1 - m_2}{m_1} \cdot 100,$$

where F is the abrasion,%;
m_1 is the mass of the sample before the test, g;
m_2 is the mass of the sample after the test, g.

Dissolution Test

Dissolution test was carried out using Apparatus 1 (rotating basket; 100 rpm). To test 1 tablet or sample of the mixture of granules (which corresponds to 40 mg of the isosorbide dinitrate) were placed in glasses filled with 500.0 ml of water. After 1 hour of dissolution 10.0 ml of solution was selected from the center of the glass, filtered through a paper filter "blue ribbon", discarding the first portions of the filtrate. The sample was diluted 1:1 with water R. After 2, 4, 6, 8, 10 and 12 hours from the beginning of the dissolution, samples undergo process in a similar manner.

A standard isosorbide dinitrate solution preparation. A sample of the isosorbide dinitrate CRS equivalent to 0.050 g of 100% isosorbide dinitrate was placed into a 50.0 ml volumetric flask. Then 2/3 of flask was filled with methanol R2. It was kept in an ultrasonic bath for 10 minutes. The volume of flask was brought by methanol R2 to the mark and stirred. The resulting solution of 1.0 ml was transferred into a volumetric flask with a capacity of 25.0 ml, bring the volume of the solution with water R to the mark and mix.

Preparation of a buffer solution with a pH of 4.7. 15.4 g of ammonium acetate R was placed in a volumetric flask with a capacity of 1000.0 ml. 300.0 ml of water R, 11.5 ml of glacial acetic acid were added, mixed and brought the volume of the solution by water R to the mark. If necessary, the pH of the solution was adjusted potentiometrically with glacial acetic acid R.

The test solution of 50 µl and the standard isosorbide dinitrate solution of 50 µl were chromatographed on a liquid chromatograph with a UV detector (wavelength 210 nm), using a column of 150 mm x 4.6 mm in size. It was filled with LS-18 sorbent and mobile phase: water R was a buffer solution with pH 4.7 and methanol R2 (350: 100: 550) with a flow rate of 1.0 ml/min.

Comparison of Dissolution Profiles

To compare the dissolution profiles of matrix granules and tableted compositions, the difference factor f1 and the similarity factor f2 were calculated according to the formulas [30]:

$$f_1 = \frac{\sum_{i=1}^{n} |R_i - T_i|}{\sum_{i=1}^{n} R_i} \cdot 100;$$

$$f_2 = 50 \cdot log\left\{\left(1 + \frac{1}{n}\sum_{i=1}^{n} |R_i - T_i|^2\right)^{-0.5} \cdot 100\right\}.$$

where n is the number of time points; R_i is the amount of active substance transferred to the solution from the comparison drug at the i-th time point (on average, %); T_i is the amount of active substance transferred to the solution from the test drug at the i-th time point (on average, %).

The value of $f1$, which is in the range from 0 to 15, reflects the degree of difference between the two curves.

The value of $f2$, which is in the range from 50 to 100, indicates to a similar dissolution kinetics of drugs.

RESULT AND DISCUSSION

Dissolution Test Validation

For simplicity and convenience of research, water itself was chosen as the dissolution medium for evaluating the compositions [31]. This approach is acceptable, since the active ingredient and dosage form have a pH-independent solubility and do not have a significant effect on the properties of the aqueous medium themselves.

The method was validated according to ICH Q2 (R2) guidelines [32]. Validation parameters were

shown in the Table 1.

All evaluated validation parameters do not exceed the established acceptance criteria. Method is validated and suitable for analysis.

Table 1. Dissolution test validation parameters

Parameter		Acceptance criterion	Result
1		2	3
Suitability of the chromatographic system	Number of theoretical plates, N	≥ 750	7645
	Peak symmetry factor, A_s	0.8 – 1.5	1.35
	Relative standard deviation, RSD,%	≤ 1.19	0.096
Linearity	Free member of linear dependence, | a |: - statistical insignificance - practical insignificance	 ≤ 0.51 ≤ 1.01	0.2057
	Critical residual standard deviation, S_o	≤ 1.58	0.4150
	Correlation coefficient, R_c	≥ 0.9994	0.99996
Convergence and correctness	Relative confidence interval, Δ, % Systematic error, δ, %	≤ maxΔ_{As} =3.0 Criterion of statistical insignificance: $\delta \leq \Delta/3$ = 0.40	1.19 0.09
Intralab accuracy	Relative confidence interval, Δ_{intra} , %	≤ maxΔ_{As} =3.0	0.18
Limit of detection (LOD),%		≤ 0.04 mg/ml	0.88 % (0.000352 mg/ml)
Limit of quantitation (LOQ),%		≤ 0.04 mg/ml	2.68 % (0.001072 mg/ml)
Specificity	Effect of placebo components	Separation of additional peaks with the peak of isosorbide dinitrate	Additional peaks separated
		No additional peaks in placebo and solvent chromatograms with retention time matching peak of isosorbide dinitrate	No peaks
Specificity	Forced degradation study	Separation of additional peaks with a peak of isosorbide dinitrate in chromatograms of solutions subjected to forced degradation	Additional peaks separated
		On all chromatograms of placebo solutions subjected to forced decomposition, there are no peaks coinciding in retention time with the peak of the main substance	No peaks
Stability of solutions over time	Relative confidence interval, Δ_t , %; - standard solution (CRS); - test solution	≤ max δ = 0.96	 0.12 0.20
Prediction of the total uncertainty of the method, Δ_{As}, %	≤ maxΔ_{As} =3.0	1.37	
Robustness	Method robustness parameters: - change in the composition of the mobile phase; - change in the pH of the aqueous component of the mobile phase; - change in flow rate; - other manufacturer of column	Obtaining a reliable result and fulfilling the requirements of the "Chromatographic system suitability test".	The chromatographic system suitability test is met. The reliability of the results of the analysis with minor changes in the parameters of the method has been proven.

Selection of Excipients and Production of Protective Granules

Among the existing commercially available products [13] that provide the function of protecting

pellets with the active substance from destruction and uncontrolled dissolution, lactose-based compositions are of particular interest. On its basis, they are formed with high density, which are also well soluble in water. Such properties are ensured by obtaining a strong multi-dose tablet with rapid effective disintegration of active pellets.

The composition of the auxiliary granules was based on the existing product StarLac (Meggle, Germany) based on lactose and starch, which accelerates the disintegration of the composition due to its leavening properties additionally [34].

The extrusion-spheronization method was also chosen to obtain auxiliary granules that will have the physical and technological properties closest to the active spheroids. The technology considered the properties of the original lactose as the main component of the auxiliary granules (Table 2).

The degree of the surface active pellets' destruction depends on the the lactose particle size used in the auxiliary granules. Maximum damping functions of the particles are achieved using the smallest lactose crystals, so a special micronized brand Lactochem® Super Fine Powder was used in the composition. Povidone Kollidon 30 in the amount of 5% was used as a binder.

Colloidal silicon dioxide in the amount of 0.5% was included in the composition. That was done to ensure efficient mixing of dry components and to achieve the most uniform distribution of the used excipients' particles.

The obtained granules were dried, then the combined number of spheroids from 10 operations was scattered on laboratory sieves. The scattering results are shown in the table 3.

Table 2. Weights of the components for one extrusion-spheronization operation

Component	**g**	**%**
Lactose monohydrate	238.5	79.5
Corn starch	45.0	15.0
Colloidal silicon dioxide	1.5	0.5
Povidone	15.0	5.0
Purified water	135.0	
Together:	***335.0***	***100.0***

Table 3. Fractional composition of the obtained auxiliary granules

Fraction of granules, mm	**g**	**%**
< 0.25	10.65 ± 2.15	3.55 ± 0.73
0.25- 0.5	53.37± 5.04	17.79 ± 1.71
0.5 – 0.8	103.35 ± 7.49	34.45 ± 2.54
0.8 – 1.0	114.36 ± 7.28	38.12 ± 2.47
> 1.0	13.11 ± 2.39	4.37 ± 0.81
Together:	***294.84 ± 3.63***	***98.28 ± 1.23***

Mass Fraction Influence on Auxiliary Granules on Release Profile

In order to select the most acceptable parameters for as multiple-unit tablets 'compression of the isosorbide dinitrate with modified release, preparation of experimental compositions were investigated. A mixture of uncoated matrix granules were used for that, namely fraction 0.8-1.0 mm and coated matrix granules fraction 0.5-0.8 mm (isosorbide dinitrate content 36.3% of mass.), in a mass ratio of 1 : 2.5 [17].

The required calculated amount of the matrix granules, which provided the required amount of active pharmaceutical ingredient in the mixture to obtain tablets at a dosage of 40 mg, was mixed with the appropriate number of auxiliary granules in a drum mixer. Magnesium stearate (0.5%) was added to the tablet mass as a glidant.

Tablet cores of the compositions 1-6 were obtained by means of compression (force 6 kN) and

the release profiles of the active substance with a mixture of unpressed pellets were compared. The content of the compositions and the obtained results are shown in the table 4.

The results of the "Dissolution" test of the obtained compositions with different ratios of active and auxiliary granules are presented in the table 5 and figure 2.

Table 4. The content of the experimental tablet compositions

Comp. №	The ratio of the isosorbide dinitrate granules to auxiliary granules	Uncoated matrix granules, mg / tab.	Coated matrix granules, mg / tab.	Auxiliary granules, mg / tab.	Magnesium stearate, mg / tab.	Tablet core weight, mg
1	40:60	30.66	76.66	159.34	1.34	268 ± 5.7
2	45:55			129.49	1.19	238 ± 5.2
3	50:50			106.60	1.08	215 ± 4.5
4	55:45			86.70	0.98	195 ± 4.4
5	60:40			70.78	0.90	179 ± 3.8
6	65:35			56.85	0.83	165 ± 3.6

Table 5. Comparative results of the dissolution of the tablet cores' compositions and unpressed pellets

Dissolution time, h	% released isosorbide dinitrate						
	Unpressed mixture of matrix granules	Tablet compositions					
		№1 (40:60)	№2 (45:55)	№3 (50:50)	№4 (55:45)	№5 (60:40)	№6 (65:35)
0	0	0	0	0	0	0	0
1	19.6 ± 2.2	20.2 ± 3.1	20.8 ± 2.9	21.5 ± 3.4	22.1 ± 3.8	26.1 ± 3.5	30.1 ± 3.2
2	30.0 ± 2.2	31.2 ± 3.6	32.3 ± 4.1	33.5 ± 3.9	34.7 ± 4.0	41.4 ± 3.9	48.2 ± 4.0
4	49.4 ± 2.5	50.8 ± 4.4	52.2 ± 4.6	53.6 ± 5.2	55.0 ± 4.8	60.9 ± 5.1	66.9 ± 5.5
6	62.1 ± 3.5	63.2 ± 4.7	64.3 ± 4.3	65.4 ± 4.8	66.5 ± 4.9	72.6 ± 4.6	78.6 ± 5.4
8	72.8 ± 4.1	73.4 ± 5.1	74.1 ± 5.1	74.7 ± 5.4	75.4 ± 5.2	79.8 ± 5.3	84.3 ± 6.1
10	81.7 ± 4.6	82.0 ± 6.1	82.2 ± 6.3	82.5 ± 6.2	82.8 ± 5.8	85.8 ± 6.2	88.9 ± 6.4
12	89.8 ± 4.3	89.5 ± 5.4	89.3 ± 5.9	89.0 ± 5.4	88.8 ± 5.3	90.0 ± 5.3	91.3 ± 5.4
f_1	-	0.91	1.28	2.39	3.67	7.71	**15.17**
f_2	-	95.04	94.28	86.84	79.55	63.93	**49.88**

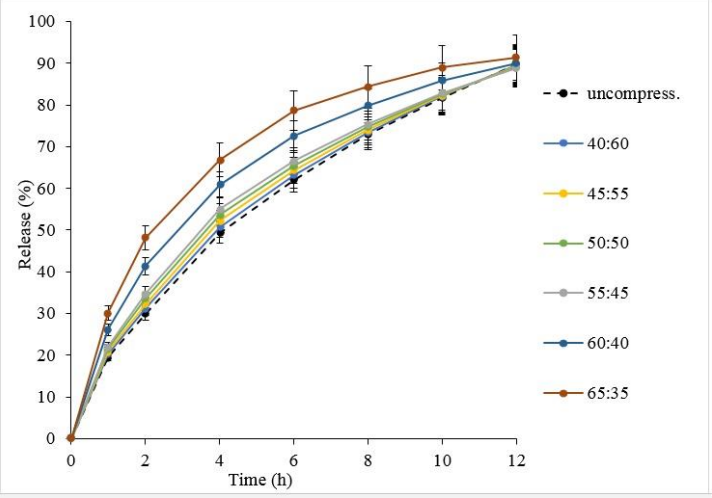

Figure 2. Dissolution profiles of tablet compositions and unpressed pellets

The ratio of active and auxiliary granules 55:45 (composition №4) is optimal because it does not lead to significant destruction of the matrix spheroids'surface and has virtually no effect on the dissolution profile of the multi-dose tablet. More reliable ratios (50:50, etc.) provide a similar result, but lead to a larger weight and size of the tablet. That is less rational from a technological and economic point of view.

Influence of Compression Force During Tabletting on the Release Profile

Composition №4 was used to study the effect of the compression force on the release profile of the isosorbide dinitrate from tablets. The tablet mass was compressed with forces from 2 to 15 kN. In addition to the dissolution kinetics, the abrasion strength of the obtained as multiple-unit tablets was investigated.

The test results and comparative data on the dissolution of the obtained tablet cores with the drug Cardiket Retard 40 mg are presented in the table 6 and on the figure 3.

Table 6. Comparative results of the dissolution of the tablet cores' composition №4 with different compression forces and the tablets Cardiket Retard 40 mg

Dissolution time, h	**% released isosorbide dinitrate**					
	Cardiket Retard 40 mg	**Pressing force, kN**				
		2	**5**	**8**	**12**	**15**
0	0	0	0	0	0	0
1	22.3 ± 2.0	22.1 ± 3.1	24.2 ± 3.8	26.3 ± 3.7	30.1 ± 3.2	32.9 ± 3.6
2	34.1 ± 2.7	34.7 ± 3.1	37.3 ± 3.4	40.0 ± 3.6	48.2 ± 3.3	54.2 ± 3.9
4	53.2 ± 2.6	55.0 ± 4.3	57.0 ± 4.4	59.1 ± 4.6	66.9 ± 4.2	69.0 ± 5.0
6	66.6 ± 3.2	66.5 ± 5.3	67.7 ± 5.3	68.8 ± 5.4	78.6 ± 5.5	77.9 ± 6.3
8	75.9 ± 3.6	75.4 ± 6.1	76.1 ± 5.9	76.9 ± 6.5	84.3 ± 6.2	82.4 ± 6.7
10	83.3 ± 4.1	82.8 ± 6.7	83.7 ± 6.6	84.6 ± 6.3	88.9 ± 6.8	86.2 ± 6.6
12	88.6 ± 4.7	88.8 ± 5.3	89.4 ± 6.1	90.0 ± 5.8	91.3 ± 5.4	87.9 ± 5.5
f_1	-	0.91	2.69	5.12	**15.17**	**16.01**
f_2	-	95.04	81.77	70.89	**49.88**	**46.66**
Abrasion, %		**>10**	**1.84**	0.59	0.15	0.12

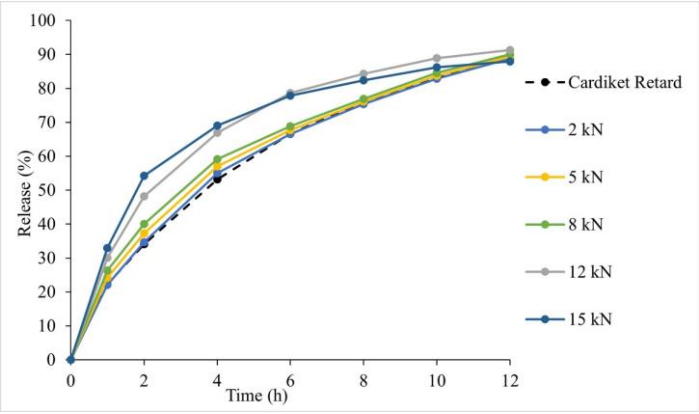

Figure 3. Comparison of the dissolution profiles' tablet cores and Cardiket Retard 40 mg

High-pressure tablet cores have satisfactory abrasion strength. But high pressure during tableting leads to partial destruction of the matrix granules' surface with the active pharmaceutical ingredient. The tablet cores' pressing with less effort prevents the destruction of the film coating, but the tablet cores have an unsatisfactory abrasion index. At a pressing force about 2 kN, the obtained tablets in the abrasion test were partially disintegrated into individual granules.

The selected composition of the protective granules under the found tableting conditions makes it possible to achieve the minimum effect of pressing on the dissolution profile of the pellets with the active component. This is ensured, on the one hand, by the acceptable cushioning properties of the auxiliary particles, and, on the other hand, by their disintegration characteristics. Rapid disintegration of the tablet into individual subunits results in dissolution similar to non-compressed pellets.

Of course, all the difficulties associated with compressing pellets could be circumvented by filling hard gelatin capsules. However, tablets are the preferred oral form because they are less expensive and less difficult to manufacture and are more acceptable to patients. A multi-dose tablet can be divided without compromising the release profile of the drug from the individual units. Such formulation features provide fewer side effects, improved bioavailability, and less variability in drug absorption.

The original prolonged preparation of isosorbide dinitrate Cardiket retard is a matrix tablet and, therefore, has all the usual disadvantages of monolithic dosage forms. Tablet release modification is achieved by hot melting of isosorbide dinitrate with a polymer and other functional excipients, which is a very specific and time-consuming technology.

Based on this, obtaining a multi-dose form of prolonged action is an urgent and promising task. The use of a combination of granules with different release kinetics provides a dissolution profile similar to the matrix tablet. At the same time, the multi-dose tablet technology provides a predictable release of the active ingredient and a consistent safety and efficacy profile of the antianginal drug.

The tablet composition obtained at a compression force about 8 kN has a satisfactory abrasion strength, and also provides the most similar release profile of the isosorbide dinitrate to the reference drug.

Summing up, a laboratory technology of the multi-dose isosorbide dinitrate tablet cores production has been developed. The optimal mass ratio of active pellets and auxiliary granules for tableting was established. The influence of the compression force of the tablet cores on the release profile of the active substance has been studied.

Invented technological conditions and parameters allow to obtain the required nature of the isosorbide dinitrate tablets dissolution, similar to the reference drug.

Generally, the approach of combining uncoated and coated spherical matrix granules makes it possible to ensure the controlled release of the active ingredient from the dosage form and to achieve its compliance with any target prototype.

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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

NEW SULFONAMIDO-BENZOXAZOLE DERIVATIVES AS ANTIMICROBIAL AGENTS: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

ANTİMİKROBİYAL AJAN OLARAK YENİ SÜLFONAMİDO-BENZOKSAZOL TÜREVLERİ: TASARIM, SENTEZ VE BİYOLOJİK DEĞERLENDİRME

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ABSTRACT

Objective: *Many investigations are conducted in the battle against infectious diseases in order to develop new drug-active ingredient candidate compounds and to identify leading compounds. The goal of this study was to synthesis a total of seven compounds, six of which are novel, with the general structure 2-(4-tert-butylphenyl)-5-(4-substitutedphenylsulfonamido)benzoxazole, to elucidate their structures, and to test their antimicrobial activities using the microdilution method.*
Material and Method: *The synthesis of the compounds was carried out in two stages. In the first stage, under PPA catalyst 2,4-diaminophenol and 4-tert-butylbenzoic acid were refluxed, and target compounds were produced in the second step by reacting 4-substitutedbenzenesulfonyl chloride with 5-Amino-2-(4-tert-butylphenyl)benzoxazole. The compounds' antimicrobial activity was determined by using Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and drug-resistant strains of these microorganisms in vitro antimicrobial activity studies. Furthermore, estimated ADME profiles were calculated using the SwissADME online software.*
Result and Discussion: *The structures of the synthesized compounds were elucidated using ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy, and also their melting points were determined. The antimicrobial activities of the compounds ranged from 64 µg/ml to >512 µg/ml and were weaker than the reference drugs. The best antimicrobial activity was reported against an isolate of E. faecalis, with all compounds having MIC values of 64 µg/ml. The fact that six of the seven synthesized compounds are novel and that their antimicrobial activity will be tested for the first time will make a significant contribution to studies to develop new or alternative antimicrobial agents.*
Keywords: *ADME, antimicrobial activity, benzoxazole, sulfonamide*

ÖZ

Amaç: *Bulaşıcı hastalıklarla mücadelede yeni ilaç-etkin madde aday bileşikleri geliştirmek ve öncü bileşiklere ulaşmak için birçok araştırma yapılmaktadır. Bu çalışmanın amacı, genel yapısı 2-(4-tert-bütilfenil)-5-(4-sübstitüefenilsülfonamido)benzoksazol olan altısı yeni olmak üzere toplam yedi bileşiğin yapılarını aydınlatmak ve mikrodilüsyon yöntemini kullanarak antimikrobiyal*

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aktivitelerini test etmektir.
Gereç ve Yöntem: *Bileşiklerin sentezi iki aşamada gerçekleştirilmiştir. İlk basamakta, PPA katalizörü altında 2,4-diaminofenol ve 4-tert-bütilbenzoik asit refluks edilmiş ve ikinci aşamada 4-sübstitüebenzensülfonil klorürün 5-Amino-2-(4-tert-bütilfenilbenzoksazol ile reaksiyona sokulmasıyla hedef bileşikler üretilmiştir. Bileşiklerin antimikrobiyal aktivitesi, Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans ve bu mikroorganizmaların ilaca dirençli suşları kullanılarak in vitro antimikrobiyal aktivite çalışmaları ile belirlenmiştir. Ayrıca, SwissADME çevrimiçi yazılımı kullanılarak tahmini ADME profilleri hesaplanmıştır.*
Sonuç ve Tartışma: *Sentezlenen bileşiklerin yapıları, ^{1}H-NMR, ^{13}C-NMR ve Kütle spektroskopisi kullanılarak aydınlatıldı ve ayrıca erime noktaları belirlenmiştir. Bileşiklerin antimikrobiyal aktiviteleri 64 µg/ml ila >512 µg/ml aralığındaydı ve referans ilaçlardan daha zayıftı. En iyi antimikrobiyal aktivite, tüm bileşiklerin 64 µg/ml MİK değerlerine sahip olduğu bir E. faecalis izolatına karşı rapor edilmiştir. Sentezlenen yedi bileşikten altısının yeni olması ve antibakteriyel aktivitelerinin ilk kez test edilecek olması, yeni veya alternatif antimikrobiyal ajanların geliştirilmesine yönelik çalışmalara önemli katkı sağlayacaktır.*
Anahtar Kelimeler: *ADME, antimikrobiyal aktivite, benzoksazol, sülfonamid*

INTRODUCTION

Bacterial and fungal diseases occur all over the world and cause quite large epidemics [1]. Unicellular organisms such as bacteria and fungi live in harmony in the bodies of multicellular organisms such as humans due to their natural habitats. When this balance is disturbed, opportunistic bacteria that take advantage of this situation cause infection [2,3]. Furthermore, drug-induced or microorganism-induced resistance of various phenotypes that develop in situations such as unnecessary drug use and multiple drug use, misdiagnosis, incorrect treatment methods, prescriptions written only to eliminate symptoms, and patients' abandonment of their treatment make fighting infectious diseases difficult [4-9]. Recent studies revealing mortality and morbidity rates increase the concern about infection cases, and microbial resistance developed by bacteria and fungi is added to this concern [10,11]. Because of the rapid development of antimicrobial drug resistance, researchers have been working hard to produce new and effective medications having these effects and agree that generating novel compounds with different structures than existing medications can help to prevent the development of resistance. For this reason, research is carried out on many compounds with different structures [12,13].

Today, benzoxazole derivative compounds are an important group on which antimicrobial activity studies are carried out intensively [14]. Compounds with the benzoxazole ring system are employed as active medicinal components in treatment due to their anticancer [15], anti-inflammatory [16], antioxidant [17], anti-alzheimer [18], and anticonvulsant [19]. Because of its structural resemblance to adenine and guanine bases, the benzoxazole ring is predicted to interact more easily with biopolymers in biological systems. So far, substitutions at the second position of the ring have been thoroughly studied, yielding a wealth of information on their structural properties and impacts.

In some previous studies, 5-ethylsulfonyl-2-(4-substitutedphenyl and/or substituted benzyl and/or phenylethyl)benzoxazole derivative compounds were synthesized, their antimicrobial activities were examined, and promising results were obtained [20]. In light of this information, the design and synthesis of compounds with the general formula 2-(4-tert-butylphenyl)-5-(4-substitutedphenylsulfonamido)benzoxazole were carried out within the scope of the study, and their structures were proven using ^{1}H-NMR, ^{13}C-NMR, and Mass spectroscopy analysis methods. The antimicrobial activities of the compounds were evaluated using Minimum Inhibitory Concentration (MIC). Finally, the estimated ADME parameters were calculated.

MATERIAL AND METHOD

Chemistry

All chemicals used in this research were purchased from Sigma Aldrich, Merck, Riedel de Haen,

and Fluka. Varian Mercury 400 MHz High-Performance Digital FT-NMR spectrometer (Palo Alto, CA, USA) was used to record the ^{1}H- and ^{13}C Nuclear Magnetic Resonance spectra. TMS was utilized as an internal standard in this spectrometer, while deuterated chloroform ($CDCl_3$) was used as a solvent. Melting points were determined using an electrothermal device, and the results were not corrected. On a Waters 2695 Alliance Micromass ZQ LC/MS spectrometer (Milford, MA, USA), mass analyses were done using the Electrospray Ionization (ESI) (+) method. In this investigation, 7 compounds were synthesized, 6 of which were novel.

General Synthesis of N2-N8

Firstly, 1 mmol 2,4-diaminophenol dihydrochloride and 1 mmol 4-tert-butyl benzoic acid were reacted at 160-190°C for around 3 h in the presence of polyphosphoric acid (PPA). At the end of the reaction, the product was poured into ice and stirred. After thoroughly mixing, it was neutralized with 10% NaOH and filtered. Finally, it was cleaned with activated charcoal and crystallized from ethanol-water. This compound (**N1**) obtained is not original [14]. The reaction of 5-Amino-2-(4-tert-butylphenyl)benzoxazole with 4-substitutedbenzenesulphonyl chloride in the presence of dichloromethane and pyridine yielded **N2-N8** in the second stage (Figure 1). Crystallization from ethanol-water was used to purify the desired products. **N2** is a commercially available product that is not original.

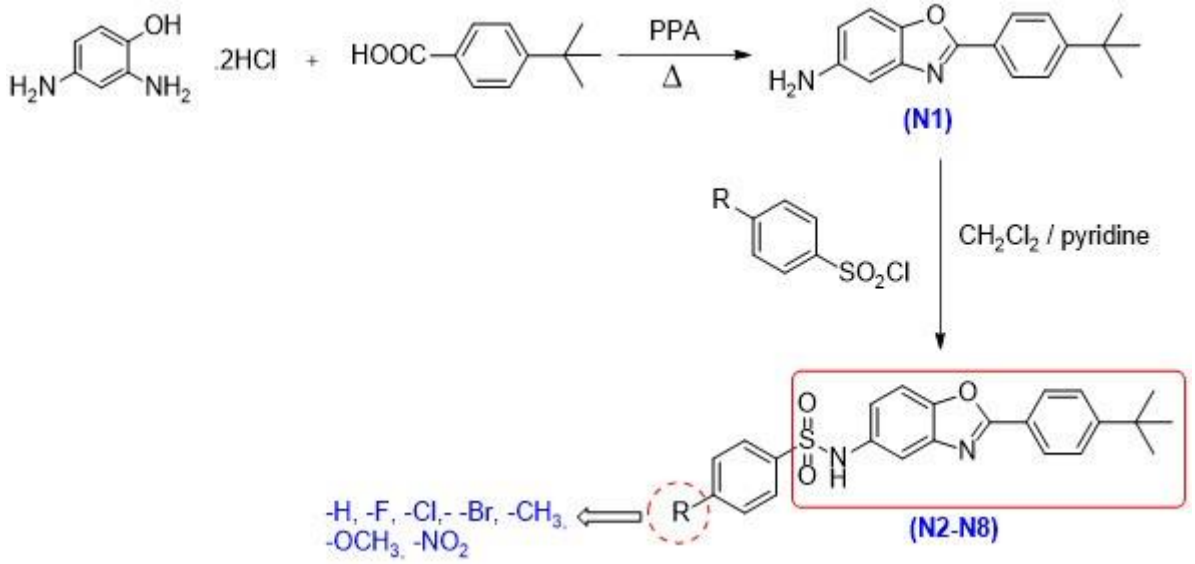

Figure 1. Synthesis of 2-(4-tert-butylphenyl)-5-(4-substitutedphenylsulfonamido)benzoxazole derivatives

Antimicrobial Activity

As in the previous investigations, the *in vitro* antimicrobial activity of compounds was appointed using the microdilution method as the minimal inhibitory concentration [14,21]. Standard strains and clinical isolates of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *Candida albicans* ATCC 10231 were employed. Standard strains and clinical isolates were obtained from the Trakya University Faculty of Medicine Microbiology Laboratory.

***In Silico* ADME Prediction**

The SwissADME web server as used to calculate some critical ADME characteristics, such as the physicochemical and pharmacokinetic features of the drugs. Some parameters of the compounds such as physicochemical properties, lipophilicity, water solubility, pharmacokinetic properties, and drug similarity were estimated.

RESULT AND DISCUSSION

Chemistry

The **N2-N8** series was synthesized in our work following the procedure described in the literature, as shown in Figure 1 [22,23]. Initially, 5-Amino-2-(4-tert-butylphenyl)benzoxazole was obtained by reaction of 2,4-diaminophenol with 4-tert-butylbenzoic acid in PPA. The target compounds were then synthesized by reacting 4-substitutedbenzenesulfonyl chlorides with 5-Amino-2-(4-tert-butylphenyl)benzoxazole. After TLC and melting point were used to determine the purity of the synthesized compounds, their structures were determined using ^{1}H-NMR and ^{13}C-NMR and Mass spectroscopy. When the compounds' ^{1}H-NMR spectra were investigated, aromatic protons were observed in the range of 7.20-8.26 ppm, tert-butyl protons as a 9H singlet at 1.36-1.37 ppm, and methoxy proton as a singlet at 3.81 ppm. The ^{13}C-NMR spectra revealed aliphatic carbon in the 21.53-55.53 ppm range and aromatic carbon in the 110.44-166.54 ppm range. Electrospray ionization positive (ESI+) method was used for mass spectral analysis and their relative intensities as (M+H)+ ions were easily monitored. In all compounds, a M+H peak was identified in the mass spectrum analysis. M+H+2 and M+H+4 peaks were also found in compounds containing elements with isotope abundances that were reasonably close or equal, such as bromine and chlorine.

2-(4-Tert-butylphenyl)-5-(phenylsulfonamido)benzoxazole (N2)

Yield: 75%, mp: 225-226°C**.** ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 1.36 (9H, s), 6.97 (1H, s), 7.11-7.14 (1H, dd, J_m=1.6, J_o=8.6), 7.40-7.45 (4H, m), 7.50-7.54 (3H, m), 7.78 (2H, d, J_o=7.2), 8.12 (2H, d, J_o=8.8). ^{13}C -NMR (100 MHz, $CDCl_3$, δ): 31.11, 35.10, 110.87, 114.45, 120.88, 123.88, 125.97, 127.31, 127.52, 129.08, 132.98, 133.07, 138.78, 142.78, 148.86, 155.56, 164.49. MS (ESI+) m/z: 407.2 (M+H)(100).

2-(4-Tert-butylphenyl)-5-(4-fluorophenylsulfonamido)benzoxazole (N3)

Yield: 55%, mp: 195-198°C**.** ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 1.36 (9H, s), 6.99 (1H, s), 7.07-7.13 (3H, m), 7.43-7.47 (2H, m), 7.53 (2H, d, J_o=8.8), 7.77-7.80 (2H, m), 8.12 (2H, d, J_o=8.4). ^{13}C -NMR (100 MHz, $CDCl_3$, δ): 31.11, 35.11, 110.98, 114.56, 116.26, 116.49, 120.93, 123.80, 125.99, 127.55, 130.04, 130.14, 132.75, 142.83, 148.96, 155.65, 164.00, 164.61, 166.54. MS (ESI+) m/z: 425.5 (M+H)(100).

2-(4-Tert-butylphenyl)-5-(4-chlorophenylsulfonamido)benzoxazole (N4)

Yield: 60%, mp: 229-230°C**.** ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 1.37 (9H, s), 6.96 (1H, s), 7.10-7.13 (1H, dd, J_m=2.4, J_o=8.8), 7.39 (2H, d, J_o=8.4), 7.43-7.47 (2H, m), 7.46 (1H, d, J_o=8.4), 7.53 (2H, d, J_o=8.4), 7.69 (2H, d, J_o=8.8), 8.13 (2H, d, J_o=8.4). ^{13}C -NMR (100 MHz, $CDCl_3$, δ): 31.11, 35.12, 111.02, 114.61, 120.95, 123.79, 126.00, 127.56, 128.76, 129.40, 132.61, 137.24, 139.68, 142.85, 149.00, 155.67, 164.64. MS (ESI+) m/z: 441.2 (M+H)(100), 443.3 (33).

2-(4-Tert-butylphenyl)-5-(4-bromophenylsulfonamido)benzoxazole (N5)

Yield: 50%, mp: 224-226°C**.** ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 1.36 (9H, s), 6.99 (1H, s), 7.10-7.12 (1H, dd, J_m=2, J_o=8.8), 7.44-7.47 (2H, m), 7.52-7.56 (4H, m), 7.62 (2H, d, J_o=8.8), 8.12 (2H, d, J_o=8.4)**.** ^{13}C -NMR (100 MHz, $CDCl_3$, δ): 31.11, 35.12, 111.03, 114.59, 120.94, 123.79, 126.00, 127.56, 128.22, 128.83, 132.39, 132.59, 137.78, 142.85, 149.00, 155.66, 164.64. MS (ESI+) m/z: 485.2 (M+H)(100), 487.2 (90).

2-(4-Tert-butylphenyl)-5-(4-methylphenylsulfonamido)benzoxazole (N6)

Yield: 70%, mp: 227-229°C**.** ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 1.37 (9H, s), 2.36 (3H, s), 6.92 (1H, s), 7.13-7.15 (1H, dd, J_m=2, J_o=8.4), 7.21 (2H, d, J_o=8.4), 7.41-7.45 (2H, m), 7.53 (2H, d, J_o=8.8), 7.66 (2H, d, J_o=8.4), 8.12 (2H, d, J_o=8.4).^{13}C -NMR (100 MHz, $CDCl_3$, δ): 21.53, 31.11, 35.10, 110.84, 114.26, 120.81, 123.89, 125.97, 127.35, 127.51, 129.70, 133.18, 135.81, 142.72, 143.93, 148.77, 155.53, 164.43. MS (ESI+) m/z: 421.5 (M+H)(100).

2-(4-Tert-butylphenyl)-5-(4-methoxyphenylsulfonamido)benzoxazole (N7)

Yield: 65%, mp: 206-207°C**.** ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 1.37 (9H, s), 3.81 (3H, s), 6.77 (1H, s), 6.87 (2H, d, J_o=9.2), 7.12-7.14 (1H, dd, J_m=2, J_o=8.4), 7.40-7.46 (2H, m), 7.53 (2H, d, J_o=8.8), 7.70 (2H, d, J_o=9.2), 8.13 (2H, d, J_o=8.4). ^{13}C -NMR (100 MHz, $CDCl_3$, δ): 22.68, 35.10, 55.53, 110.83, 114.22, 114.32, 120.87, 123.90, 125.97, 127.51, 129.50, 130.33, 133.25, 142.75, 148.78, 155.53, 163.14, 164.44. MS (ESI+) m/z: 437.4 (M+H)(100).

2-(4-Tert-butylphenyl)-5-(4-nitrophenylsulfonamido)benzoxazole (N8)

Yield: 45%, mp: 239-240°C**.** ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 1.37 (9H, s), 6.96 (1H, s), 7.10-7.13 (1H, dd, J_m=2, J_o=8.8), 7.44-7.49 (2H, m), 7.53 (2H, d, J_o=8.4), 7.93 (2H, d, J_o=8.4), 8.12 (2H, d, J_o=8.4), 8.26 (2H, d, J_o=8.8). ^{13}C -NMR (100 MHz, $CDCl_3$, δ): 31.08, 35.11, 111.19, 115.03, 121.14, 123.66, 124.28, 126.01, 127.61, 128.60, 131.90, 143.03, 144.54, 149.32, 150.31, 155.85, 164.91. MS (ESI+) m/z: 452.4 (M+H)(100).

Antimicrobial Evaluation

The MIC (μg/ml) values for the antimicrobial activity of the synthesized compounds and reference drugs were given in Table 1. When the antimicrobial activities of the synthesized compounds were evaluated as MIC values, they were found in the range of 64 ->512 μg/ml . According to the study's results, the best antimicrobial activity was observed against *E. faecalis* isolate (VREF), and all compounds showed MIC value of 64 μg/ml. The synthesized compounds have MIC of 128 μg/ml against *S. aureus* and its isolate (MRSA), *E. faecalis, E. coli* and its isolate, *P. aeruginosa* and its isolate. The antifungal activities of the compounds against *C. albicans* were >512 μg/ml, and were much less effective than amphotericin B (0.5 μg/ml). As a result, it was shown that the compounds in the study showed moderate antibacterial, and very low antifungal activity and the change of substituents had no effect on the activity. These derivatives will make an essential addition to research to develop new or alternative antibacterial agents due to the uniqueness of the compounds in our study and the first evaluation of their antimicrobial activity.

Table 1. *In vitro* antimicrobial MIC values (μg/ml) of **N2-N8** and reference drugs

Compound	S. a.	S.a.*	E.f.	E.f. *	E. c	E. c*	P. a	P. a*	C. a
N2	128	128	128	64	128	128	128	128	>512
N3	128	128	128	64	128	128	128	128	>512
N4	128	128	128	64	128	128	128	128	>512
N5	128	128	128	64	128	128	128	128	>512
N6	128	128	128	64	128	128	128	128	>512
N7	128	128	128	64	128	128	128	128	>512
N8	128	128	128	64	128	128	128	128	>512
Vancomycin	<0,0625	<0,0625	<0,0625	>8 (R)	-	-	-	-	-
Ampicillin	2	>8 (R)	2	>8 (R)	2	2	-	-	-
Meropenem	<0,0625	>8 (R)	-	-	<0.0625	<0.0625	0.5	0.5	-
Ciprofloxacin	0,5	0,5	2	2	<0.0625	<0.0625	1	2 (R)	-
Gentamicin	1	2 (R)	-	-	0.25	0.25	0.5	>8 (R)	-
Amphotericin B	-	-	-	-	-	-	-	-	0.5

S. a: *Staphylococcus aureus* ATCC 29213; S. a.*: Methicillin-Resistant *Staphylococcus aureus* (MRSA); E.f. : *Enterococcus faecalis* ATCC 29212; E.f.*: Vancomycin resistant *E. faecalis* (VREF); E. c.: *Escherichia coli* ATCC 25922; E.c.*: *E. coli* isolate; P.a.: *Pseudomonas aeruginosa* ATCC 27853; P.a.*: *P. aeruginosa* isolate; C.a.: *Candida albicans* ATCC 1023. **(R)**: Resistant (according to CLSI and EUCAST)

***In Silico* ADME Calculation**

Data on ADME properties should be supplied as early as possible in the drug research process to identify prospective candidates. Many drug candidates fail early in the drug development process due to poor ADME characteristics such as inadequate absorption and extensive first-pass metabolism. By identifying the best drug development candidates and excluding those who are unlikely to succeed, early

assessment of ADME features minimizes screening, trial time, and costs. It also offers the information required for proper dosage forms and formulation. Predicting ADME features in silico is therefore critical for drug research and development. As a result, some of the compounds' physicochemical properties were determined using the SwissADME program, and these guidelines, as well as the compliance of the synthesized compounds with these rules, are shown in Table 2. The compounds' molecular weights ranged between 406.5 and 485.39 g/mol. All compounds had poor water solubility. There was low GI absorption and BBB permeability. It obeyed all the limiting rules of Lipinski and Veber. PAINS, Brenk, and Leadlikeness values were suitable for all compounds, and synthetic accessibility was in the easy class.

Table 2. Calculated SwissADME parameters of **N2-N8**

	N2	N3	N4	N5	N6	N7	N8
Physicochemical Properties							
Molecular weight	406.50	424.49	440.94	485.39	420.52	436.52	451.49
Num. heavy atoms	29	30	30	30	30	31	32
Num. arom. heavy atoms	21	21	21	21	21	21	21
Num. rotatable bonds	5	5	5	5	5	6	6
Num. H-bond acceptors	4	5	4	4	4	5	6
Num. H-bond donors	1	1	1	1	1	1	1
Molar Refractivity	115.83	115.79	120.84	123.53	120.79	122.32	124.65
TPSA	80.58	80.58	80.58	80.58	80.58	89.81	126.40
Lipophilicity							
Log Po/w(iLOGP)	3.70	3.63	3.23	3.90	3.76	3.94	3.07
Log Po/w(XLOGP3)	5.62	5.72	6.25	6.31	5.98	5.59	5.45
Log Po/w(WLOGP)	6.48	7.04	7.14	7.25	6.79	6.49	6.39
Log Po/w(MLOGP)	3.60	3.97	4.08	4.18	3.54	3.00	2.69
Log Po/w(SILICOS-IT)	4.28	4.70	4.92	4.96	4.81	4.35	2.12
Consensus Log Po/w	4.74	5.01	5.12	5.32	4.98	4.67	3.94
Water Solubility							
Log S (ESOL)	-6.11	-6.26	-6.70	-7.01	-6.40	-6.17	-6.16
Class	Poorly	Poorly	Poorly	Poorly	Poorly	Poorly	Poorly
Log S (Ali)	-7.08	-7.18	-7.73	-7.79	-7.45	-7.24	-7.86
Class	Poorly	Poorly	Poorly	Poorly	Poorly	Poorly	Poorly
Pharmacokinetics							
GI absorption	Low	Low	Low	Low	Low	Low	Low
BBB permeant	No	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	No	No	No	No	No	No
CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	Yes	Yes	Yes	Yes	No	Yes	No
CYP3A4 inhibitor	Yes	Yes	Yes	No	Yes	Yes	Yes
Log Kp	-4.79 cm/s	-4.83 cm/s	-4.55 cm/s	-4.78 cm/s	-4.62 cm/s	-4.99 cm/s	-5.18 cm/s
Druglikeness							
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ghose	No	No	No	No	No	No	No
Veber	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Egan	No	No	No	No	No	No	No
Muegge	No	No	No	No	No	No	No
Medicinal Chemistry							
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	2 alerts
Leadlikeness	No	No	No	No	No	No	No
Synthetic accessibility	3.52	3.50	3.52	3.55	3.64	3.58	3.67

The study attempts to find new drug-active candidate compounds to fight infectious diseases or to reveal the investigations required to find the leading compounds. As a result, 7 sulfonamido-benzoxazole derivative compounds, 6 of which were novel, were developed, and synthesized in two steps, their structures validated by ^{1}H-NMR, ^{13}C-NMR, and Mass spectroscopy, and their antibacterial properties were tested on 9 distinct microorganisms. Standard strains of nosocomial infectious pathogens and drug-resistant isolates in people were used to create the test microorganisms. The synthesized compounds were shown to have lower antibacterial activity than the reference drugs. In

addition, some estimated ADME parameters have been calculated and are estimated to show low gastrointestinal absorption, although generally complying with the limiting rules. The compounds are essential as they are newly synthesized and tested for the first time against selected bacterial, fungal, and clinical isolates. The development of resistance against existing treatment methods will significantly contribute to the studies conducted to develop new or alternative drugs with promising antimicrobial activities.

AUTHOR CONTRIBUTIONS

Concept: M.E., C.A., Ö.T.A.; Design: M.E., C.A., Ö.T.A.; Control: M.E., C.A., G.K., A.S.S., Ö.T.A.; Sources: M.E., C.A., G.K., A.S.S., Ö.T.A.; Materials: M.E., C.A., G.K., A.S.S., Ö.T.A.; Data Collection and/or Processing: M.E., C.A., G.K., A.S.S., Ö.T.A.; Analysis and/or Interpretation: M.E., C.A., G.K., A.S.S., Ö.T.A.; Literature Review: M.E., C.A., G.K., A.S.S., Ö.T.A.; Manuscript Writing: M.E., C.A., G.K., A.S.S., Ö.T.A.; Critical Review: M.E., C.A., G.K., A.S.S., Ö.T.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

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

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ÖZGÜN MAKALE / ORIGINAL ARTICLE

RUŞEYM YAĞININ DİYABETE BAĞLI TESTİKÜLER HASAR ÜZERİNDEKİ ETKİSİNİN OKSİDATİF STRES PARAMETRELERİ YÖNÜNDEN İNCELENMESİ

INVESTIGATION OF THE EFFECT OF WHEAT GERM OIL ON DIABETES-RELATED TESTICULAR DAMAGE IN TERMS OF OXIDATIVE STRESS PARAMETERS

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ÖZ

Amaç: *Ruşeym yağının (RY) diyabetin neden olduğu testiküler hasar üzerinde oksidatif stres aracılı etkisinin araştırılması amaçlanmıştır.*
Gereç ve Yöntem: *42 erkek Wistar albino sıçan randomize 6 gruba ayrılmıştır: kontrol, kontrol düşük doz (100 mg/kg/gün), kontrol yüksek doz (1000 mg/kg/gün), diyabet kontrol, diyabet düşük doz (100 mg/kg/gün), diyabet yüksek doz (1000 mg/kg/gün). Diyabet ve kontrol gruplarına 28 gün süre ile gavajla RY uygulanmıştır. Her hafta kan glukoz düzeyleri ölçülmüştür. Ötenazinin ardından testis dokuları çıkartılmıştır. Dokular homojenize edilmiş ve Bradford yöntemi ile total protein düzeyleri ölçülmüştür. Lipit peroksidasyonunun göstergesi olarak dokularda MDA düzeyleri, oksidatif stresin göstergesi olarak glutatyon düzeyleri ölçülmüştür.*
Sonuç ve Tartışma: *Kontrol grubunda hem düşük, hem de yüksek doz RY uygulaması rölatif testis ağırlığında azalmaya neden olmuştur. Diyabetik yüksek doz RY grubunun rölatif testis ağırlığı diyabet kontrol grubuna göre azalmıştır. Kan glukoz düzeyleri ve vücut ağırlıklarında RY'ye bağlı olarak anlamlı farklılık görülmemiştir ($p>0.05$). Diyabetik hayvanlar arasında en düşük testis MDA düzeyleri diyabet yüksek doz grubunda bulunmuştur. Kontrol düşük doz grubunda glutatyon düzeyleri artmıştır. Ancak gruplar arasında MDA ve glutatyon düzeyi bakımından anlamlı farklılık bulunmamıştır ($p>0.05$). Halk arasında kullanımı ve antioksidan aktivitesi olan RY'nin seksüel hormonlar ve diğer moleküler yolaklar üzerindeki etkisinin araştırılacağı yeni çalışmalara ihtiyaç vardır.*
Anahtar Kelimeler: *Buğday ruşeymi, oksidatif stres, ruşeym yağı, testiküler hasar*

ABSTRACT

Objective: *Our study aimed to investigate the oxidative stress-mediated effect of wheat germ oil (WGO) on testicular damage caused by diabetes.*
Material and Method: *42 male Wistar albino rats were randomly divided into 6 groups: control, control low-dose (100 mg/kg/day), control high-dose (1000 mg/kg/day), diabetes control, diabetes low-dose (100 mg/kg/day), diabetic high-dose (1000 mg/kg/day). RY/carrier cornoil was applied to the diabetes and control groups by gavage for 28 days. Blood glucose levels were measured every week. Following euthanasia, testicular tissues were removed. Tissues were homogenized and total protein levels were measured by Bradford method. MDA levels were measured in tissues as an*

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indicator of lipid peroxidation, and glutathione levels were measured as an indicator of oxidative stress.

Result and Discussion: *Both low and high dose WGO administration in the control group caused a decrease in relative testicular weight. The relative testicular weight of the high dose diabetic group decreased compared to the diabetic control group. There was no significant difference in blood glucose levels and body weights depending on WGO ($p>0.05$). Among diabetic animals, the lowest testicular MDA levels were found in the high -dose group. Glutathione levels increased in the control low-dose group. However, there was no significant difference between the groups in terms of MDA and glutathione levels ($p>0.05$). Further research is needed to investigate the effectiveness of WGO, which has a traditionally usage and antioxidant activity, on sexual hormones and other molecular pathways.*

Keywords: *Germ oil, oxidative stress, testicular damage, wheat germ*

GİRİŞ

Diyabet, pankreasın yeterli insülin üretememesi ya da üretilen insülinin vücut tarafından kullanılamamasıyla ortaya çıkan; zamanla kalpte, kan damarlarında, gözlerde, böbreklerde, sinir sistemi ve üreme sisteminde ciddi hasarlara yol açabilen, yüksek kan glukoz düzeyleri ile karakterize edilen, kronik, metabolik bir hastalıktır [1]. Türkiye Endokrinoloji ve Metabolizma Derneği diyabeti, insülin eksikliği ya da periferik dokularda insülin etkisine karşı gelişmiş olan, "insülin direnci" nedeniyle ortaya çıkan, organizmanın karbonhidrat, yağ ve proteinlerden yeterince yararlanamadığı, pek çok organı etkileyerek multisistemik tutuluma neden olan, hiperglisemi ile karakterize, sürekli tıbbi bakım gerektiren, kronik ve geniş spekturumlu bir metabolizma hastalığı olarak tanımlamaktadır [2]. Uluslararası Diyabet Federasyonu (International Diabetes Federation, IDF) 2021 yılı verilerine göre dünya genelinde yaklaşık 537 milyon yetişkin (20-79 yaş) diyabet hastası bulunmaktadır. Bu sayının 2030 yılında 643 milyon, 2045 yılında 783 milyon olacağı tahmin edilmektedir [3]. 2022 IDF verileri Türkiye'de erişkinlerin (20-79 yaş) %15.9'unun diyabet hastası olduğunu ve bu sayının yaklaşık 9 milyon hastayı ifade ettiğini göstermektedir [4].

Diyabet; tipleri, nedenleri ve altında yatan mekanizmalara göre tip 1 diyabet (T1D), tip 2 diyabet (T2D), diğer spesifik diyabet tipleri, gestasyonel diyabet (GDM), bozulmuş glukoz toleransı (BGT) ve bozulmuş açlık glukozu (BAG) olarak sınıflandırılabilmektedir [2]. Vücutta insülin üretiminin yetersizliği ile karakterize bir sendrom olan T1D; insülin bağımlı, çocukluk çağında başlayan jüvenil diyabettir [1,2]. T2D insülin bağımlı olmayan ya da yetişkinlik döneminde başlayan diyabet olarak bilinmektedir. Uzun yıllar boyunca T2D'nin sadece yetişkinlerde görüldüğü düşünülmüş, ancak son zamanlarda çocukluk çağında da ortaya çıkabileceği saptanmıştır. T2D, vücudun bünyesindeki insülini etkili bir şekilde kullanamamasıyla ilişkili bir hastalıktır [1,2]. Diğer spesifik diyabet tipleri β-hücre fonksiyonlarının genetik defekti (monogenik diyabet formları), insülin etkisindeki genetik defektler, diğer genetik defektler, pankreasın ekzokrin doku hastalıkları, endokrinopatiler, ilaç veya kimyasal ajanlar veya immun aracılı nadir diyabet formlarıdır. GDM, gebelikte değişen fizyolojik koşullara bağlı olarak ortaya çıkan, uzun dönemde T2D riski taşıyan bir hastalıktır. Kan glukoz düzeylerinin normal sınırların üzerinde, ancak diyabet tanısı konamayacak kadar düşük düzeyde olduğu durum olarak ifade edilebilir. GDM'li gebelerde ve doğacak bebeklerinde komplikasyon görülme riski artmaktadır [1,2]. BGT ve BAG kan glukoz düzeyleri normalin üstünde olmasına rağmen, diyabet tanısı konulacak kadar yükselmemesi durumudur. Bazı bireylerde diyabete geçiş dönemi olarak değerlendirilir. BGT ve BAG olan bireylerde kalp krizi ve felç riskinin arttığı bilinmektedir [1,2].

Kontrol altında tutulamayan diyabet kişinin sağlığını tehdit eden ve yaşam kalitesini düşüren çeşitli komplikasyonlara yol açar. Kan glukoz düzeylerindeki ani yükselmelere bağlı olarak ortaya çıkan diyabetin akut komplikasyonları, T1D ve T2D'de diyabetik ketoasidoz, T2D'de hiperosmolar koma olarak görülebilmektedir. Kan glukoz düzeylerindeki ani düşüşlere bağlı olarak tüm diyabet tiplerine bağlı olarak bilinç kaybı veya nöbetler görülebilir [5,6]. Diyabetin uzun dönem komplikasyonları; retinopati, periferal nöropati, nefropati, kardiyovasküler semptomlar ve seksüel disfonksiyon olarak sıralanabilir. Diyabetli hastalarda Alzheimer hastalığı, aterosklerotik kardiyovasküler, periferal arteriyel ve serebrovasküler hastalıkların insidansının arttığı bilinmektedir. Bu hastalarda zaman içinde diyabete bağlı lipoprotein metabolizması bozuklukları ve hipertansiyon

komplikasyon olarak görülebilir [7-9].

Diyabetin erkek üreme sistemi üzerine erektil disfonksiyon, azalmış cinsel dürtü ve boşalma sorunları gibi bir dizi olumsuz etkileri olduğu bilinmektedir. Bu etkilerin üreme sistemi organlarını besleyen kan damarları ve işlevlerini düzenleyen sinir ağında meydana gelen hasara bağlı olabileceği düşünülmektedir [10,11]. Ayrıca diyabete bağlı hormon düzeyi bozuklukları, testis doku hasarı, spermatogenezis aşamasındaki problemlere bağlı olarak infertiliteye neden olabileceği bilinmektedir [10,12]. Erkek üreme sistemindeki bozuklukların dokulardaki oksidatif hasar, hipotalamus-hipofiz-testis aksında bozulma ve germ hücrelerindeki apoptoz ve otofaji kaynaklı olabileceğini gösteren insan ve hayvan çalışmaları bulunmaktadır [10,11,13-15]. Diyabetin neden olduğu erkek üreme sistemi hasarı ile ilgili yapılan birçok çalışma oksidatif hasarın etkiden sorumlu önemli bir mekanizma olduğunu ve bu hasarın antioksidan kullanımı ile önlenebileceğini göstermiştir [11,15-17].

Günümüzde oldukça yaygın bir şekilde görülen diyabet gibi metabolik hastalıkların önlenmesi, tedavi edilmesi ve neden olabileceği ikincil komplikasyonların oluşumunun önlenmesinde etkili doğal bileşiklerin kullanılması oldukça dikkat çeken bir konudur. Ruşeym yağı (RY), ülkemizde ve dünyada tüketilen birçok besin maddesinin temel hammaddesi olan buğday (Triticum aestivum L.)'dan elde edilmektedir. Buğday çekirdeğinin embriyosu olarak adlandırılan buğday rüşeyminin %8-14'ünü sabit bir yağ olan RY oluşturmaktadır. RY, E vitamini içeriği bakımından en zengin bitkisel kaynaklardan biridir [18]. Zengin vitamin ve mineral içeriği ve doymamış yağ asitlerinden dolayı RY'nin antioksidan kapasitesinin yüksek olduğu, tüketiminin oksidatif strese karşı koruyucu rol oynayabileceği bildirilmiştir [19-21]. Literatürde RY'nın diyabetin neden olduğu testiküler hasar üzerindeki etkisinin araştırıldığı çalışmaların yeterli olmadığı görülmektedir.

Çalışmamızda RY'nin diyabetin testiküler dokularda neden olduğu hasar üzerindeki etkisinin oksidatif stres aracılı yolaklar yönünden incelenmesi amaçlanmıştır. Araştırmada hem yüksek doz RY'nin, hem de düşük doz RY'nin etkisi oksidatif stresin temel biyogöstergelerinden olan glutatyon düzeyleri, lipit peroksidasyonun biyogöstergesi olan malondialdehit (MDA) düzeyleri ve kan glukozu düzeyleri ölçülerek incelenmiştir.

GEREÇ VE YÖNTEM

Kimyasallar

Diyabet modeli oluşturmak için kullanılan streptozosin (STZ) "AG Scientific (San Diego, Amerika)" firmasından, RY "Zadevital (Konya, Türkiye)" firmasından satın alınmıştır. Doku homojenizasyonu tamponu (tris, dietilentriaminpentaasetik asit (DTPA) ve fenilmetansülfonil florür (PMSF)) ve sitrat tamponu (sitrik asit ve sodyum sitrat) hazırlanırken "Sigma Aldrich (St. Louis, Amerika)" firmasının kimyasalları kullanılmıştır. Doku MDA düzeylerinin ölçümünde kullanılan tiyobarbitürik asit reaktif maddeler (TBARS) kiti "Cayman Chemical (Ann Arbor, Amerika)" firmasından, total protein ölçüm kiti (Bradford) Quick Start™ "Bio-Rad (Amerika)" firmasından satın alınmıştır. Doku glutatyon düzeyleri ölçülürken kullanılan MOPS tamponu, sodyum bikarbonat, 5,5 ditiyobis 2-nitrobenzoik asit (DTNB, Ellman reaktifi), nikotinamid adenin dinükleotit fosfat (NADP) ve glutatyon redüktaz enzim karışımı "Sigma Aldrich (St. Louis, Amerika)" firmasından satın alınmıştır. Tüm spektrofotometrik ölçümler, SpectraMax M2 spektrofotometre (Molecular Devices, Sunnyvale, CA) kullanılarak yapılmıştır.

Çalışma Gruplarının ve Diyabet Modelinin Oluşturulması

Çalışmamız Kobay Deney Hayvanları Laboratuvarı'nda, sağlıklı 12 haftalık (250-350 gram) Wistar Albino erkek sıçanlar kullanılarak yapılmıştır. Sıçanların yem ve su tüketimi sınırlandırılmamıştır. Sıçanlar çalışma boyunca sıcaklık 22°C (± 3°C), nem oranı en az %30, en fazla %70, 12 saat aydınlık 12 saat karanlık bir odada tutulmuştur [22]. Literatür verisi ve G*Power programı kullanılarak yapılan Güç Analizi sonuçları dikkate alınarak gruplardaki hayvan sayısı diyabet modeli oluşturulan gruplarda 8, oluşturulmayan gruplarda 6 olarak planlanmıştır [22,23].

Diyabet gruplarında hastalık modeli, literatürde sıklıkla kullanılan ve kabul görmüş bir yöntem olan STZ kullanılarak oluşturulmuştur [24,25]. Bu modele göre randomize seçilen sağlıklı sıçanlara 0.1 M sitrat tampon içinde (pH:4.5) hazırlanmış tek doz 45 mg/kg STZ çözeltisi intraperitoneal (ip) yoldan

uygulandı. STZ injeksiyonundan sonraki 72 saat boyunca hipoglisemi ve mortaliteyi engellemek amacıyla, içme suyu olarak (%10) glukoz içeren su verilmiştir. Diyabet indüksiyonundan 72 saat sonra kan glukozu düzeyleri ölçülmüş ve 250 mg/dl'nin altında olan sıçanlar çalışma dışı bırakılmıştır [24,25].

Literatürdeki benzer çalışmalar ve kemirgenlere uygulanabilecek dozlar dikkate alındığında uygulama oral yoldan, 28 gün süre ile, günlük 1000 mg/kg (yüksek doz olarak) ve 100 mg/kg (düşük doz olarak maksimum dozun 1/10'u) olarak planlanmıştır [19,21,22,26,27]. Kemirgenlere oral uygulama ile ilgili prosedürler göz önünde bulundurularak hayvan başına en fazla 0.5 ml olacak şekilde (250 g ağırlıktaki sıçana 0.5 ml yağ çözeltisi oral yoldan verilecek şekilde) dozlama yapılmış ve taşıyıcı olarak mısır özü yağı seçilmiştir [28].

Çalışma grupları aşağıdaki şekilde oluşturulmuştur.

Kontrol grubu (K): Randomize seçilen sağlıklı sıçanlara oral yoldan ile 28 gün boyunca mısır özü yağı uygulanmıştır (n=6).

Kontrol+Düşük doz RY grubu (KDD): Randomize seçilen sağlıklı sıçanlara oral gavajla 28 gün boyunca 100 mg/kg/gün RY uygulanmıştır (n=6).

Kontrol+Yüksek doz RY grubu (KYD): Randomize seçilen sağlıklı sıçanlara oral gavajla 28 gün boyunca 1000 mg/kg/gün RY uygulanmıştır (n=6).

Diyabet grubu (D): Randomize seçilen, diyabet modeli oluşturulan sıçanlara oral gavajla 28 gün boyunca mısır özü yağı uygulanmıştır (n=8).

Diyabet+Düşük doz RY grubu (DDD): Randomize seçilen, diyabet modeli oluşturulan sıçanlara oral gavajla 28 gün boyunca 100 mg/kg/gün RY uygulanmıştır (n=8).

Diyabet+Yüksek doz RY grubu (DYD): Randomize seçilen, diyabet modeli oluşturulan sıçanlara oral gavajla 28 gün boyunca 1000 mg/kg/gün RY uygulanmıştır (n=8).

Çalışma süresi boyunca her sabah, gavajdan önce, tüm sıçanların vücut ağırlıkları ölçülmüş ve uygulanacak RY dozu hesaplanmıştır. 28 günlük dozlama süresinin sonunda hayvanlar tartılmış, ağırlıklarına uygun dozda anestezi uygulanmış ve ağırlıkları kaydedilmiştir. Anestezi altındaki hayvanlara kalpten kan alma yöntemi ile ötenazi işlemi uygulanmıştır.

Kan Glukoz Düzeyleri

Kan glukoz düzeyleri; glikoz oksidaz emdirilmiş test çubukları (stripler) aracığıyla, strip üzerine kuyruk veninden damlatılan kanda glukometre kullanılarak ölçülmüştür. İlk dozlama gününden başlayarak haftada bir hayvanların açlık kan düzeyleri kaydedilmiştir.

Doku Ağırlıkları

Ötenazi işlemi sonrası hayvanların sağ testisleri alınarak serum fizyolojik ve deiyonize suda yıkamayı takiben kurutulmuş, hassas terazide tartılmış ve kaydedilmiştir. Ağırlığı kaydedilen organlar hızlıca sıvı azota atılarak dondurulmuştur. Sıvı azottan çıkarıldıktan sonra oksidatif stres parametrelerinin analizleri için deneysel işlemler yapılana kadar -80°C'de saklanmıştır.

Doku Homojenizasyonu

Testis dokuları yapılacak analizler öncesinde homojenize edilmiştir. Tartılan doku örneklerine 1:5 (a/h) olacak şekilde Tris (10 mM), DTPA (1 mM) ve PMSF (1 mM; pH 7.4'e ayarlanmış) tamponu eklenmiştir. Mekanik homojenizatör cihazı kullanılarak yapılan homojenizasyonu takiben homojenat 10 dk 1500 x g'de ve 4^{0}C'de santrifüj edilmiştir. Süpernatant kısmı alikotlarına ayrılarak analize dek -80°C'de saklanmıştır.

Total Protein Düzeyleri

Testis homojenatlarının total protein düzeyleri Bradford yöntemini kullanan ticari bir kit ile ölçülmüştür (Quick Start™ Bradford Protein Assay kit, Bio-Rad, USA). Bradford protein ölçüm yöntemi asidik koşullar altında protein moleküllerinin Coomassie boyasına bağlanması ve renk değişimine neden olması esasına dayanmaktadır. Doku total protein düzeyleri kit standartları (0.125-1.5 mg/ml) ile hazırlanan kalibrasyon doğrusu kullanılarak mg/ml cinsinden hesaplanmıştır.

Lipit Peroksidasyon Düzeyleri

Lipit peroksidasyonunun göstergesi olarak testis dokularında MDA seviyeleri ölçülmüştür. Dokular homojenize edildikten sonra deney günü muhafaza edildiği -80°C'den çıkarılmıştır. Doku MDA düzeyleri ticari bir kit yardımı ile ölçülmüştür (TBARS Assay Kit, Item No. 10009055, Cayman, ABD). Kit, lipit peroksidasyonu sonucu oluşan MDA'in uygun koşullar altında tiyobarbitürik asit (TBA) ile renkli bir kompleks oluşturması ve MDA-TBA kompleksinin renk yoğunluğunun spektrofotometrik olarak 530 nm'de ölçülmesi esasına dayanmaktadır. Kit içerisinde yer alan MDA standartları kullanılarak elde edilen kalibrasyon doğrusu ile doku MDA miktarı hesaplanmış ve sonuçlar µM/mg protein olarak verilmiştir.

Glutatyon Düzeyleri

Testis doku homojenatlarında oksidatif stresin önemli bir göstergesi olan glutatyon düzeyleri ölçülmüştür. Glutatyon düzeyleri Sedlak ve Lindsay'in kullandığı yöntem ile ölçülmüştür [29]. Bu yöntem, Ellman Reaktifinin sülfhidril gruplarıyla indirgenmesi ve reaksiyon sonucunda 1 mol sülfidril grubu için 1 mol 2-nitro-5-merkaptobenzoik asit oluşması esasına dayanmaktadır. Oluşan sarı renkli nitromerkaptobenzoik asitin renk yoğunluğu 412 nm'de spektrofotometrik olarak ölçülmüştür. Standartlar yardımı ile elde edilen kalibrasyon doğrusu kullanılarak doku glutatyon düzeyleri hesaplanmış ve sonuçlar uM/mg protein olarak ifade edilmiştir.

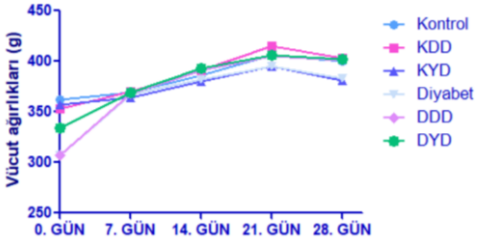
İstatistiksel Analizler

Çalışma kapsamında elde edilen sonuçlar GraphPad Prism 5 software (Boston, USA) ve IBM SPSS version 17.0 (Chicago, IL) istatistik paket programı kullanılarak değerlendirilmiştir. Kontrol ve deney gruplarındaki tüm veriler ortalama±standart sapma olarak ifade edilmiştir. Verilerin dağılım profilleri Shapiro wilk testi ile değerlendirilmiştir. Gruplar arasındaki farklılıklar, Kruskal-Wallis tek yönlü varyans analizi ve ardından post hoc Dunn's testi ile değerlendirilmiştir. $p<0.05$ değeri istatistiksel olarak anlamlı kabul edilmiştir.

SONUÇ VE TARTIŞMA

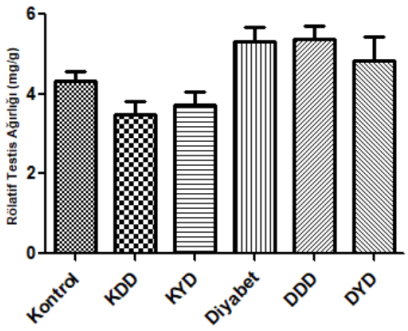
Hayvan Vücut ve Doku Ağırlıkları

Çalışma süresince her gün dozlama işleminden önce hayvanların vücut ağırlıkları ölçülmüştür. 0. gün, 7. gün, 14. gün, 21. gün ve 28. gün vücut ağırlıkları Şekil 1'de gösterilmiştir. Hem kontrol, hem de diyabet gruplarında 7., 14. ve 21. günde vücut ağırlıkları artma eğilimi gösterirken, 21. günde azalma eğilimine geçmiştir.



Şekil 1. Kontrol ve diyabet grubundaki hayvanların vücut ağırlıkları (g)
KDD: kontrol düşük doz, KYD: kontrol yüksek doz, DDD: diyabet düşük doz, DYD: diyabet yüksek doz

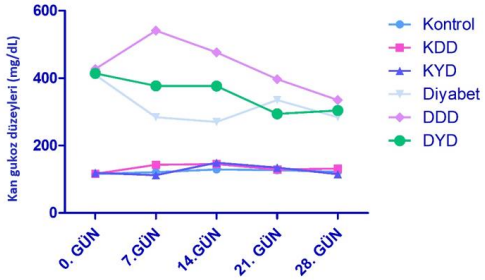
Testis doku ağırlıkları ölçülmüş ve bu değerler kullanılarak rölatif testis ağırlıkları hesaplanmıştır. Sonuçlar Şekil 2'de şematize edilmiştir. Diyabet grubunda rölatif testis ağırlığının kontrole göre arttığı görülmüştür. Kontrol grubunda hem düşük, hem de yüksek doz RY uygulamasının rölatif testis ağırlığında azalmaya neden olduğu görülmüştür. Yüksek doz RY uygulanan diyabetik grubun rölatif testis ağırlığının diyabet kontrol grubuna göre azaldığı belirlenmiştir.



Şekil 2. Kontrol ve diyabet grubundaki hayvanların rölatif testis ağırlığı (mg/g)

Kan Glukoz Düzeyleri

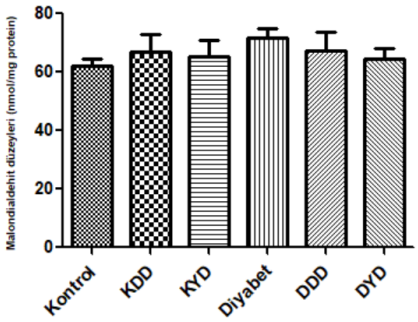
Diyabet grubuna kan glukoz düzeyleri 250 mg/dl ve üzerinde olan hayvanlar dahil edilmiştir. Kontrol ve diyabet grubundaki hayvanların kan glukoz düzeyleri Şekil 3'te gösterilmiştir. Düşük doz RY uygulanan diyabet grubunda 7. günde artan kan glukoz düzeyinin, 28. güne kadar azaldığı görülmüştür. Diyabet grubu ve kontrol grupları kendi içinde değerlendirildiğinde kan glukozu düzeylerinde RY uygulamasına bağlı olarak anlamlı bir farklılık bulunmamıştır ($p>0.05$).



Şekil 3. Kontrol ve diyabet grubundaki hayvanların kan glukoz düzeyleri (mg/ml)
KDD: kontrol düşük doz, KYD: kontrol yüksek doz, DDD: diyabet düşük doz, DYD: diyabet yüksek doz

Lipit Peroksidasyon Düzeyleri

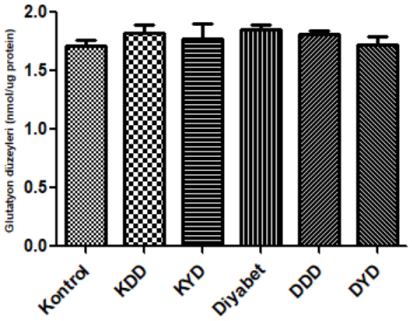
Testis dokularında lipid peroksidasyonun göstergesi olarak MDA düzeyleri ölçülmüştür (Şekil 4). Diyabet grubuunda kontrole kıyasla testis doku MDA düzeylerinde artış olduğu saptanmıştır ($p>0.05$). Diyabetik hayvanlar arasında en düşük testis MDA düzeylerinin yüksek doz RY uygulanan grupta olduğu ve düşük doz RY uygulanan grupta da MDA düzeylerinin diyabet kontrol grubuna göre azaldığı görülmüştür. Ancak gruplar arasındaki farklılık istatistiksel olarak anlamlı bulunmamıştır ($p>0.05$).



Şekil 4. Testis MDA düzeyleri (nmol/mg protein)
KDD: kontrol düşük doz, KYD: kontrol yüksek doz, DDD: diyabet düşük doz, DYD: diyabet yüksek doz

Glutatyon Düzeyleri

Testis dokularında glutatyon düzeyleri ölçülmüş ve sonuçlar Şekil 5'te şematize edilmiştir. Kontrole kıyasla diyabet grubunda glutatyon düzeyleri artmış olmakla birlikte RY uygulanan diyabet gruplarında glutatyon düzeylerinin kontrol grubu düzeylerine yakın olduğu saptanmıştır. Gruplara ait testis glutatyon düzeylerindeki bu değişimler istatistiksel olarak anlamlı bulunmamıştır. ($p>0.05$).



Şekil 5. Testis glutatyon düzeyleri (nmol/ug protein)
KDD: kontrol düşük doz, KYD: kontrol yüksek doz, DDD: diyabet düşük doz, DYD: diyabet yüksek doz

DM, kan glukoz düzeylerinde kontrolsüz artış ile karakterize, karbonhidrat, lipit ve protein yapılarında bozulmaya neden olabilen, kronik, metabolik bir hastalıktır. Kronik hiperglisemi ve kontrolsüz diyabet oküler sistem, boşaltım sistemi, endokrin sistem, sinir sistemi, kalp-damar sistemi ile üreme sisteminde hasarla birlikte mikrovasküler ve makrovasküler komplikasyonlara neden olabilir [30]. İnsülin eksikliği ve insülin direnci hipotalamus, hipofiz bezi, gonadlar ve perigonadlara zarar vererek seks hormonlarının salgılanmasını bozabilir. Bu süreç testis ve stromal hücre atrofisine, seminifer tübül hasarına, spermatojenik hücre hasarına neden olabilir [31]. Bu nedenle diyabet oluşumunun önlenmesinde, progresyonunun yavaşlatılmasında ve tedavi edilmesinde beslenme yaklaşımlarının değiştirilmesi, oksidatif stresin azaltılması ve antioksidan kullanımının arttırılmasına yönelik araştırmalar yürütülmektedir [32,33].

Buğday; zengin vitamin ve mineral içeriği, yüksek verim potansiyeli, ekmek, makarna, erişte ve diğer gıda ürünlerine dönüştürülmesine olanak tanıyan viskoelastik özellikleri, taşıma ve depolama kolaylığı nedeniyle dünya nüfusunun temel gıda maddelerinden biridir [34]. Yapısında yüksek düzeyde karbonhidrat, protein, lipit, B ve E grubu vitaminler, bakır, magnezyum, çinko, fosfor, demir gibi mineraller bulunmaktadır [35,36]. Buğday tanesi temel olarak endosperm (%83), perikarp (%14), tohum (%3) kısımlarından oluşmaktadır [37]. Buğday çekirdeğinin embriyosu olan buğday ruşeymi, vitamin, mineral ve diğer besin maddeleri bakımından buğdayın en zengin kısımlarından biridir [38]. Buğday ruşeyminin sabit yağı olan RY, en zengin bitkisel E vitamini kaynağıdır. Yaklaşık 1 servis kaşığı RY'nin porsiyon başına 20.3 mg alfa-tokoferol içerdiği, bu miktarın tüketimi günlük E vitamini ihtiyacını karşıladığı ifade edilmiştir [39]. Çoklu doymamış yağ asitleri bakımından zengin olan RY'nin yapısında yüksek oranda linoleik asit, palmitik asit ve oleik asit bulunmaktadır. RY ayrıca polikosanoller, fitosteroller, tokoferoller, karotenoidler, tiamin, riboflavin, flavonoidler, steroller, oktakosanoller, glutatyon, steril ferulatlar ve çeşitli enzimler bakımından zengindir [40-43].

RY'nin zengin antioksidan içeriği ve bitkisel protein kaynağı olması sayesinde, oksidatif stres ve ilişkili hastalıklarda koruyucu etki gösterebileceği bildirilmiştir. RY'nin farklı hayvan türlerinin beyin, karaciğer, dalak, kalp, böbrek gibi organlarında tokoferol ve diğer biyoaktif bileşenlerin düzeyini arttırdığı ve oksidasyon sürecine karşı koruma sağladığı gösterilmiştir [44]. L-arjinin indüklü akut pankreatit oluşturulan sıçanlarda 3 gün oral RY (3 ml/kg/gün) uygulamasının olumlu etki sağladığı, ancak pankreatit oluşumundan önce RY uygulamasının, sonradan oluşturulan hastalık etkileri üzerine koruyucu etki göstermediği bildirilmiştir [45]. STZ indüklü diyabetik sıçanlara 3 hafta oral 0.4 g/kg/gün 1000 mg balık yağı+100 mg RY uygulamasının over fonksiyonlarını düzenlediği; GSH, katalaz (CAT), superoksit dismutaz (SOD), glutatyon peroksidaz (GPx), folikül stimüle edici hormon (FSH), estradiol (E2), luteinize edici hormon (LH), anti-Müllerian (AMH) düzeyleri ve folikül sayısını arttırdığı, MDA

düzeylerini azalttığı bildirilmiştir [20]. Hepatotoksik tiyoasetamid (TAA) indüklü karaciğer ve böbrek hasarına karşı RY'nin etkinliği araştırılmış, 5 gün oral RY (1400 mg/kg) uygulamasının kreatin kinaz düzeylerinde iyileşme sağladığı, tek başına RY ve birlikte tedavi grubunun (TAA+olmutinib +RY), lezyon veya karyoliz olmaksızın normal hepatik ven ve normal hepatositler gösteren kontrol grubuna benzer duruma geldiği, böbrek tübüllerindeki tüm dejeneratif değişikliklerin düzeldiği, normal küboidal hücrelerin belirgin çekirdeklere sahip olduğu ifade edilmiştir [46].

En zengin bitkisel E vitamin kaynağı olan RY halk arasında üreme sağlığını koruyucu ve erkek üreme sistemine bağlı hastalıklarda destekleyici olarak kullanılmaktadır [47]. RY'nin dişi ve erkek üreme sistemi üzerine etkinliğinin araştırıldığı çalışma sayısı oldukça azdır. Ayrıca etki mekanizmasının aydınlatıldığı yeterli çalışma da bulunmamaktadır. Dişi albino sıçanlara 28 ve 42 günlük oral RY uygulamasının (900 mg/kg/gün) böbrek TBARS düzeyinde anlamlı azalış, GSH düzeyinde artışa neden olduğu, karaciğer ve böbreklerde oksidatif hasarı engellediği bildirilmiştir. İçme suyunda nitrat konsantrasyonunun artması serum östradiol düzeyinde kontrole göre önemli bir düşüşe neden olurken, RY'nin nitratla kombine olarak uygulanmasının, her iki zaman aralığında östradiol düzeyini arttırdığı ifade edilmiştir [48]. Başka bir araştırmada, Wistar sıçanlara 28 gün oral RY uygulamasının (68.75 mg/kg/gün), sertralinin neden olduğu testiküler DNA hasarını, testiküler dokularda lipid peroksidasyonu ve artmış serum testosteron konsantrasyonunu azalttığı bildirilmiştir [17]. Wistar sıçanlarda 5 hafta oral RY uygulamasının, diyabetin indüklediği erektil ve endotelyal disfonksiyon üzerine etkisi araştırılmış; in vitro vasküler fonksiyon, *in vivo* erektil fonksiyon, aort ve penis oksidatif stres parametreleri incelenmiştir. Aortta asetilkolin aracılı gevşeme ve erektil fonksiyonların diyabet grubunda anlamlı derecede azaldığı ($p = 0.018$, $p=0.005$), 3 ml/kg ve 6 ml/kg RY'nin diyabet gruplarında vasküler fonksiyonları iyileştirdiği, ($p=0.001$, $p=0.014$), vasküler veya erektil disfonksiyonda düzelme sağladığı ifade edilmiştir. Ancak yüksek doz RY'nin penis dokusunda MDA düzeylerini arttırdığı, SOD düzeylerinde anlamlı değişikliğe neden olmadığı, dolayısıyla bu iyileşmelerin antioksidan etkinlikle ilişkili olmadığı, mekanizmanın aydınlatılması için yeni araştırmalara ihtiyaç olduğu ifade edilmiştir [27].

Çalışmamızda diyabetik sıçanlara ve kontrol grubuna 4 hafta süre ile düşük (100 mg/kg/gün) ve yüksek doz (1000 mg/kg/gün) oral RY uygulanmıştır. RY uygulamasının kan glukoz düzeylerinde incelendiğinde, DDD ve DYD gruplarında RY uygulamasının bu düzeylerde düşüşe neden olduğu görülmüştür. Bu düşüş RY'nin antidiyabetik etkinliğini destekler niteliktedir [32]. Diyabet kontrol grubunun rölatif testis ağırlığının kontrol grubuna göre arttığı; hem düşük, hem de yüksek doz RY uygulanan kontrol gruplarında rölatif testis ağırlığının kontrole göre azaldığı belirlenmiştir. Diyabetik grupta ise yalnızca yüksek doz RY uygulamasının rölatif testis ağırlığını diyabet kontrol grubuna göre azalttığı belirlenmiştir. RY'nin bu etkinliğinin östrojenik aktivitesi olan ve metabolizmayı değiştirebilen fitosteroller içermesine bağlı olabileceği düşünülmektedir [38,43]. RY'nin yapısı ve içeriği değerlendirildiğinde steroid sentezinde yer alan diğer enzimleri de inhibe edebileceği düşünülmektedir. Çalışmamızda testis dokularında oksidatif stresin değerlendirilmesi için glutatyon düzeyleri, lipid peroksidasyonun değerlendirilmesi için MDA düzeyleri ölçülmüştür. Ayrıca kan glukoz düzeyleri, vücut ağırlıkları ve testis ağırlıkları kayıt altına alınmıştır. Diyabet grupları arasında en düşük testis MDA düzeyleri yüksek doz RY grubunda bulunmuştur. Diğer kontrol ve diyabet gruplarının testis glutatyon ve MDA düzeylerinde farklılıklar bulunsa da, bu düzeyler istatistiksel olarak anlamlı bulunmamıştır. Çalışma sonuçlarımız bu yönüyle Guven ve arkadaşlarının (2022) verilerini destekler niteliktedir [27]. Yayınlanan literatür verileri, halk arasında kullanımı ve araştırma sonuçlarımız bir arada değerlendirildiğinde RY'nin testiküler hasara karşı koruyucu etkinliğinin mekanizmasının oksidatif stres kaynaklı olmayabileceği sonucu çıkarılabilir. Erkek üreme sistemi hasarına karşı koruyucu etkilerini serum testesteron düzeylerini değiştirerek hormon aracılı ya da hipotalamik-hipofiz yolundaki bozulmayı önleyerek gösterebileceği düşünülebilir. Öte yandan çok daha yüksek kan glukoz düzeyleri ile seyreden diyabetik hastalarda görülebilen ciddi oksidatif hasarda RY kullanımının oksidatif hasara karşı koruyucu etkisinin daha belirgin olabileceği ihtimali de bulunmaktadır.

Sonuç olarak diyabete bağlı görülen testiküler hasara karşı akut ve kronik RY uygulamasının koruyucu etkinliğinin hormonal ya da diğer moleküler yolaklar aracılığıyla incelendiği yeni araştırmalara da ihtiyaç vardır.

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ÖZGÜN MAKALE / ORIGINAL ARTICLE

POPÜLER DİÜRETİK İLAÇ HİDROKLOROTİAZİDİN KROMATOGRAFİK İYONLAŞMA SABİTİ DEĞERİNİN BELİRLENMESİ

DETERMINATION OF THE CHROMATOGRAPHIC IONIZATION CONSTANT VALUE OF THE POPULAR DIURETIC DRUG HYDROCLOROTHIAZIDE

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ÖZ

Amaç: *Hidroklorotiazid, idrar çıkışının hızını ve hacmini arttırırken, Na^+ ve Cl^- içeriğini azaltarak kan basıncını düşüren bir ilaçtır. Bu ilaç, tek başına ya da diğer hipertansif ilaçlarla beraber kullanılmaktadır. Ancak, bu kadar önem arz eden bir ilacın etki mekanizması tam olarak bilinmemekle birlikte, bileşiğin hidro-organik sistemlerdeki iyonlaşma sabiti değeriyle alakalı literatürde sadece bir çalışma ve bileşiğin sudaki iyonlaşma sabiti değeriyle alakalı hiç çalışma yoktur. Bu nedenle bu çalışmada, farklı asetonitril-su ikili karışımlarında hidroklorotiazidin iyonlaşma sabiti değerlerinin tayini ve bu değerler kullanılarak çeşitli ekstrapolasyon yöntemleriyle hidroklorotiazidin su ortamındaki iyonlaşma sabiti değerinin tayini amaçlanmıştır.*

Gereç ve Yöntem: *Hidroklorotiazidin iyonlaşma sabiti tayini %11, %13 ve %15 (h/h) asetonitril-su ikili karışımında 30°C'de, 1 ml/dakika akış hızında ve X Terra C18 kolonda gerçekleştirilmiştir. Hidroklorotiazidin iyonlaşma sabitinin belirlenmesi için pH-alıkonma ilişkisi, lineer solvasyon enerjisi ilişkisi (LSER) modeliyle değerlendirilmiştir. Sudaki iyonlaşma sabiti değerlerinin tayini için LSER modelinden elde edilen veriler, Yasuda-Shedlovsky ve mol kesri-$^s_spK_a$ yöntemiyle değerlendirilmiştir.*

Sonuç ve Tartışma: *Her iki metotla hesaplanan termodinamik $^w_wpK_{a_1}$ değerlerinin birbiriyle uyumu dikkat çekicidir ve literatürdeki organik çözücü-su ortamında elde edilen değerle uyumludur. Bu bilgiler ışığında, hidroklorotiazidin tayin edilen $^w_wpK_{a_1}$ değerinin doğruluğu ve tekrarlanabilirliği açıkça görülmektedir. Ayrıca, tayin edilen değerler hidroklorotiazidin herhangi bir analitik yöntemle tayin edilen literatürdeki ilk termodinamik $^w_wpK_{a_1}$ verileridir.*

Anahtar Kelimeler: *Hidroklorotiazid, iyonlaşma sabiti tayini, LSER modeli, RPLC*

ABSTRACT

Objective: *Hydrochlorothiazide is a drug that lowers blood pressure by decreasing Na^+ and Cl^- levels while increasing the rate and volume of urine excretion. This drug is used alone or in combination with other antihypertensive medications. However, the action mechanism of such an important drug is unknown, and in the literature, there is only one study on the value of the ionization constant of the compound in hydroorganic systems and no study on the value of the ionization constant of the compound in water. Therefore, in this study, an attempt was made to determine the*

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ionization constant of hydrochlorothiazide in various binary acetonitrile-water mixtures and to determine the ionization constant of hydrochlorothiazide in water by using these values through various extrapolation methods.

Material and Method: *The ionization constant of hydrochlorothiazide was determined in a binary mixture of 11%, 13%, and 15% (v/v) acetonitrile and water at 30°C, a flow rate of 1 ml/min, and an X Terra C18 column. To determine the ionization constant of hydrochlorothiazide, the pH retention relationship was evaluated using the linear solvation energy relationship (LSER) model. To determine the ionization constant values in water, the data obtained from the LSER model were evaluated using the Yasuda-Shedlowsky method and the mole fraction-* $^{s}_{s}pK_a$ *method.*

Result and Discussion: *The agreement between the thermodynamic* $^{w}_{w}pK_{a_1}$ *values calculated by the two methods is remarkable and consistent with those found in the literature for the organic solvent-water environment. Considering this information, the accuracy and reproducibility of the determined* $^{w}_{w}pK_{a_1}$*value of hydrochlorothiazide becomes clear. Moreover, the determined values are the first thermodynamic* $^{w}_{w}pK_{a_1}$*data in the literature obtained by any analytical method for hydrochlorothiazide.*

Keywords: *Hydrochlorothiazide, LSER model, RPLC, the determination of the ionization constant*

GİRİŞ

Hidroklorotiazid (6-kloro-3,4-dihidro-2*H*-1,2,4- benzotiazidin-7-sülfonamid 1,1-dioksit) tiazid diüretikleri grubuna ait bir prototipik ilaç etken maddesidir [1,2]. Tiazid diüretikleri, benzendisülfonamid moleküllerinden bir halka oluşması sonucunda meydana gelmiş bileşiklerdir [1]. Bu bileşiklerin diüretik etki gösterebilmeleri için, yapılarında serbest bir sülfonamid grubunun bulunması gereklidir. Tiazid diüretikler, idrar çıkışının hacmini ve hızını arttırırken, distal kıvrımlı tübülün ilk kısımlarında (Henle kısımına yakın yerde) Na^+ ve Cl^- içeriğini azaltarak, hücre dışı sıvı hacmini azaltıp (hipovolemi) kan basıncını düşüren ilaçlardır [1,3]. Ancak, hidroklorotiazid ve diğer tiazidlerin etki mekanizmaları tam olarak henüz belirlenememiştir. Bu ilaçlar, hafif derecede hipertansiyon olgularının tedavisinde tek başına kullanılırken, orta ve ileri olgularda diğer hipertansif ilaçlar ile kullanılırlar [1,3]. Hidroklorotiazid, suda zayıf çözünen, etil ve metil alkolde çözünürlüğü sudakinden daha da az olan bir bileşiktir.

İlaçların hücre zarlarından geçişi ve dokularda absorbsiyonu ve dağılımı, pasif difüzyon ile ilacın moleküler halde olduğu formda gerçekleşir. Bir ilacın iyonlaşmamış formu, iyonize formuna kıyasla lipidlerde daha fazla çözünür olduğundan, ilacın hücre zarından geçişi ve dokularda absorbsiyonu ve dağılımı bu formda gerçekleşir. Bundan dolayı, ilaçların absorpsiyon, dağılım, metabolizma ve atılım (ADME) özelliklerinin belirlenmesinde iyonlaşma, büyük bir etkiye sahiptir [4-7]. Ayrıca, fizyolojik pH değerinde (pH 7.4) bir ilacın iyonlaşma durumunu belirleyebilmek, ilacın kimyasal yapısı ve onun biyolojik aktivitesi arasındaki ilişkinin belirlenmesine imkân sağladığından, enzim aktif bölgeleriyle ilacın etkileşimi hakkında da bilgi verebilir [4,6]. Bir molekülün iyonlaşma durumunun belirlenmesinde kullanılan fizikokimyasal değere iyonlaşma/protonasyon sabiti (pK_a) adı verilir [7,8].

İyonlaşma/protonasyon sabitinin belirlenmesinde potansiyometrik, kapiler elektroforez, spektrofotometre ve sıvı kromatografi gibi birçok analitik yöntem kullanılmasına rağmen [9-15] ters faz sıvı kromatografi yöntemi (RPLC), uygulama kolaylığı, tekrarlanabilirliği, yüksek doğruluk ve kesinlik değerlerine sahip olması, ayrıca, az kimyasal tüketimi ve analiz edilecek maddenin saf olmasının zorunlu olmaması gibi avantajlarından dolayı çoğunlukla tercih edilmektedir [9,10].

RPLC yöntemle bileşiklerin pK_a değerlerinin tayininde, yapısında iyonlaşabilen fonksiyonel grup bulunduran bileşiklerin sabit kolon sıcaklığı ve mobil faz derişiminde, farklı mobil faz pH değerleri için elde edilen alıkonma değerleri arasındaki değişim incelenir. Buna göre; bileşiklerin farklı mobil faz pH değerlerinde elde edilen alıkonma değerleri, pH değerlerine karşı grafiğe geçirilirse bir sigmoidal ilişki elde edilir [7,8,16]. Bu sigmoidal ilişkiyi matematiksel olarak ifade etmek için zaman içinde birçok model ileri sürülmüşse de içlerinde en çok rağbet gören model, lineer solvasyon enerji ilişkisi (LSER) modelidir [17,18]. Bu model, bileşiklerin alıkonma değerini (t_R) mobil faz pH değeri ve çalışılan mobil faz derişiminde elde edilen bileşiğin pK_a değerinin ($^{s}_{s}pK_a$) bir fonksiyonu olarak matematiksel bir eşitlikle ifade edebilmekte ve bu sayede, bileşiğin $^{s}_{s}pK_a$ değeri kolaylıkla belirlenebilmektedir [7,8].

Gerçekleştirilen çalışmada hidroklorotiazid için yapılan literatür taramasında, bileşiğin çeşitli

analitik yöntemlerle tablet formülasyonlarında ve birkaç bileşikle beraber eş zamanlı tayiniyle alakalı çalışmaların literatürde mevcut olduğu görülmüştür [19-22]. Ancak, hidroklorotiazidin bir çalışma dışında iyonlaşma sabiti değeriyle alakalı bir çalışma bulunmamaktadır. Bu çalışmada hidroklorotiazidin ve yapısında asidik, bazik ve hem asidik hem de bazik fonksiyonel grubu bulunduran (zwitteriyonik) diğer bileşiklerin iyonlaşma sabitleri potansiyometrik yöntemle tayin edilmiştir. İyonlaşma sabiti tayini sadece %80 metanol-su ortamında gerçekleştirilmiştir [23]. Literatürde hidroklorotiazidin su ortamındaki pK_a ($^{w}_{w}pK_a$) tayiniyle ilgili hiçbir çalışma olmamakla birlikte, organik çözücü su karışımlarındaki pK_a ($^{s}_{s}pK_a$) değerleriyle alakalı da veri eksikliği bulunmaktadır. Bundan dolayı, bu çalışma hidroklorotiazidin çeşitli asetonitril-su ikili karışımlarında $^{s}_{s}pK_a$ değerinin RPLC yöntemle tayinine ve bu veriler kullanılarak, bileşiğin $^{w}_{w}pK_a$ değerinin tayinine odaklanmıştır. Ayrıca, bu çalışmayla elde edilen pK_a verisi bileşiğin farmakokinetik çalışmalarına ışık tutacağı gibi, aynı zamanda bileşiğin asetonitril-su ikili karışımlarında gerçekleştirilen optimizasyon çalışmalarında da yararlı olabilecektir.

GEREÇ VE YÖNTEM

Çalışmada Kullanılan Kimyasallar

Çalışmada pK_a tayininde incelenecek bileşik olan hidroklorotiazid, Sigma-Aldrich (St. Louis, MO, ABD)'den temin edilmiştir. Mobil faz hazırlamada kullanılan asetonitril (ACN), *o*-fosforik asit (o-H_3PO_4), sodyum hidroksit (NaOH), amonyum klorür (NH_4Cl), amonyak (NH_3) ve mobil faz pH ayarlamasında kullanılan elektronun kalibrasyonu için kullanılan potasyum hidrojen ftalat, (KHP) Merck (Darmstadt, Germany)'den tedarik edilmiştir.

Kullanılan Cihazlar

Hidroklorotiazidin pK_a tayini için gerçekleştirilen deneyler, bir pompa (LC -20AD), bir UV dedektör (SPD-20A), bir kolon fırını (CTO -20A) ve bir gaz giderme ünitesinden (DGU-20A3) oluşan Shimadzu yüksek performans sıvı kromatografi cihazında gerçekleştirilmiştir. Analizi yapılacak bileşiğin tüm sıvı kromatografik çalışmaları X Terra C18 (250 mm × 4.6 mm i.d., 5 µm) kolonda gerçekleştirilmiştir. Bu kolon, özellikle bazik bileşiklerin analizi için geliştirilmiş ve geniş pH aralığında (pH 1-12) çalışma imkanı sunan bir kolondur. Mobil faz pH ayarlamalarında Mettler Toledo InLab 413 Ag/AgCl cam elektrotla birlikte Metleer Toledo MA 235 pH/ion analiz cihazı kullanılmıştır. Çalışmada mobil faz hazırlamada kullanılan saf su, Direct-Q®3 UV su saflaştırma ünitesi kullanılarak günlük olarak tedarik edilmiştir.

Ters Faz Sıvı Kromatografi (RPLC) Yöntemi

Bu çalışmada, hidroklorotiazidin pK_a değerlerinin tayini için asetonitril-su ikili karışımlarında çalışılmıştır. İlk olarak, incelenen bileşikten 0.0010 g tartılıp 10 ml mobil fazda çözülerek hidrolorotiazidin 100 ppm'lik çözeltisi elde edilmiştir. Bu çözelti, günlük olarak hazırlanmış, kullanımına kadar güneş ışığından korunarak +4°C'de saklanmıştır. Sıvı kromatografik çalışmaya başlamadan önce bileşiğin maksimum absorbans gösterdiği dalga boyunu belirleyebilmek amacıyla, 190-400 nm aralığında UV-Vis spektrofotometre cihazıyla spektral davranışı belirlenmiştir. Bu inceleme neticesinde, hidroklorotiazidin maksimum absorbans gösterdiği dalga boyu 270 nm olarak belirlenmiş ve sıvı kromatografik cihazın dedektörü bu dalga boyuna ayarlanmıştır.

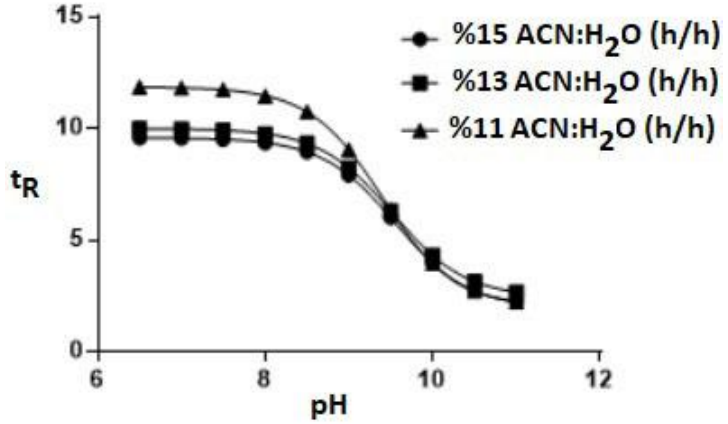
Çalışma, pH 6.5-11 aralığında ve yapısında %11, %13 ve %15 (h/h) asetonitril içeren ACN-su hidroorganik-su karışımlarında gerçekleştirilmiştir. pH 6.5 ila 8.0 aralığında hazırlanan mobil fazlarda 50 mM o-fosforik asit kullanılmış ve istenilen pH değerine mobil fazı getirebilmek için saf su kullanılarak 1 M derişimde hazırlanmış NaOH çözeltisi kullanılmıştır. pH 8.5 ila 11 aralığında hazırlanan mobil fazlarda 50 mM MH_4Cl kullanılmış ve istenilen pH değerine mobil faza derişik NH_3 ilave edilerek getirilmiştir. Hazırlanan hidroklorotiazid çözeltisi, her çalışmada RPLC sisteme 20 µl hacminde enjekte edilmiş ve bu prosedür üç kez tekrar edilerek ortalama alıkonma zamanı değeri elde edilmiştir. Bileşiğin RPLC tayini için belirlenen sıvı kromatografik çalışma koşulları 30°C ve 1 ml/dakika akış hızında sabitlenmiştir.

SONUÇ VE TARTIŞMA

Bir bileşiğin RPLC ya da herhangi bir analitik yöntemle iyonlaşma/protonasyon sabiti tayinini gerçekleştirebilmek için, öncelikle bileşiğin protik ya da aprotik bir çözücüde çözünmesi gereklidir. Çalışılacak bileşiğin çözeltisinin hazırlanmasında birçok araştırmacının ilk tercih ettiği çözücü, çevreye en az toksik özelliklerinden dolayı sudur [9,10]. Ancak, günümüzde geliştirilen birçok bileşiğin daha çok lipofilik özellikte olması ve bundan dolayı sudaki çözünürlüklerinin yetersiz olması, bu bileşiklerin herhangi bir analitik yöntemle analizini zorlaştırmaktadır. Bu handikap, sudaki çözünürlüğü yetersiz bileşiklerin su-organik çözücü ikili karışımlarında çalışılmasıyla aşılmaktadır [24,25]. Başta RPLC olmak üzere, analitik çalışmalarda iyonik numuneleri ve tamponları çözme yeteneği, düşük toksisiteye sahip olma gibi sahip olma özellikleri sayesinde sudan sonra araştırmacılar tarafından en çok tercih edilen hidro-organik ikili karışımları asetonitril-su ve metanol-sudur [9,10]. Bu hidro-organik çözücü sistemleri içerisinde de asetonitril, çoğu organik asiti çözebilmesinin yanı sıra, nispeten yüksek dielektrik sabiti (ε=36) ve düşük otoproliz sabiti (pK_s=33.9) sayesinde iyonlaşma/protonasyon sabiti tayini için daha uygun bir çözücüdür [26]. Bu sebeplerden dolayı gerçekleştirilen bu çalışmada, asetonitril-su ikili karışımı hidroklorotiazidin iyonlaşma sabiti tayininde tercih edilmiştir.

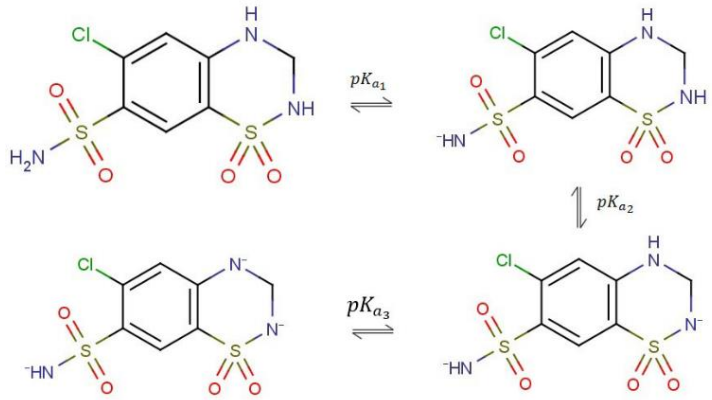
Gerçekleştirilen bu çalışmada hidroklorotiazidin yapısındaki sülfonamit gruplarından dolayı polar özellikte olması dolayısıyla (logP=-0.58) kolonda bileşiğin yeterli tutunumunu sağlamak ve gerçekleştirilen analiz süresini kontrol altında tutmak amacıyla çalışılan mobil faz organik modifiyer derişimi %11-%15 (h/h) aralığında seçilmiştir. Ayrıca, mobil faz pH'ları bileşiğin tamamen moleküler ve tamamen iyonik formlarda olduğu pH aralığı olarak seçilmiştir.

Hidroklorotiazidin çalışılan asetonitril ve su hidro-organik sisteminde farklı mobil faz pH'larına karşı elde edilen alıkonma değerleri (t_R) grafiğe geçirildiğinde, asidik fonksiyonel gruba özgü bir sigmoidal davranış gözlemlenmiştir (Şekil 1). Ancak, bileşiğin iyonlaşma dengesi incelenildiğinde, bileşiğin asidik merkezlerinden biri yapısındaki benzotiazidin halkasına bağlı sülfonamid grubundan, bir diğeri de yapısındaki benzotiazidin halkasındaki azottan kaynaklanmaktadır (Şekil 2). Bu durumda bileşiğin yapısındaki iki asidik merkezden dolayı, asidik fonksiyonel gruba özgü 2 sigmoidal davranış gözlemlenmesi beklenirdi. Bu durum, bileşiğin asidik merkezlerinin iyonlaşma sabiti değerlerinin (pK_{a_1} ve pK_{a_2}) arasında 2 birimden daha az fark olmasından dolayı asidik merkezlerin iyonlaşmalarının birbirinden ayrılamamasından kaynaklanmaktadır [7,9].



Şekil 1. Hidroklorotiazidin çalışılan asetonitril:su ikili karışımlarındaki pH-t_R ilişkisi

Şekil 2'de görülen bir diğer denge, benzotiazidin halkasındaki bazik azotun protonasyonundan (pK_{a_3}) kaynaklanmaktadır. Bu fonksiyonel grubun protonasyonunu ifade eden kromatografik veriyi elde edebilmek için, çalışılan sıvı kromatografik kolonun sınırları dışında çalışmak gerektiğinden, bu fonksiyonel grubun ilişkili olduğu pK_{a_3} değeri, bu çalışma kapsamı dışında bırakılmıştır.



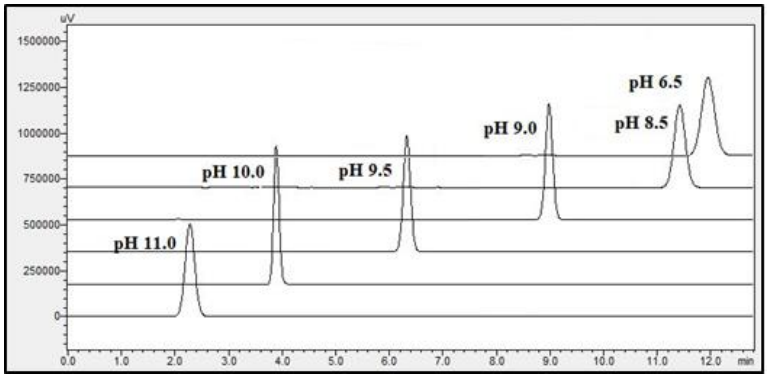
Şekil 2. Hidroklorotiazidin iyonlaşma ve protonasyon davranışı

RPLC yöntemle iyonlaşma sabiti tayini (pK_a), yapısında iyonlaşabilen fonksiyonel grup bulunduran bileşiğin farklı pH değerlerinde farklı alıkonma değerlerine (t_R) sahip olması temeline dayanır (Şekil 3). Bu pH-t_R ilişkisi sigmoidal bir davranış sergiler ve LSER modeliyle aşağıdaki eşitliğe göre matematiksel olarak ifade edilebilir (Eşitlik 1).

$$logt_R = logt_{R_{HA}} + \frac{U}{1 + \gamma . V . 10^{\pm(pH - pK_a)}} \quad (1)$$

Bu eşitlikte U ve V ayarlama katsayıları, γ ise molar aktivite katsayısını ifade eder. Bu katsayılardan U, $log(f)$'yi ifade ederken $\left(f = \frac{t_{R_{A^-}}}{t_{R_{HA}}}\right)$, V ise yapısında organik modifiyer içeren bir mobil fazda $\mp(pH - pK_a)$ değişimini ifade etmektedir.

Bu denklemde teorik log t_R değerleri, bir başlangıç pK_a değeri için U ve V değerleri sırasıyla 1 ve -4 alınarak hesaplanır [27]. Daha sonra, deneysel t_R değerlerinin logaritması ($logt_{R_{den.}}$) ve teorik olarak hesaplanan t_R değerlerinin logaritması ($logt_{R_{teo.}}$) kullanılarak artık değerler hesaplanır. LSER modeli, çalışılan her hidro-organik çözücü sistemi ve çalışılan tüm pH değerlerinde elde edilen deneysel t_R değerleri için tekrarlanır. En uygun logt_R değerleri, Microsoft Excel SOLVER programı kullanılarak artık kareler toplamının (RSS) en aza indirilmesiyle elde edilir. Tüm $logt_{R_{den.}}$ ve $logt_{R_{teo.}}$ değerleri için RSS değerini en aza indiren pK_a değeri, bileşiğin çalışılan mobil fazı için iyonlaşma sabitinin değeri olarak belirlenir [7,8].

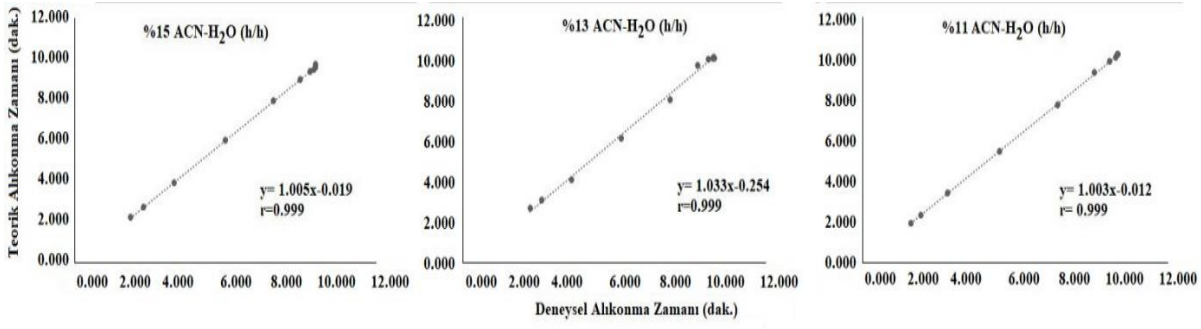


Şekil 3. Hidroklorotiazidin %11 ACN-Su ortamında (h/h) farklı pH değerlerinde elde edilen alıkonma değerlerini gösteren üst üste bindirilmiş kromatogram

LSER modelinde kullanılan formüle, mobil fazın her pH'sı için hesaplanan molar aktivite katsayılarının eklenmesiyle bileşiğin termodinamik iyonlaşma sabitinin değeri elde edilir. [7,8,16,24]. Asetonitril-su ortamlarında bu yöntem kullanılarak belirlenen $logt_{R_{den.}}$ ve $logt_{R_{teo.}}$ değerleri Tablo 1'de hidroklorotiazid için verilmiştir. Tablo 1'deki veriler incelendiğinde, RSS değerlerinin çok düşük olduğu görülmektedir. Ayrıca, çalışılan her asetonitril-su ortamı için deneysel t_R ve teorik t_R değerlerine göre çizilen grafiklerin eğim ve kesişim değerlerinin sırasıyla 1 ve 0'a yakın olması ve grafiklerin korelasyon katsayılarının en az 0.99 olması, doğrusal bir ilişkinin varlığını göstermektedir (Şekil 4). Bu kriterler, LSER modelinin deneysel verilerle tutarlı olduğunu ve hidroklorotiazidin iyonlaşma sabitini hesaplamak için kullanılabileceğini açıkça göstermektedir.

Tablo 1. Çalışılan tüm mobil fazlar için LSER modelinde kullanılan deneysel ve hesaplanan veriler

%15 ACN-H_2O (h/h)							
pH	**Hidroklorotiazid**						
	t_R (den.)	**t_R (teo.)**		**Molar Aktivite Katsayısı (ɣ)**	**logt_R (den.)**	**logt_R (teo.)**	**(log$t_{Rden.}$-log$t_{Rteo.}$)^2**
6.5	9.578	9.571	**U=-0.687; V=4.001 f=0.242**	0.784	0.981	0.981	1.014x10^{-07}
7	9.688	9.555		0.769	0.986	0.980	3.582x10^{-05}
7.5	9.479	9.505		0.747	0.977	0.978	1.409x10^{-06}
8	9.368	9.346		0.730	0.972	0.971	1.054x10^{-06}
8.5	8.953	8.948		0.808	0.952	0.952	6.837x10^{-08}
9	7.931	7.901		0.830	0.899	0.898	2.731x10^{-06}
9.5	6.012	6.001		0.866	0.779	0.778	6.292x10^{-07}
10	3.930	3.959		0.908	0.594	0.598	9.900x10^{-06}
10.5	2.747	2.728		0.942	0.439	0.436	8.936x10^{-06}
11.0	2.221	2.227		0.966	0.347	0.348	1.386x10^{-06}
***RSS: Artık Kareler Toplamı**					**RSS***		**6.203x10^{-05}**
%13 ACN-H_2O (h/h)							
6.5	10.000	9.992	**U=-0.614; V=4.000 f=0.265**	0.788	1.000	1.000	1.076x10^{-07}
7	10.059	9.976		0.772	1.003	0.999	1.305x10^{-05}
7.5	10.018	9.921		0.751	1.001	0.997	1.769x10^{-05}
8	9.969	9.751		0.734	0.999	0.989	9.197x10^{-05}
8.5	9.629	9.327		0.813	0.984	0.970	1.913x10^{-04}
9	7.975	8.231		0.834	0.912	0.915	8.672x10^{-06}
9.5	6.099	6.305		0.870	0.792	0.800	5.379x10^{-05}
10	4.078	4.317		0.911	0.638	0.635	5.594x10^{-06}
10.5	3.089	3.149		0.944	0.504	0.498	2.944x10^{-05}
11.0	2.649	2.675		0.967	0.423	0.427	1.772x10^{-05}
***RSS: Artık Kareler Toplamı**					**RSS***		**4.293x10^{-04}**
%11 ACN-H_2O (h/h)							
6.5	11.858	11.845	**U=-0.768; V=4.000 f=0.198**	0.791	1.074	1.074	2.192x10^{-07}
7	11.884	11.817		0.775	1.075	1.073	6.054x10^{-06}
7.5	11.680	11.725		0.754	1.067	1.069	2.836x10^{-06}
8	11.463	11.441		0.738	1.059	1.058	7.023x10^{-07}
8.5	10.851	10.749		0.821	1.035	1.031	1.690x10^{-05}
9	8.995	9.048		0.845	0.954	0.957	6.585x10^{-06}
9.5	6.346	6.348		0.883	0.803	0.803	1.601x10^{-08}
10	3.948	3.938		0.922	0.596	0.595	1.291x10^{-06}
10.5	2.700	2.707		0.952	0.431	0.432	1.285x10^{-06}
11.0	2.251	2.249		0.972	0.352	0.352	2.213x10^{-07}
***RSS: Artık Kareler Toplamı**					**RSS***		**2.044x10^{-05}**



Şekil 4. 6.5-11.0 pH aralığında teorik alıkonma zamanı ve deneysel alıkonma zamanı değerlerine göre çizilen grafik

Asetonitril-su ikili karışımlarında LSER modeli kullanılarak hesaplanan mobil faz termodinamik iyonlaşma sabiti değerleri ($^{s}_{s}pK_a$), Tablo 2'de verilmiştir. Tablo 2'de farklı asetonitril-su ikili karışımları için elde edilmiş termodinamik $^{s}_{s}pK_a$ değerleri, bileşiğin yapısındaki benzotiazidin halkasındaki azotun ve benzotiazidin halkasına bağlı sülfonamid grubundaki azotun iyonlaşmasından kaynaklanmaktadır. Yukarıda da ifade edildiği gibi, bileşiğin asidik merkezlerinin pK_a değerleri arasındaki farkın 2 pH biriminden düşük olmasından dolayı bu merkezler birlikte iyonlaşmaktadır. Bu durumun en önemli kanıtı, farklı asetonitril-su ikili karışımlarında bileşiğin pH-t_R ilişkilerine göre elde edilen tek sigmoidal davranıştır [7,9].

Tablo 2. Hidroklorotiazidin farklı asetonitril-su ikili karışımlarında elde edilen mobil faz termodinamik iyonlaşma sabiti değerleri

Parametre	%11 ACN-H_2O (h/h)	%13 ACN-H_2O (h/h)	%15 ACN-H_2O (h/h)	Literatür
$^{s}_{s}pK_{a_1}$	9.214	9.274	9.337	9.97 (27°C) [23] 9.93 (37°C) [23]

Tablo 2'deki $^{s}_{s}pK_a$ değerleri incelenildiğinde, asetonitril-su ikili karışımlarının organik modifiyer derişimi arttıkça $^{s}_{s}pK_a$ değerlerinin de bir miktar arttığı görülmektedir. Bu durum, asidik fonksiyonel gruba sahip bileşikler için beklenen bir durumdur [9,25,26]. Asetonitril-su ve diğer su-organik çözücü karışımlarında organik çözücü ilavesiyle suyun yapısı bozulduğundan analit solvasyonu azalır. Bu durumda denge kinetiği de azalacağından, analitin iyonlaşması da azalır. Sonuç olarak, bileşiğin iyonlaşma sabiti değeri artar [9,26]. Bu durum düşünüldüğünde, potansiyometrik olarak tek %80 (h/h) metanol içeren metanol-su ortamında iki farklı sıcaklıkta elde edilen $^{s}_{s}pK_a$ değerlerinin de bu çalışmada belirlenen değere yakınlığı görülmektedir [23]. Bu bilgiler ışığında, hesaplanan $^{s}_{s}pK_{a_1}$ değerlerinin literatür verisiyle yakınlığı açıkça görülmektedir. Hidroklorotiazidin termodinamik $^{s}_{s}pK_{a_1}$ değerlerini hesaplamada kullanılan molar aktivite katsayıları (γ), her mobil faz ve mobil faz pH değeri için klasik Debye-Hückel bağıntısı kullanılarak hesaplanmıştır [7,8,24,26].

Vücut ortamı su ortamı olduğundan, ilaçların vücuttaki iyonlaşma durumunu belirlemek adına, su ortamındaki iyonlaşma sabiti ($^{w}_{w}pK_a$) tayini ilaç araştırma ve geliştirme aşamasında mutlaka gereklidir. Su ortamında çözünürlüğü yetersiz bileşikler için ilk olarak bir su-organik çözücü ikili karışımında bileşiğin $^{s}_{s}pK_a$ değeri hesaplanır. Daha sonra, elde edilen $^{s}_{s}pK_a$ değerleri kullanılarak $E_T^N - {}^{s}_{s}pK_a$, $X_{\text{çözücü}} - {}^{s}_{s}pK_a$ ya da Yasuda-Shedlovsky yöntemi gibi çeşitli makroskopik çözücü parametreleri ile bileşiğin $^{s}_{s}pK_a$ değeri arasındaki ilişkiyi kullanan ekstrapolasyon metotlarıyla bileşiğin $^{w}_{w}pK_a$ değerleri hesaplanabilir [27].

Bu çalışmada elde edilen $^{s}_{s}pK_a$ değerleri, Yasuda-Shedlovsky ve $X_{ACN} - {}^{s}_{s}pK_a$ metotları ile değerlendirilerek hidroklorotiazidin $^{w}_{w}pK_{a_1}$ değeri hesaplanmıştır. Bu metotlardan $X_{ACN} - {}^{s}_{s}pK_a$ ilişkisi, asetonitril-su ikili karışımlarındaki asetonitrilin mol kesri değeri (X_{ACN}) ile bileşiğin bu hidro-

organik ortamda elde edilen ${}^{s}_{s}pK_a$ değerleri arasındaki doğrusal ilişkiyi temel alır. Bu elde edilen doğrusal ilişkinin kesim noktası bileşiğin termodinamik ${}^{w}_{w}pK_a$ değerini vermektedir.

Bu çalışmada tercih edilen bir diğer metot olan Yasuda-Shedlovsky ekstrapolasyon metodu, suca zengin bölgelerde yapılan çalışmalarda çok tercih edilmektedir. Bu metotta bileşiğin ${}^{w}_{w}pK_a$ değeri, çalışılan asetonitril-su ikili karışımının dielektrik sabitinin tersinin $(1/\varepsilon)$, ${}^{s}_{s}pK_a + \log[H_2O]$ değerine karşı grafiğe geçirilmesiyle elde edilen doğrusal eşitlikten hesaplanır. Elde edilen bu doğrusal eşitlikte, ilgili yerlere saf suyun dielektrik sabiti (ε=78.3) ve derişim değeri (log [H_2O]=log 55.5) yerlerine konularak bileşiğin ${}^{w}_{w}pK_{a_1}$ değeri elde edilir. Her iki metodun uygulanabilmesi için en az üç farklı derişimde asetonitril içeren asetonitril-su ortamında elde edilmiş ${}^{s}_{s}pK_a$ değerlerine ihtiyaç vardır [27,28]. Bu çalışmada $X_{ACN} - {}^{s}_{s}pK_{a_1}$ metodundan elde edilen doğrusal eşitliğin verileri Tablo 3'te, Yasuda-Shedlovsky metodundan elde edilen doğrusal eşitliğin verileri Tablo 4'te standard sapma değerleriyle birlikte verilmiştir. Ayrıca her iki yöntemden elde edilmiş ${}^{w}_{w}pK_a$ değerleri, Tablo 5'de verilmiştir.

Tablo 3. $X_{ACN} - {}^{s}_{s}pK_a$ yönteminden elde edilen doğrusal eşitlik ile ilgili veriler

Bileşik	%ACN(h/h)	Eşitlik	r	N
Hidroklorotiazid	11-15	${}^{s}_{s}pK_{a_1} = 7.693.X_{ACN}\ (0.044) + 8.904\ (0.002)$	0.999	3

Tablo 4. Yasuda-Shedlovsky yönteminden elde edilen doğrusal eşitlikle ilgili veriler

Bileşik	%ACN (v/v)	ε	${}^{s}_{s}pKa_1 + \log[H_2O] = a_e\,\varepsilon^{-1} + b_\varepsilon$		r	N
			a (eğim)	B (kesim)		
Hidroklorotiazid	11-15	74.655-73.101	425.4 (0.048)	5.256 (0.001)	0.999	3

Tablo 5. Hidroklorotiazidi iki farklı yöntemle hesaplanan termodinamik ${}^{w}_{w}pK_{a_1}$ değerleri

Bileşik	Parametre	$X_{ACN} - {}^{s}_{s}pK_a$	Yasuda-Shedlovsky metot
Hidroklorotiazid	${}^{w}_{w}pK_{a_1}$	8.904	8.945

Tablo 5'de deneysel olarak belirlenen ${}^{s}_{s}pK_{a_1}$ değerlerinin farklı ekstrapolasyon yöntemleriyle değerlendirilerek hesaplanan termodinamik ${}^{w}_{w}pK_{a_1}$ değerleri arasındaki uyum dikkat çekicidir. Bu bilgiler ışığında, hesaplanan ${}^{s}_{s}pK_{a_1}$ değerlerinin doğruluğu ve tekrarlanabilirliği açıkça görülmektedir. Ayrıca, Tablo 5'de sunulan değerler hidroklorotiazidin herhangi bir analitik yöntemle tayin edilen literatürdeki ilk termodinamik ${}^{w}_{w}pK_{a_1}$ verileridir. Bu çalışmayla elde edilen sonuçlar bileşiğin farmakokinetik çalışmalarına ışık tutacağı gibi, aynı zamanda bileşiğin asetonitril-su ikili karışımlarında gerçekleştirilen optimizasyon çalışmalarında da yararlı olabilecektir.

YAZAR KATKILARI

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ÇIKAR ÇATIŞMASI BEYANI

Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler.

ETİK KURUL ONAYI

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

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PREPARATION AND *IN-VITRO* CHARACTERIZATION OF CONTACT LENSES CONTAINING COENZYME Q10 LOADED MICELLES

KOENZİM Q10 YÜKLÜ MİSEL İÇEREN KONTAKT LENSLERİN HAZIRLANMASI VE İN-VİTRO KARAKTERİZASYONU

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ABSTRACT

Objective: *Coenzyme Q10 (CoQ10) offers potential therapeutic benefits for ocular health, yet faces challenges of poor solubility and bioavailability when applied to the eye. This study aimed to enhance CoQ10 delivery using contact lenses by incorporating CoQ10-loaded polymeric micelles, using Pluronic F127 and solvent evaporation technique.*
Material and Method: *Polymeric micelles encapsulating CoQ10 were produced via solvent evaporation with Pluronic F127. Commercial contact lenses were subsequently loaded with these micelles. Characterization of the loaded lenses included assessments of light transmittance, swelling behavior, and drug release profile under non-sink conditions, simulating the constraints of the ocular surface.*
Result and Discussion: *The unloaded lenses exhibited a light transmittance of 91.78±3.29% and swelling percentage of 47.51±4.45% while micelle-loaded lenses demonstrated high light transmittance levels (95.31±0.80%), ensuring optical clarity. Swelling studies showed a slight increase in size to 48.1±4.4%. The lenses effectively encapsulated 403.6±21.8 µg of CoQ10. In vitro release profile exhibited controlled release over six hours, indicating potential for sustained drug delivery. These results highlight the feasibility of micelle-loaded contact lenses for efficient ocular drug delivery, warranting further exploration into their long-term effectiveness and safety.*
Keywords: *Coenzyme Q10, contact lens, polymeric micelle, solubility enhancement*

ÖZ

Amaç: *Koenzim Q10 (CoQ10), göz sağlığı için potansiyel terapötik faydalar sunmakta, ancak göze uygulandığında düşük çözünürlük ve biyoyararlanım sorunlarıyla karşı karşıya kalmaktadır. Bu çalışma, CoQ10 taşıyan polimerik miselleri Pluronic F127 ve çözücü buharlaştırma tekniği kullanarak kontakt lenslere yükleyerek CoQ10 teslimatını artırmayı amaçlamaktadır*
Gereç ve Yöntem: *Pluronic F127 ile çözücü buharlaştırma yöntemi kullanılarak CoQ10 kapsüllenmiş polimerik miseller üretildi. Ticari kontakt lensler daha sonra bu misellerle yüklendi. Yüklenmiş lenslerin karakterizasyonu, ışık geçirgenliği, şişme davranışı ve göz yüzeyinin kısıtlamalarını taklit eden olmayan emilim koşulları altında ilaç salım profili değerlendirmelerini içermektedir.*
Sonuç ve Tartışma: *Yüklenmemiş lensler %91.78±3.29 ışık geçirgenliği ve %47.51±4.45 şişme oranı sergilerken, misel yüklü lensler yüksek ışık geçirgenliği seviyeleri (%95.31±0.80) göstererek optik berraklığı sağlamıştır. Şişme çalışmaları boyutta hafif bir artışa (%48.1±4.4) işaret*

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etmektedir. Lenslere, etkili bir şekilde 403.6±21.8 µg CoQ10 yüklenmiştir. İn vitro salım profili altı saat boyunca kontrollü salım göstermiş, sürekli ilaç salımı için potansiyel olduğu belirtilmiştir. Bu sonuçlar, etkin oküler ilaç taşıma için misel yüklü kontakt lenslerin uygulanabilirliğini vurgulamakta ve uzun vadeli etkinlik ve güvenliklerinin daha fazla araştırılmasını gerektirmektedir.
Anahtar Kelimeler: *Çözünürlük arttırma, koenzim Q10, kontakt lens, polimerik misel*

INTRODUCTION

Drug-loaded contact lenses represent a novel and promising approach in ocular drug delivery, offering several advantages over conventional eye drops. These specialized contact lenses are designed to provide sustained and controlled release of therapeutics directly to the eye, enhancing drug bioavailability and reducing systemic side effects. The incorporation of drugs into contact lenses can be achieved through various techniques, such as soaking the lenses in drug solutions, embedding drugs within the lens matrix, or attaching drug-loaded nanoparticles to the lens surface. This technology is particularly beneficial for treating chronic eye conditions like glaucoma, dry eye syndrome, and infections, as it maintains a therapeutic level of the drug in the tear film for extended periods, ensuring more effective treatment. Additionally, drug-loaded contact lenses improve patient compliance by reducing the frequency of drug administration and eliminating the discomfort often associated with eye drops [1-3].

Coenzyme Q10 (CoQ10), often referred to as ubiquinone, is a lipid-soluble compound similar to vitamins, essential for the generation of energy at the cellular level and known for its strong antioxidant capabilities. Although its benefits in cardiovascular and neurological health is widely recognized, CoQ10 has also garnered attention in the field of ophthalmology, particularly for its potential in treating eye conditions such as age-related macular degeneration (AMD) and glaucoma [4,5]. However, the application of CoQ10 in ocular therapies is challenging due to its poor water solubility, high molecular weight, chemical sensitivity, and therefore limited bioavailability [6,7]. The molecule's lipophilic nature hampers its effective absorption and penetration into the eye, necessitating the development of innovative delivery systems. To address these challenges, researches have focused on various formulation strategies, including lipid-based nanocarriers, surfactant-aid solubilization, and liposomes, to enhance the solubility, and ocular bioavailability of CoQ10 [5,8]. These advanced delivery systems aim to improve the penetration of CoQ10 into the eye, thereby maximizing its therapeutic potential. In this study, we encapsulated it into the polymeric micelles to increase the solubility and therapeutic activity.

Polymeric micelles are nanoscopic structures formed by the self-assembly of amphiphilic block copolymers in an aqueous solution. These unique structures, typically in the range of 10 to 100 nm, consist of a hydrophobic core and a hydrophilic shell. The hydrophobic core enables the encapsulation of lipophilic drugs, improving their solubility, and bioavailability, which is a significant advantage in drug delivery applications [9,10]. The hydrophilic shell, usually composed of polyethylene glycol (PEG), imparts stealth characteristics to the micelles, reducing opsonization and prolonging circulation time in the bloodstream. This feature is particularly advantageous in passive targeting of tumors via the enhanced permeability and retention (EPR) effect [11]. Polymeric micelles are also explored for their potential in targeted drug delivery, capable of being functionalized with ligands to recognize and bind to specific cell types. Additionally, stimuli-responsive polymeric micelles, which disassemble or change properties in response to pH, temperature, or enzymatic activity, have shown promise in achieving controlled and site-specific drug release [12,13].

In the micelle preparation, Pluronic F127 copolymer was used. Pluronic F127, a triblock copolymer composed of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) blocks, exhibits unique micellization properties due to its hydrophilic-hydrophobic balance. The physical properties of Pluronic F127, characterized by a higher number of hydrophobic PPO units and a higher ratio of hydrophobic PO to hydrophilic EO units, contribute to its micellization behavior and stability in aqueous solutions[14]. The stability and micellization properties of Pluronic F127 are crucial factors in its potential applications for drug delivery and other biomedical uses [15,16].

In this study, we utilized hydrophobic CoQ10 for its therapeutic potential in ocular applications, acknowledging the challenge posed by its high lipophilicity in drug delivery to the eye. Main formulation strategies are incorporating a significant proportion of surfactants or polymeric micelles, known for their solubility-enhancing ability to enhance solubility and bioavailability. However, given the inherent issue of rapid drainage through the nasolacrimal duct with aqueous micellar dispersions, we innovatively employed contact lenses as a delivery system. This approach not only mitigates the drainage issue but also potentially increases the residence time and bioavailability of CoQ10 on the ocular surface. The study extensively characterizes both the CoQ10-loaded micelles and their integration into contact lenses, offering new insights into the effectiveness of this novel ocular drug delivery system.

MATERIAL AND METHOD

Materials

All solutions in this study were meticulously prepared using reagent grade chemicals and ultra-pure water (18 MΩ cm). The HPLC grade Acetonitrile and Ethanol were procured from Supelco, Germany, and Aklar Kimya, Turkey, respectively. Coenzyme Q10 was sourced from Abcr, Germany. Pluronic F127, used as a surfactant, was obtained from Merck, Germany. Contact lenses were (Dailies Total 1®) were bought from optic market. Dailies Total 1 water gradient contact lenses are designed with a 14.1mm diameter, a base curve of 8.5mm, and a central thickness measuring 0.09mm for a -3.00D prescription. Lenses made from a substance known as delefilcon A, these lenses possess a modulus of 0.7MPa. Delefilcon A is composed of a silicone hydrogel core with a 33% water content and an outer hydrogel layer with an 80% water content. The inorganic salts, sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), Calcium chloride ($CaCl_2$), and potassium chloride (KCl), also from Merck, Germany, were used to prepare physiological buffer solution for dissolution test.

Preparation of CoQ10-Loaded Polymeric Micelles

The CoQ10-loaded polymeric micelles were prepared using a reverse-phase evaporation method [10]. Initially, CoQ10 and Pluronic F127 were dissolved in 3 ml of chloroform to form a homogenous solution (Table 1). This solution was then added dropwise to 10 ml of distilled water under continuous stirring. The purpose of this gradual addition was to ensure the formation of micelles with CoQ10 encapsulated in the core surrounded by the Pluronic F127 corona. After the complete addition of the chloroform solution, the mixture was left to stir 700 rpm overnight. This step was critical to facilitate the evaporation of the organic solvent, leading to the self-assembly of micelles. The stirring process was carefully monitored to ensure a gentle and consistent mixing, which is crucial for the formation of uniform micelles. Following the evaporation of chloroform, the micelle suspension was further processed to remove any undissolved active substance. This was achieved by filtering the suspension through a 0.22 µm membrane filter. The filtration process not only removed the undissolved CoQ10 but also helped in obtaining a clear micelle solution, which is essential for subsequent characterization and application. Finally, the obtained micelle suspension was subjected to thorough characterization. The characterization process aimed to assess the size, zeta potential and encapsulation efficiency of CoQ10 of the prepared micelles. This step is crucial to ensure that the micelles meet the required standards for their intended use in drug delivery applications.

Table 1. Formulation components

Components	F1	F2	F3
Pluronic F127 (%)	2.00	2.00	2.00
CoQ10 (%)	0.50	0.75	1.00

Drug Loading onto Contact Lenses

The process of drug loading onto contact lenses was meticulously conducted using commercially

available Dailies Total 1® contact lenses. The procedure initiated with the preparation of the lenses, which were left to dry overnight. This drying step is crucial as it ensures the removal of any moisture that could potentially interfere with the subsequent drug loading process. Once the lenses were adequately dried, they were immersed in the F1 formulation for diffusion-mediated loading. The drug loading process was facilitated by placing the lenses in a shaking water bath set at 37°C with a speed of 75 rpm over the night. This controlled environment, including the temperature and agitation, was meticulously maintained to optimize the drug loading onto the lenses.

Following the incubation, the drug-loaded lenses were carefully retrieved and gently dried using a clean tissue. This step is important to remove any excess formulation from the lens surface, preparing them for subsequent characterization. The final step involved a comprehensive characterization of the drug-loaded lenses. The characterization of the lenses is a crucial phase, as it ensures the suitability and safety of the lenses for ocular use.

Characterization of Polymeric Micelles

In this study, the characterization of micelles was conducted with a focus on analyzing the particle sizes, distributions, and zeta potentials of the micelles. The size, size distribution, and polydispersity index (PDI) of the micelles were determined using Dynamic Light Scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments, UK). These measurements were performed at 25°C using a He-Ne laser (633 nm) at a scattering angle of 173 degrees (n=4). Disposable Zetasizer cuvettes were employed micelles and each sample was measured four times to ensure accuracy and reproducibility of the data. Additionally, the zeta potential of the micelle formulations was also assessed using the Zetasizer. This involved the use of specific cuvettes designed for zeta potential measurements, with each formulation being measured three times.

Characterization Studies Micelle-Loaded-Contact Lenses

Light Transmittance: In this study, the light transmittance of micelle loaded and unloaded contact lenses was quantitatively assessed using a UV-Vis spectrophotometer. Each lens type was fully immersed in deionized water and placed optical path of the UV. Transmittance was measured at a wavelength of 480 nm, within the visible light spectrum. An air was used as a blank to calibrate the spectrophotometer for each set of measurements. To ensure reliability, each transmittance measurement was performed in triplicate [17].

Water Retention and Swelling Determination: The dry weight of the lenses (W_{dry}) was initially measured. The lenses were then immersed in water and incubated for 24 hours. Subsequently, the surface water was blotted off with a tissue, and the final weights of the swollen lenses ($W_{swollen}$) were recorded. The percentage of swelling was calculated using the following equation:

Swelling Percentage = $[(W_{swollen} - W_{dry}) / W_{dry}] \times 100$ [17].

The encapsulation efficiency of CoQ10 within the polymeric micelles was quantitatively analyzed using a UV spectrophotometer. A 100 µl aliquot of the micelle solution was first diluted with 1 ml of acetonitrile to ensure the proper dissolution of CoQ10 for analysis. This step was done to disrupt the micellar structure, releasing the encapsulated CoQ10 into the acetonitrile. The diluted samples were then subjected to UV analysis following a validated method specific for CoQ10 quantification at 274 nm [18]. The encapsulation efficiency was calculated using Equation 1 [16].

$$\text{E. E. (\%)} = \frac{\text{Loaded CoQ10 in micelles}}{\text{Total CoQ10 weight}} \times 100 \quad (1)$$

***In Vitro* CoQ10 Release Study from Contact Lenses**

The study focused on determining the release profile of the active substance from contact lenses loaded with CoQ10 micelle formulations. After successfully loading the micelles onto the contact lenses, the lenses were subjected to active substance release studies conducted in a shaking water bath. The

release studies were performed at 37°C with a shaking speed of 75 rpm, using 20 ml of simulated tear fluid as the dissolution medium. The simulated tear fluid was prepared by dissolving 0.68 g of NaCl, 0.22 g of $NaHCO_3$, 0.008 g of $CaCl_2$, and 0.14 g of KCl in 1000 ml of water, mimicking the natural ocular environment [19]. The dissolution studies were carried out under non-sink conditions to closely replicate physiological conditions.

At predetermined time points – specifically at 0.5, 1, 2, 3, 4, and 6 hours – aliquots of 0.5 ml were withdrawn from the dissolution medium. These samples were then diluted with acetonitrile and analyzed using UV spectrophotometry to determine the concentration of the released active substance at 274 nm ($\lambda_{acetonitrile}$). To maintain the volume consistency in the dissolution medium, an equivalent volume of fresh simulated tear fluid was added back after each sampling. The entire process was replicated thrice (n=3) for each formulation to ensure the reliability and reproducibility of the results.

Statistical Analysis

Statistical evaluations were carried out using a one-way analysis of variance (ANOVA) using Stat-Ease's Design Expert software version 13.0.5.0 (Minneapolis, MN, USA). Differences between formulations were deemed statistically significant at a p-value threshold of less than 0.05.

RESULT AND DISCUSSION

Preparation of Polymeric Micelles

In our study, polymeric micelles were formulated using Pluronic F127 polymer. Pluronic F127 is a triblock copolymer that is well-recognized for its thermoresponsive properties and its ability to form stable micelles with a hydrophobic core and hydrophilic shell in aqueous solutions. The core provides a reservoir for hydrophobic drugs, like CoQ10, enhancing their solubility and stability within biological systems [16,20]. The solvent evaporation technique is particularly advantageous for encapsulating hydrophobic drugs. This method involves dissolving both the drug and the polymer in a common volatile organic solvent, followed by the gradual removal of the solvent, leading to the self-assembly of the polymer into micelles with the drug encapsulated within the core [21]. This approach allows for the fine-tuning of micelle size and drug loading, crucial for ensuring efficient drug delivery and release kinetics [22].

The results presented in Table 2 suggest that components influence on the characteristics of CoQ10 loaded polymeric micelles. Across the three formulations, F1, F2, and F3, with increasing concentrations of CoQ10 from 0.50% to 1.00%, a trend of increasing particle size is observed ($p<0.05$). Specifically, the average size of the micelles ranges from 93.62 nm in F1 to 110.52 nm in F3, with corresponding standard errors, indicating a measure of consistency in particle size distribution within each formulation batch.

The PDI values for all formulations are relatively low, with a range of 0.146 to 0.214, suggesting a uniform size distribution among the micelle populations (Figure 1). Uniformity in micelle size is desirable in drug delivery systems for predictable bio-distribution, and drug release profiles.

Zeta potential values, which provide insight into the surface charge and colloidal stability of the micelles, are relatively similar across all formulations, hovering around -15 mV. This negative surface charge is indicative of the stability of the micelle in suspension, as particles with zeta potentials beyond ±30 mV typically exhibit strong electrostatic repulsion, preventing aggregation [23].

Encapsulation efficiency, an important parameter indicating the proportion of CoQ10 successfully incorporated into the micelles, appears to decrease with increasing CoQ10 concentration. F1 shows the highest encapsulation efficiency at 86.02%, while F3, with double the CoQ10 content, shows a reduced efficiency of 54.79%. This trend might suggest a saturation point in the micelle's capacity to encapsulate CoQ10, highlighting the need for a balance between drug load and micellar stability.

These findings provide valuable insights for optimizing CoQ10-loaded polymeric micelle formulations. According to results, F1 formulation was selected in terms of size and encapsulation efficiency.

Table 2. Characterization results of micelles

Formül	Pluronic F127 (%)	CoQ10 (%)	Size±SE (nm) (n=4)	PDI±SE (n=4)	Zeta Potential±SE (mV) (n=4)	Encapsulation Efficiency %±SE (n=3)
F1	2	0.50	93.62±0.44	0.214±0.01	-15.4±0.30	86.02±1.94
F2	2	0.75	103.7±2.11	0.198±0.01	-16.0±0.07	68.41±2.31
F3	2	1.00	110.5±2.70	0.146±0.01	-15.1±0.45	54.79±0.09

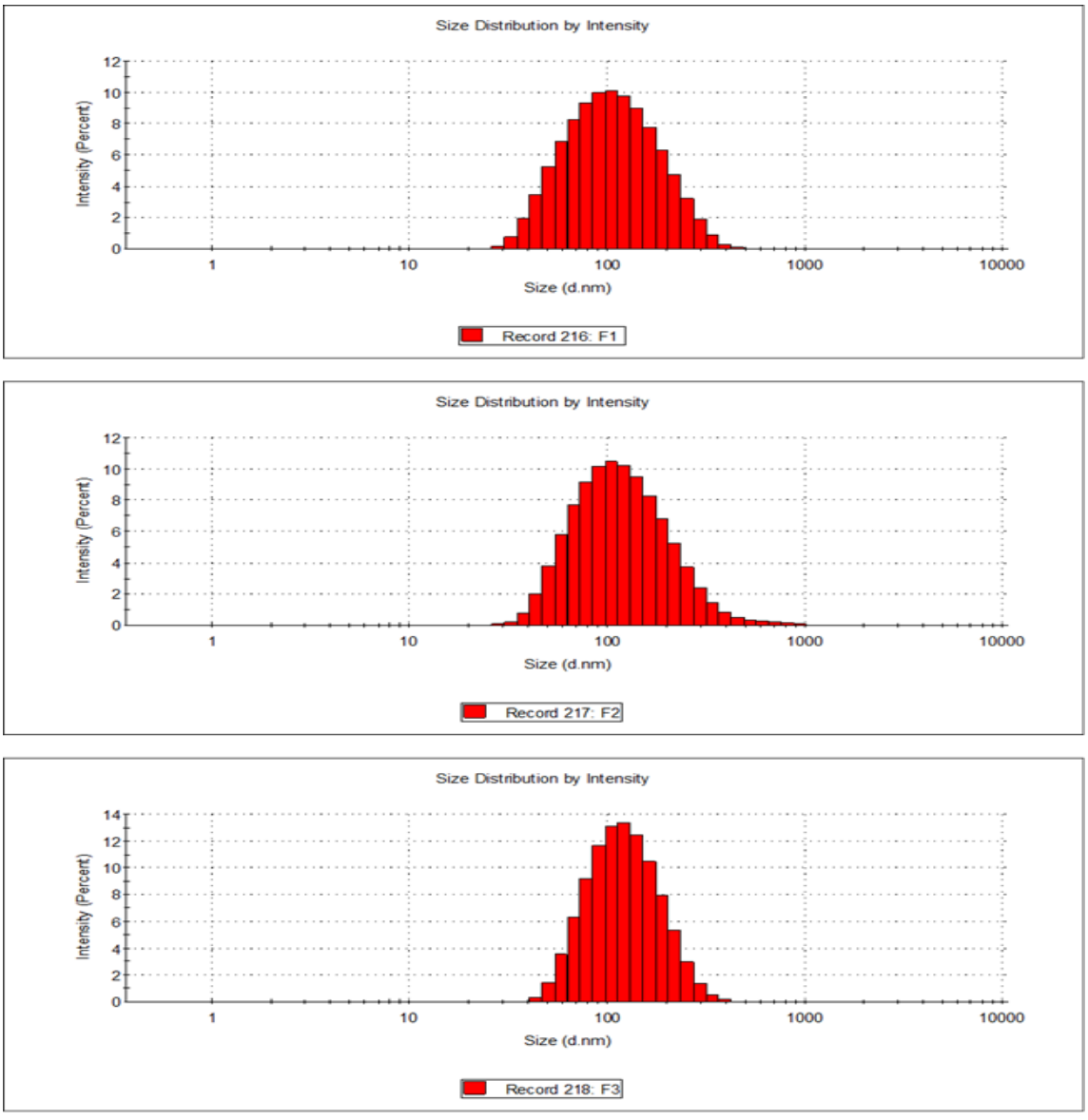

Figure 1. Particle size distribution of micelles

Characterization of Contact Lenses

Characterization studies in contact lenses involve a range of physical characterization techniques to assess their properties. These techniques include transparency, oxygen permeability, mechanical investigation, glass transition temperature, wettability, and water content [24-26]. Furthermore, the release of active pharmaceutical ingredients from drug-eluting contact lenses can be studied using techniques such as UV-Vis spectroscopy to evaluate drug release kinetics. Biocompatibility studies involving cell culture and tissue interaction assays are also essential to assess the safety and

compatibility of contact lenses with ocular tissues [27-29]. These physical characterization techniques provide comprehensive insights into the properties of contact lenses, ensuring their suitability for ophthalmic applications. However, we have used two of them transmittance (transparency) and water content (swelling) like the vast majority studies [25,30,31].

In the characterization studies performed on contact lenses, the F1 formulation was incubated with dried contact lenses to facilitate the loading of the formulation. The study presents an investigation into the loading efficiency and optical clarity of a drug formulation designated as F1 on contact lenses. The incubation of the dried lenses with the F1 formulation resulted in a substantial uptake of the drug, quantified at 403.6±21.8 µg per lens, showcasing the potential of these lenses as a medium for drug delivery.

The pure contact lens showed a swelling percentage of 47.51±4.45%. After loading with CoQ10 micelles, this increased marginally to 48.1±4.4%. This small increase suggests that the incorporation of the micelles into the lens matrix slightly enhances its ability to absorb and retain moisture. The change is relatively minimal and not significant statistically.

There is a more noticeable improvement in light transmittance from 91.78±3.29% in the pure lenses to 95.31±0.80% in the micelle-loaded lenses. This increase in light transmittance suggests that the incorporation of CoQ10 micelles improves the clarity of the lenses. Higher light transmittance in contact lenses is generally desirable as it implies better visibility for the wearer (Figure 2).

Figure 2. Contact lenses containing CoQ10-loaded polymeric micelles

The increase in light transmittance observed in the micelle-loaded contact lenses may be attributed to the unique properties of the micelles. This can be explained that micelles might have a refractive index that is closer to the contact lens material compared to the air or moisture in the pores of the pure lens. This closer match can reduce the scattering of light, allowing more light to pass through the lens, thus increasing its transmittance. This results were in concordance with previous study conducted by Mun et al. [32].

This balance between drug loading capacity and the retention of optical properties underscores the potential of the F1-loaded contact lenses as a promising platform for ocular drug delivery [33].

The release profile of the F1 formulation from contact lenses demonstrates a biphasic drug release over a six-hour period (Figure 3). An initial burst release occurs within the first hour, with approximately 40% of the drug being released, which is typical for systems where the drug is near the surface or readily diffusible. Subsequently, the release rate reaches a plateau between the first and fourth hours, indicating a transition to a more controlled release phase. This suggests that the remaining drug is being released more slowly, likely from deeper within the contact lens matrix. Towards the sixth hour, there is a notable increase in drug release, possibly due to increased hydrogel matrix swelling, enhancing the diffusion of the drug. The standard deviation represented by the error bars implies some variability but overall consistency in the release mechanism, indicating a reproducible drug release system. Such a profile is advantageous for providing sustained therapeutic levels of medication in ocular applications.

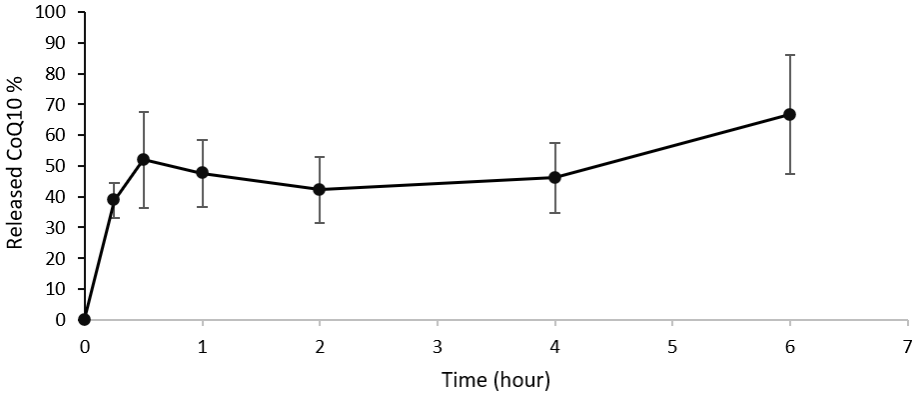

Figure 3. CoQ10 release from contact lenses

In vitro release study was performed with only CoQ10 micelle-loaded contact lenses. Because of the highly lipophilicity and using non-sink condition, pure CoQ10 can not dissolve. The release profile observed from the F1 formulation-laden contact lenses under non-sink conditions in this study are crucial for ocular drug delivery systems, providing insights into the *in vivo* behavior of sustained-release formulations. The non-sink conditions, characterized by the limited volume and dynamic nature of the ocular fluid, lead to a significant initial burst release, which may offer an immediate therapeutic effect upon administration [34]. This is followed by a plateau phase, indicative of a controlled release that could maintain therapeutic drug levels over an extended period. The final uptick in release at later hours could suggest a secondary release mechanism, possibly influenced by lens swelling within the tear film.

As a conclusion, this study successfully demonstrates the potential of contact lenses as a novel delivery system for CoQ10, utilizing polymeric micelles to overcome the challenges associated with its hydrophobic nature. The formulation of CoQ10 within Pluronic F127-based micelles, prepared through the solvent evaporation technique, effectively enhanced its solubility. The subsequent loading of these micelles onto contact lenses resulted in a promising delivery platform, as evidenced by the controlled and sustained release profile observed under non-sink conditions. The swelling behavior of the lenses also indicated a moderate increase in size, which is crucial for comfort and functionality. Overall, these findings highlight the viability of using CoQ10-loaded micelle contact lenses for ocular drug delivery, presenting an innovative approach that could potentially improve therapeutic outcomes in eye-related treatments.

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AUTHOR CONTRIBUTIONS

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

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

DEVELOPMENT AND VALIDATION OF A NEW UHPLC-DAD APPROACH FOR ATOMOXETINE DETECTION IN SEVERAL MEDICINAL PLANTS

BAZI TIBBİ BİTKİLERDE ATOMOKSETİN TAYİNİ İÇİN YENİ BİR UHPLC-DAD YÖNTEMİNİN GELİŞTİRİLMESİ VE VALİDASYONU

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ABSTRACT

Objective: *Atomoxetine (ATX) is a medication that is extensively used to treat attention deficit hyperactivity disorder in children, adolescents, and adults. The goal of this work was to create a speedy, easy, and sensitive ultra high performance liquid chromatographic method (UHPLC) for the measurement of atomoxetine in various medicinal plants. (Salvia officinalis L., Rosmarinus officinalis L., Melissa officinalis L., Ginkgo biloba L.).*
Material and Method: *Prior to chromatographic separation, liquid-liquid extraction was applied, which is currently the preferred extraction technique due to its simple, fast and efficient procedure for sample preparation. The chromatographic separation was achieved by reversed phase C18 (5 µm × 4.6 mm × 150 mm) analytical column and a mobile phase consisting of monobasic potassium dihydrogen orthophosphate (pH=6.8) and acetonitrile (50:50 v/v) at flow rate of 0.8 ml/min and diode array detector (DAD) detecting at 215±2 nm.*
Result and Discussion: *The envisioned method's linear behavior was tested in the 0.5-20 µg/ml range (r^2=0.09990). In compliance with International Conference on Harmonisation (ICH) criteria, the method received validation by means of accuracy, precision, repeatability, specificity, robustness, and detection and quantification boundaries. LOD and LOQ values were determined as 0.16 and 0.5 µg/ml. RSD values for hourly and daily measurements are found to be below 2.5% for both assays. The proposed method can be used effectively for quantification of atomoxetine in medicinal and aromatic plants. The proposed analytical procedure represents an efficient method for the quantification and routinee analysis of atomoxetine in medicinal and aromatic plants.*
Keywords: *Atomoxetine, attention deficit hyperactivity disorder, diode array detector (DAD), medicinal plants, ultra high performance liquid chromatography (UHPLC)*

ÖZ

Amaç: *Atomoksetin (ATX) çocuklarda, ergenlerde ve yetişkinlerde dikkat eksikliği hiperaktivite bozukluğunun tedavisinde yaygın olarak kullanılmaktadır. Bu çalışmada, bazı tıbbi bitkilerde (Salvia officinalis L., Rosmarinus officinalis L., Melissa officinalis L., Ginkgo biloba L.) atomoksetin analizi için hızlı, basit ve hassas bir ultra yüksek performanslı sıvı kromatografik yöntem (UHPLC) geliştirilmesi amaçlanmıştır.*
Gereç ve Yöntem: *Kromatografik ayırmadan önce, numune hazırlama için basit, hızlı ve verimli prosedürü nedeniyle günümüzde tercih edilen ekstraksiyon tekniği olan sıvı-sıvı ekstraksiyonu uygulanmıştır. Kromatografik ayırma, ters fazlı C18 (5 µm × 4.6 mm × 150 mm) analitik kolon ve*

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monobazik potasyum dihidrojen ortofosfat (pH=6.8) ve asetonitrilden (50:50 h/h) oluşan bir mobil fazda 0.8 ml/dk akış hızında ve 215±2 nm'de diyot dizisi dedektörü (DAD) tespiti ile sağlandı.
Sonuç ve Tartışma: *Önerilen yöntemin doğrusallığı 0.5-20 µg/ml (r^2=0.9990) aralığında incelenmiştir. Yöntem, Uluslararası Uyumlaştırma Konferansı (ICH) yönergelerine uygun olarak doğruluk, kesinlik, tekrarlanabilirlik, özgüllük, sağlamlık ve dedeksiyon ve kantitasyon limitleri açısından doğrulanmıştır. LOD ve LOQ sırasıyla 0.16 ve 0.5 µg/ml olarak bulundu. Gün içi ve günler arası RSD değerleri her iki test için de %2.5'in altındadır. Önerilen yöntem, tıbbi ve aromatik bitkilerde atomoksetin miktarının belirlenmesi için etkin bir şekilde kullanılabilir. Önerilen analitik prosedür, tıbbi ve aromatik bitkilerde atomoksetinin miktarının belirlenmesi ve rutin analizi için etkili bir yöntemi temsil eder.*
Anahtar Kelimeler: *Atomoksetin, dikkat eksikliği hiperaktivite bozukluğu, diyot dizisi dedektörü (DAD), tıbbi bitkiler, ultra yüksek performanslı sıvı kromatografisi (UHPLC)*

INTRODUCTION

The condition that is most frequently identified and treated in children is attention-deficit hyperactivity disorder (ADHD), which affects 5-12% of children and adolescents globally. It has been linked with significant morbidity and worse results later in life [1,2]. The first non-stimulant oral selective norepinephrine reuptake inhibitor is atomoxetine hydrochloride (ATX) [(-)-N-methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride] shown in first figure. In November 2002, the US Food and Drug Administration legally adviced it positively as a usable drug ingredient for ADHD in people aged 6 and up [3,4].

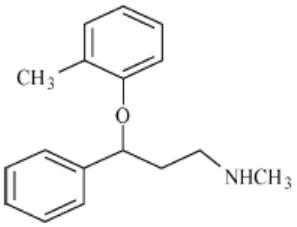

Figure 1. Chemical structure of ATX

There is no known precise mechanism through which ATX exerts its therapeutic effects in ADHD. The ATX has minimal affinity for other neural transporters or neurotransmitter receptor sites and increases norepinephrine function by blocking the presynaptic norepinephrine transporter in a highly selective manner. This retains a larger level of norepinephrine active in the brain's inter-neuron junctions [5-7]. The clearance of ATX displayed a bimodal distribution in studies with this molecule in healthy human volunteers, indicating that an enzyme with a genetic variation was involved in the metabolism of ATX [8].

Many ailments have been attempted to be treated with plants throughout the history of mankind. According to the World Health Organization (WHO), 80% of the world's population- roughly 4 billion people- first tried using herbal medicines to treat their health issues. Additionally, active compounds with a plant origin make up around 25% of prescription medications in affluent nations (e.g., vimbilastine, reserpine, quinine, aspirin). discovering new applications for medicinal and aromatic plants, rising the need for natural goods; the use of these plants is expanding daily [9].

So far, the reported analytical methods for atomoxetine include GC [10], LC-MS [11], and HPLC-UV [12]. None of these studies included the determination of atomoxetine in medicinal plant extracts using the HPLC-DAD method. In this study, a DAD-detection UHPLC approach was established to analyze ATX at a level of µg/ml in a number of medicinal herbs. Liquid-liquid (LLE) extraction is used as a sample pretreatment process because medicinal plants have a complex matrix. LLE features a quicker and less complicated technique than solid-phase extraction (SPE) and ultrasonic assisted extraction. A straightforward analytical technique, LLE combination to UHPLC, has been devised in the proposed investigation for ATX in several medicinal plants (*Salvia officinalis* L., *Rosmarinus*

officinalis L., *Melissa officinalis* L., *Ginkgo biloba* L.).

MATERIAL AND METHOD

Plant Materials and Chemicals

Plant samples (*Salvia officinalis* L., *Rosmarinus officinalis* L., *Melissa officinalis* L., *Ginkgo biloba* L.) were purchased from local markets (herbalist).

Shanghai Yingxuan Pharmaceutical Science & Technology (China) provided the ATX. Solvents other than plant samples and reagents were analytical quality from Merck (Germany), with the exception of acetonitrile, phosphate buffer, NaOH, and ethanol which were HPLC grade. The water that has been purified using the Milipore Direct-Q system.

Plants and Treatment Solution Preparation

A standard solution of 0.5 to 20 µg/ml of ATX was created by further diluting a stock solution of 200 µg/ml (calculated as free base) of ATX in water. Through the course of the study, the stable stock standard and used solutions were continuously maintained at +4ºC.

1 g of plant materials were incubated in 20 ml solvent at room temperature in a shaking water bath at 100-150 rpm for 3 hours to prepare ethanol extracts. The produced extracts were paper filtered for the filtration process combined 1:1 with the mobile phase for liquid-liquid extraction, and the solution underwent filtering through a 0.45 mm filter prior being measured using UHPLC.

Instrument and Chromatographic Condition

The HPLC studies were performed on an Agilent brand 1260 Infinity mode, HPLC-DAD system for ATX detection. To chromatography, a Phenomenex-C18 (5 µm × 4.6 mm × 150 mm) column was utilized. WTW pH 526 digital pH Meter was used to monitor pH. Various conditions, such as C8 and C18 columns and varying flow rates, were examined in order to determine the best suited approach. To achieve the most efficient chromatographic separation, multiple mobile phase, column type, and stationary phase size combinations were tested at various flow rates and column temperatures.

Validation of the Method

The new approach has been examined using the International Conference on Harmonisation (ICH) guidelines [13].

Linearity and detection and quantification boundaries

The graph used for calibration was constructed using standard samples with concentrations ranging from 0.5 to 20 µg/ml of ATX. The calibration curve for peak area versus ATX concentration was created. Limit of detection (LOD) is the minimum level of a component in a sample analyte which we can detect but can not quantify at a satisfactory level where as the significant variable limit of quantification (LOQ) which is the minimum concentration level that can be measured by the experimental system.

Selectivity of the suggested approach

Selectivity has been referred to the ability of an analysis to be performed correctly considering the presence of the elements that may impact the analysis or be in interference with the substance. These parameters had no effect on the outcome of the analysis throughout the investigation.

Accuracy

For the determination of ATX in plant samples extracts; quality control (QC) samples were prepared in several concentration (2.5, 5.0 and 10 µg/ml) which could be categorized as low, medium and high concentration levels (n=3). The accuracy was indicated by the recovery values and the accuracy of the recovery study was determined by the relative standard deviation (RSD) values of the recovery results in three repeated studies. The amount recovered of the chemical was calculated after the produced plant samples were filtered.

Precision of the method

Precision is used for the ability of an analysis to be performed with the alike samples and/or solvents with identical conditions across various different durations. The precision experiments included hourly and daily examinations.

Robustness of the method

Overall this method is supposed to be robust and it was tested by altering the flow rate, composition, as well as column temperature. The mobile phase proportions were changed from 50:50 (v/v) (acetonitrile-phosphate buffer) to 45:55, however originally the the ratio was 55:45. The flow rate and the column temperature were adjusted to 0.7 and 0.9 ml/min and 25 and 35ºC correspondingly.

Stability

Working standard ATX solutions' stability was examined under various storing settings at three different QC levels. The three storage methods being tested include keeping the samples in autosampler conditions for 24 hours, storing in dark and room temperature for 24 hours, and maintaining in a refrigerator at 4°C for one month. Following are the rates of recovery percentages for the conditions that were tested: 98.7%, 96.5%, and 99.1%, respectively. For all of these demonstrations, the greatest RSD% was 1.27%. It would be accurate to say that ATX was determined to be stable given the entire test settings.

RESULT AND DISCUSSION

Chromatographic Process

It was preferred to employ reversed phase (RP) UHPLC and a use of column (5 µm x 4.6 mm x 150 mm, Phenomenex-C18). Acetonitrile-monobasic potassium dihydrogen orthophosphate (pH=6.8) was put in service as the mobile phase for a flow rate of 0.8 ml/min and an isocratical elution pattern. To get the best chromatogram resolution, the column temperature was fixed at 30ºC. The delay lasts around 1.476±0.004 minutes. In Figure 2 illustrative chromatograms are displayed. Table 1 indicates the chromatographic system suitability parameters.

Table 1. Chromatographic system suitability parameters

Capacity factor*	Resolution*	HETP*	Tailing factor*	Asymmetry factor*	Theoretical plates (N)
7.66	3.30	0.08	1.2	1.1	2420

*Mean values of the parameters of all the points in calibration study are mentioned

Validation of the Analytical Method

Sensitivity and Linearity

By graphing the peak zones of the derivatives with the respective ATX concentrations, linear least-squares regression analysis was used to generate curves for calibration. The value calculated from the calibration curve's equation (n=5) was y=102.8 x - 5.101 (correlation coefficient=0.9990), where y stands for the peak zone of ATX and x represents the ATX concentration.

LOD, limit of detection and LOQ, limit of quantitation were calculated by the use of formulae LOD= 3 SDa/m and LOQ= 10 SDa/m. SDa is the intercept's standard deviation, while b is being the slope. Table 2 provides a summary of the variables necessary for the analytical efficacy of the suggested approach. LOD is 0.16 and LOQ is 0.5 µg/ml.

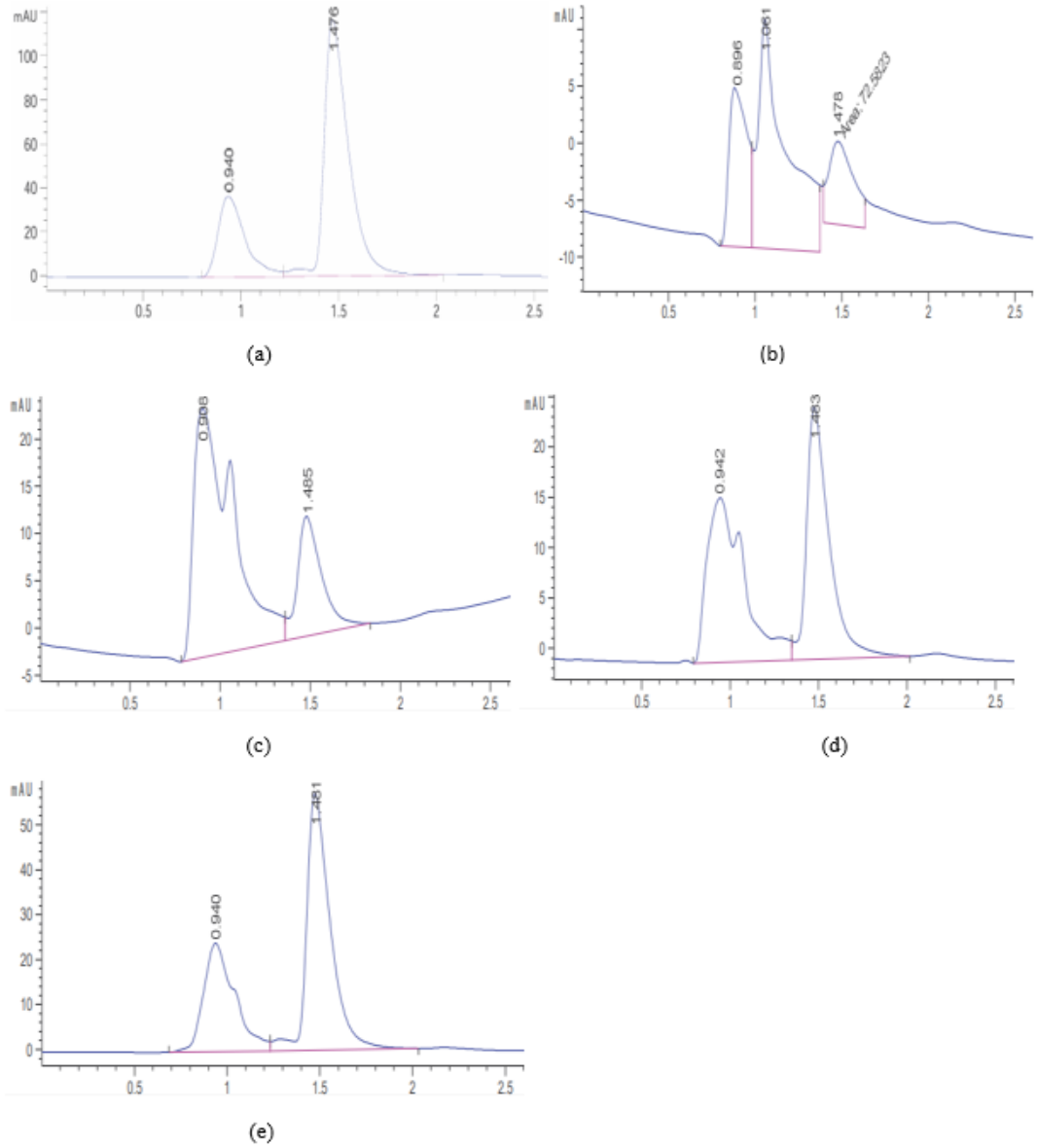

Figure 2. a: standard solution (10 µg/ml standard ATX solution), b: real sample 1 (*Melissa officinalis* L.), c: real sample 2 (*Salvia officinalis* L.), d: real sample 3 (*Rosmarinus officinalis* L.), e: real sample 4 (*Ginkgo biloba* L.)

Table 2. Results for the analytical variables of the suggested approach

Variables	**Results**
Concentration range [a] (µg ml^{-1})	0.5-20.0
Regression equation[b]	y= 102.8x – 5.101
Intercept± SD	5.101 ± 0.457
Slope± SD	102.8 ± 9.22
Correlation coefficient (r^2)	0.9990
LOD (µg ml^{-1})	0.16
LOQ (µg ml^{-1})	0.5

[a] The mean value of three analysis

[b] *C represent contcentrationn in* µg/ml and y represents the peak area for *y=xC* +b

Accuracy and Precision

In order to determine the precision and accuracy values, the samples were examined at three distinct dilution levels. Quality control (QC) specimens were prepared as low, medium, and high concentrations (n=3) at 2.5, 5.0, and 10.0 µg/ml. Standard addition used here for the determination of revocery. Standard addition is a recovery estimation approach. Three identical specimens at each QC concentration in order to be examined for various tests on three consecutive days for daily precision and the same day for hourly precision to display the accuracy of the subjected procedure. Table 3 represents the accuracy and precision results fort he real sample (*Ginkgo biloba* L.) with the highest relative amount of ATX.

Table 3. The outcomes of the precision and accuracy experiments for *Ginkgo biloba* L.

Existant concentration (µg ml^{-1})	**Added concentration (µg ml^{-1})**	**Found concentration (µg ml^{-1}) (Mean±SD[1])**	**Recovery (%)**	**RSD of recovery**	**RSD of intraday variation**	**RSD of interday variation**
10	2.5 5.0 10.0	12.48±0.04 15.01±0.05 20.07±0.06	99.86 100.08 100.35	0.35 0.32 0.27	0.35 0.32 0.27	0.39 0.36 0.33
Mean relative recovery = 100.09						

n=3 for every single concentration

Robustness

By making minor adjustments to the flowrate, column oven temperature, and acetonitrile and water phase concentrations of the mobile phase, robustness was assessed. Temperature column was changed from 30°C to 25°C and 35°C, the mobile phase proportions were modified from (50:50 v/v) (acetonitrile-phosphate buffer) to 45:55 and 55:45; and the flow rate was rised from 0.7 to 0.9 ml/min. Used modifications did not impact the peak areas. Evaluations for robustness are shown in Table 4. The recovery% figures of 104.32, 105.19, and 107.35 are better than those from our earlier study [14].

Table 4. Outcomes of the robustness

Condition	**Value**	**Recovery %**	**RSD %**
Flow rate (ml min^{-1})	0.7 0.9	102.75 104.32	1.76 1.31
Mobile phase composition (ACN:Phosphate buffer)	45:55 55:45	105.19 102.41	2.43 3.21
Column temperature	25 35	107.35 104.63	2.53 4.53

n=3 for each Quality Control samples

The Method's Application to the Identification of ATX from Plant Extracts

After the plant samples were extracted as described in the method section, they were prepared for analysis for UHPLC and the amount of ATX they contained was determined. The relative amounts of ATX in extracts *Salvia officinalis* L., *Rosmarinus officinalis* L., *Melissa officinalis* L., *Ginkgo biloba* L. were determined as 63%, 66%, 42% and 79%, respectively.

Conclusion

Secondary metabolites, unlike primary metabolites, are not directly related to the essential vital activities of the plants. Plants are complex matrices because of the secondary metabolites they contain.

The extraction of target analytes in plant extracts is a difficult process. Liquid liquid extraction is simple and fast compared to other extraction techniques for drug analysis.

Although many medicinal plants are widely preferred by healthcare professionals, patients and the public, no method has been found in the literature to determine ATX in medicinal plants. For the purpose of finding ATX in several medicinal plants, a new sample the development and quantification technique was devised in this work.

The procedure we created is quiet, straightforward, quick, and less expensive. The procedure offers a straightforward mobile phase with isocratic flow and does not call for any derivatization reaction. The developed method can be practically applied in the routine analysis of ATX in herbal extracts, food products and nutraceuticals.

AUTHOR CONTRIBUTIONS

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

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANTIMICROBIAL COMBINATION OF *LAVANDULA ANGUSTIFOLIA* L. ESSENTIAL OIL WITH KOJIC ACID

KOJİK ASİT İLE LAVANDULA ANGUSTIFOLIA L. UÇUCU YAĞININ ANTİMİKROBİYAL KOMBİNASYONU

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ABSTRACT

Objective: *Lavandula angustifolia L. (Lavender) is one of the most plants essential oils used in the cosmetic, food and biological activities. Kojic acid has been used in cosmetics for its whitening effect and pharmaceutical activity. The aim of this study to determine the composition of the Pharmacopoeia quality L. angustifolia essential oil (EO). Moreover, antimicrobial activities against skin pathogens and synergistic antibacterial activity were also examined of EO and kojic acid.*
Material and Method: *In this work, chemical composition of the EO was defined. Linalyl acetate (43.3%) and linalool (38.6%) were determined as the major components by GC-MS and GC-FID, simultaneously. The antimicrobial activity was evaluated against Candida albicans, C. glabrata, Staphylococcus aureus and Salmonella typhimurium.*
Result and Discussion: *EO and kojic acid showed weak antimicrobial effects. MIC values were determined as the EO 10 mg/ml and kojic acid 1.25 mg/ml against S. typhimurium. To assess the synergistic activity was evaluated by the checkerboard microdilution assay, EO was combined with kojic acid against S. typhimurium. Among the tested skin pathogen microorganisms, S. typhimurium was more sensitive to kojic acid. Therefore, synergic activity was investigated against S. typhimurium and found indifferent effect.*
Keywords: *Antimicrobial activity, kojic acid, Lavandula angustifolia, synergistic activity*

ÖZ

Amaç: *Lamiaceae familyasına ait bir bitki olan Lavandula angustifolia L. (Lavanta), kozmetik, yiyecek ve biyolojik etkilerinden dolayı en çok kullanılan uçucu yağlardan birisidir. Kojik asit beyazlatıcı etkisi için kozmetikte ve antimikrobiyal, antibakteriyel, antiviral gibi farmasötik etkilerinden dolayı kullanılmaktadır. Bu çalışmanın amacı Farmakope kalitesindeki Lavandula angustifolia uçucu yağının kimyasal kompozisyonunu belirlemektir. Buna ek olarak cilt patojenlerine karşı antimikrobiyal aktivitesi ve kojik asit ile uçucu yağın aktivite olarak antibakteriyel sinerjisi incelenmiştir.*
Gereç ve Yöntem: *Bu çalışmada, uçucu yağın kimyasal bileşimi araştırılmıştır. Uçucu yağın içerdiği ana bileşenler GK-AİD ve GK-KS ile linalil asetat (%43,3) ve linalol (%38,6) olarak tespit edilmiştir. Antimikrobiyal aktivite Candida albicans, C. glabrata, Staphylococcus aureus ve Salmonella typhimurium'a karşı değerlendirildi.*
Sonuç ve Tartışma: *Uçucu yağ ve kojik asit C. albicans ve S. aureus'a karşı zayıf antimikrobiyal*

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etki gösterdi. MİK değerleri S. typhimurium'a karşı uçucu yağda 10 mg/ml ve kojik asit 1,25 mg/ml olarak hesaplandı. Sinerjik aktiviteyi değerlendirmek için dama tahtası mikrodilüsyon yöntemi ile uçucu yağ kojik asit ile S. typhimurium'a karşı birleştirildi. Test edilen cilt patojen mikroorganizmalar arasında S. typhimurium, kojik aside karşı daha duyarlıdır. Bu nedenle S. typhimurium'a karşı sinerjik aktivite araştırıldı ve bağımsız etki gözlemlenmiştir.
Anahtar Kelimeler: *Antimikrobiyal aktivite, kojik asit, Lavandula angustifolia, sinerjik aktivite*

INTRODUCTION

The overuse and misuse of antibiotics were resulted in the creation of antibiotic-resistant microorganisms, making treating infectious diseases more challenging. However, essential oils have emerged as a potential solution to combat bacterial antibiotic resistance. Essential oils are multi-component and have been observed to have a synergistic effect when combined with antibiotics, decreasing the minimal effective dose of antibiotics, and limiting their adverse effects. Studies have shown that essential oils can affect both the external envelope of the cytoplasm and cell of microorganisms, leading to a disturbance in their structures and functions, ultimately rendering them permeable [1]. Essential oils have been found to inhibit multidrug-resistant bacteria independently of their antibiotic resistance profile, and their effectiveness against common pathogens depends on the concentration of active phenolic compounds [2]. Therefore, the essential oils' combination with conventional antibiotics has great promise for the development of novel treatments and the treatment of infectious disorders caused by multidrug-resistant pathogens [1].

Lavandula angustifolia L. (Lavender), plant is family name Lamiaceae, is used in the cosmetics, food, pharmaceuticals, and biological activities, such as antioxidant effect. Among its biological activities beside the central nervous system are antimicrobial, antioxidant and anti-inflammatory activities [3,4].

Kojic acid is one of the popular ingredients used in various cosmetic products due to its whitening effects on the skin and against sunspots, inhibits catecholase activity of tyrosinase and pharmaceutical activity. Kojic acid is a natural product by several species of fungi like *Penicillium* and *Aspergillus.* Whereas the natural kojic acid is used in cosmetics for skin whitening purposes and reducing sunspots. It also functions as an antioxidant caused by free radicals on the skin along with a broad spectrum of antimicrobial effects. It is a potent natural antioxidant, mild anti-inflammatory safe compound without genotoxic properties [5,6].

The current investigation aimed to identify the antimicrobial properties of pharma-grade *Lavandula angustifolia* essential oil with kojic acid as a combination against human skin pathogens. Initially the EO was confirmed as linalyl acetate (43.3%) and linalool (38.6%) were identified as the major components. The antimicrobial activity was evaluated against *Escherichia coli, Candida albicans, C. krusei, Staphylococcus aureus* and *Salmonella typhimurium.* Both the oil and kojic acid showed weak antimicrobial effects against *C. albicans* and *S. aureus*. To assess the antimicrobial combination activity a checkerboard microdilution assay was conducted. To the best our knowledge, the synergistic activity of EO and kojic acid was observed for the first time against pathogens like *Salmonella typhimurium.*

MATERIAL AND METHOD

General Experimental Procedures

Lavandula angustifolia (Pharma Grade, Davenne, France) analytical compounds and microbiological media were obtained from Sigma-Aldrich, Merck, Fluka if not otherwise stated in high purity.

GC-FID and GC/MS Analysis

The essential oil was analysed to confirm its quality by GC-FID and GC-MS, at the same [7-9]. Main components were compared with European Pharmacopoeia 8th Edition.

An Agilent 5975 GC-MSD equipment was used for the GC-MS analysis. Helium (0.8 ml/min)

was utilized as the carrier gas. The gas chromatograph's oven degree was kept constant at 60-240°C at a rate of 1°C/min. The mass spectrum was *m/z* 35 to 450.

An Agilent 6890N GC equipment was used for the GC analysis. To achieve the same elution order using GC-MS, simultaneous auto-injection on the same column under the same operating conditions was performed. FID chromatograms were used to compute the relative percentage quantities of the separated compounds.

Identification of the Volatile Components

The volatile components were identified by comparing their relative retention durations to those of authentic samples or their relative retention index (RRI) to a *n*-alkanes' series. Computerized scanning against commercial databases (Wiley GC-MS and MassFinder 4.0 Libraries) [7,8] and the in-house "Başer's Essential Oil Constituents Library" comprised of actual compounds and components of recognized oils.

Microorganisms

The human skin test pathogens used in the study were *Salmonella typhimurium* American Type Culture Collection (ATCC) 14028, *Escherichia coli* Research Service Culture Collection (NRRL) B-3008, *Candida albicans* ATCC 90028 *and Candida krusei* ATCC 6258.

Antimicrobial Activity

Minimum inhibitory concentrations of the oil and kojic acid that producing 50% of inhibition (MIC50) were determined using Clinical and Laboratory Standards Institute (CLSI) methods with adaptations for aerobic microorganisms (M07-A7 and M27-A2), also European Committee of Antimicrobial Susceptibility Testing (EUCAST E.DEF 7.2) was used [10,11].

Synergistic Antibacterial Activity

The Checkerboard microdilution assay was used in 96-well plates to study the interaction of the test samples. There were eight successive dilutions of *Lavandula angustifolia* essential oil and antibiotic tetracycline (128-0.25 g/ml) produced. The broth microdilution checkerboard technique was utilized, and the fractional inhibitory concentration index (∑FIC) was calculated as the total of the MICs of each sample when used in combination divided by the MIC of the sample when used alone [12]. As a result, the following activity kinds were defined [13]:

Synergism: $\sum FIC \leq 0.5$

Additive effect: $\sum FIC\ 0.5 \leq 1$

Indifferent effect: $\sum FIC > 1–4$

Antagonism: $\sum FIC \geq 4$.

RESULT AND DISCUSSION

Chemical Composition

In this present study, Pharmacopoeia grade essential oil from commercial sources was evaluated for its broad antibacterial properties. The essential oil was analysed to confirm its quality. Linalyl acetate (43.3%) and linalool (38.6%) were determined as major components. Other constituents were listed in Table 1. According to result, the essential oil was complied with the supplier's quality and Pharmacopoeia Monograph (2014). Furthermore, the main constituents are oxygenated monoterpenes.

The chemical composition of *Lavandula angustifolia* essential oil can vary depending on various factors such as the plant genotype, environmental conditions, and part of the plant used. However, some common components were identified in the *L. angustifolia*'s oil. The main components of this essential oil are often linalool, linalyl acetate, geraniol, *β*-caryophyllene, and lavandulyl acetate. The percentage of each component can vary, but linalool and linalyl acetate are typically present in significant amounts.

Table 1. The volatile components of *L. angustifolia*

No	**RRI[a]**	**Compound**	**%[b]**
1	1032	*α*-Pinene	0.1
2	1076	Camphene	0.3
3	1118	*β*-Pinene	0.1
4	1132	Sabinene	tr
5	1146	*δ*-2-Carene	0.3
6	1174	Myrcene	0.3
7	1203	Limonene	0.2
8	1213	1,8-Cineole	0.1
9	1230	*n*-Butyl-*n*-butyrate	0.1
10	1246	*(Z)-β*-Ocimene	0.8
11	1265	3-Octanone	1.5
12	1266	*(E)-β*-Ocimene	0.3
13	1280	*p*-Cymene	0.1
14	1282	Hexyl acetate	0.6
15	1386	1-Octenyl acetate	0.7
16	1393	3-Octanol	0.3
17	1424	Hexyl butyrate	0.4
18	1450	*trans*-Linalool oxide (*furanoid*)	0.3
19	1452	1-Octen-3-ol	0.4
20	1478	*cis*-Linalool oxide (*furanoid*)	0.3
21	1532	Camphor	0.6
22	1553	**Linalool**	**38.6**
23	1565	**Linalyl acetate**	**43.3**
24	1583	*α*-Santalene	0.9
25	1595	*trans-β*-Bergamotene	0.2
26	1612	*β*-Caryophyllene	3.8
27	1695	*(E)-β*-Farnesene	1.4
28	1706	*α*-Terpineol	0.6
29	1719	Borneol	2.0
30	1733	Neryl acetate	0.2
31	1765	Geranyl acetate	0.4
32	1808	Nerol	tr
33	1857	Geraniol	0.1
34	2008	Caryophyllene oxide	0.7
		Monoterpene hydrocarbons	**2.5**
		Oxygenated monoterpenes	**87.4**
		Sesquiterpene hydrocarbons	**6.3**
		Oxygenated sesquiterpenes	**0.7**
		Others	**3.1**
		Total	**100**

tr: Trace (<0.1 %); [a]: Relative retention indices calculated against *n*-alkanes; [b]: calculated from FID data

For example, one study reported linalool at 30.6% and linalyl acetate at 14.2%, while another study found linalyl acetate at 27.5% and linalool at 24.1% [14,15]. These components contribute to the characteristic aroma and potential therapeutic properties of *Lavandula angustifolia* essential oil.

Antimicrobial Activity

The antimicrobial abilities of essential oils and their volatile components are critical in utilizing them. Thus, in the frame of our study, the antimicrobial effect of the *Lavandula* essential oil and kojic acid were tested on different *in vitro* antimicrobial assays. MIC values were listed on Table 2. According to the results, kojic acid was showed higher antibacterial and antifungal properties than essential oil. When the results were compared, it was observed that both kojic acid and essential oil had inhibitory effects against *Salmonella typhimurium*.

Table 2. Antimicrobial activities of Kojic acid and *L. angustifolia* as MIC (mg/ml)

Bacterial strains	**Kojic acid**	***L. angustifolia***	**Ampicilin**	**Clarithromycin**	**DMSO**
E. coli NRRL B-3008	2.5	2.5	0.01	0.02	-
S. typhimurium ATCC 14028	1.25	5	1.3	0.04	-
Yeast isolates			**Ketoconazole**	**Itraconazole**	**Fluconazole**
Candida albicans ATCC 90028	>1.25	10	0.01	0.04	0.05
C. krusei ATCC 6258	>1.25	2.5	0.01	0.01	0.04

The antimicrobial effect of kojic acid against several microorganisms has been reported. Previous research explored kojic acid's antibacterial and anti-biofilm action against some foodborne pathogens like *Listeria monocytogenes*, and *S. typhimurium*. The study showed that *Escherichia coli* was significantly susceptible, with the lowest MIC (10 mM) and MBC (20 mM) [16]. Another study found that kojic acid had antibacterial effect against *P. aeruginosa*, *S. aureus*, and *Micrococcus luteus* with minimum inhibitory concentration values between 0.125 and 1.0 mg/ml [17]. These findings suggest that kojic acid has potential as an antimicrobial agent against various bacteria.

Lavandula essential oil was demonstrated to have considerable antibacterial properties against a wide range of microorganisms. In previous research evaluated the antimicrobial activity of EOs from different *Lavandula* cultivars and found that they showed antimicrobial activity against all microorganisms analysed, indicating the broad-spectrum antimicrobial potential of *Lavandula* essential oil [18]. Another study found that Lavandula angustifolia essential oil had an antibacterial effect against *Staphylococcus* species in a hospital setting, decreasing germs in all hospital places [19]. These findings highlight the significant antimicrobial activity of *Lavandula* essential oil, indicating its potential for various applications in antimicrobial formulations.

Synergistic Antibacterial Activity

The essential oil and kojic acid were combined varying proportions to determine the synergistic antibacterial potential. Antibacterial combination results were expressed as the fractional inhibitory concentration index (ΣFIC). Combined with kojic acid essential oil has an additive effect (ΣFIC: 0.5078) (Table 3.).

Kojic acid has been found to have synergistic antibacterial activity when combined with other compounds. For example, a study found that kojic acid and tea polyphenols had a strong synergistic antibacterial effect against spoilage bacteria in refrigerated sea bass fillets [20]. Another study investigated the potential antibacterial properties of kojic acid in conjunction with metal cations by co-crystallizing them and reported that co-crystallization of kojic acid with silver(I), copper (II), zinc (II), and gallium (III) demonstrated increased antibacterial activity [21]. Furthermore, kojic acid-grafted

konjac glucomannan oligosaccharides were reported to have high antimicrobial effects against bacteria like *S. aureus* in a study [22]. These findings suggest that kojic acid can enhance the antibacterial activity of other compounds, making it a potentially useful ingredient in antibacterial formulations.

Table 3. Fractional Inhibitory Concentration Index (ΣFIC) (µg/ml)

Bacteria	**Kojic Acid**			***L. angustifolia* Essential Oil**				
	***A**	****C**	**FIC**	**A**	**C**	**FIC**	**ΣFIC**	**RESULT**
S. tymphirium	1.25	0.625	0.5	5	0.039	0.0078	0.5078	**Additive effect**

*A: MIC values of alone, **C: MIC values in combination

Lavandula essential oil was found to have synergistic antibacterial activity when combined with other compounds. In previous study found that the combination of *L. latifolia* essential oil and camphor had a synergistic antibacterial effect [23].

Another study examined the possible synergy of essential oils from *Lavandula angustifolia*, *Artemisia herba alba*, and *Rosmarinus officinalis*. The study found that combining these essential oils exhibited synergistic antibacterial effects against various bacterial strains. [24].

These findings indicate that *Lavandula* essential oil can enhance the antibacterial activity of other compounds, making it a potentially helpful ingredient in antibacterial formulations.

The literature did not return specific information about the synergistic effects of kojic acid and *Lavandula* essential oil. However, it is known that both kojic acid and *Lavandula* essential oil have antimicrobial and antioxidant activities. Kojic acid has been found to possess antifungal activity, while *L. angustifolia* essential oil has demonstrated antimicrobial and antioxidant activities. Our research has shown that the additive effect of kojic acid and *Lavandula* essential oil was determined.

Among the tested the pathogen microorganisms, *S. typhimurium* was more sensitive to kojic acid and *L. angustifolia*. Therefore, synergic activity was investigated against *S. typhimurium* and found additive effect. In the present work, to the best of knowledge there is no report for kojic acid and combination with *L. angustifolia*. Further studies are required for the safe therapeutic *in vivo* and clinical studies.

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AUTHOR CONTRIBUTIONS

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

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

TOWARDS UNDERSTANDING NATURAL ALPHA-GLUCOSIDASE INHIBITORS: A COMPUTATIONAL STUDY

DOĞAL ALFA-GLUKOSİDAZ İNHİBİTÖRLERİNİ ANLAMAYA DOĞRU: HESAPLAMALI BİR ÇALIŞMA

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ABSTRACT

Objective: *Diabetes mellitus is a metabolic disorder affecting hundreds of millions of people around the world. It is characterized by hyperglycemia caused by impaired glucose homeostasis that results from insufficient insulin production or insulin resistance. There are clinically available α-glucosidase inhibitor drugs that are used to decrease postprandial blood glucose level. However, these drugs have side effects that necessitated the discovery of new α-glucosidase inhibitors with less side effects and high potency. The interest in the use of natural products to deal with diabetes has been increasing. Therefore, the potential of natural α-glucosidase inhibitors to inhibit the enzyme was investigated through computational methods.*

Material and Method: *The binding potential of selected natural α-glucosidase inhibitors was investigated through molecular docking. Thereafter, the stability of the complexes with the highest binding potential were assessed through molecular dynamics (MD) simulation.*

Result and Discussion: *The molecular docking demonstrated that compound 2 had better binding potential than the standard drug, acarbose. Compound 7 had comparable binding potential to the standard drug. Furthermore, all the tested compounds exhibited a reasonable binding potential towards the enzyme but were weaker than the standard drug. The MD simulation demonstrated that compounds 2 and 7 gave complexes with similar stability to the standard drug. The overall computational results revealed that the natural inhibitors investigated had the ability to bind to the enzyme and formed stable complexes. Therefore, these compounds could be potential α-glucosidase inhibitors for clinical use. For this reason, further in vitro investigations on compounds with the highest binding potential is recommended.*

Keywords: *Diabetes, α-glucosidase, MD simulation, molecular docking, natural inhibitors*

ÖZ

Amaç: *Şeker hastalığı dünya çapında yüz milyonlarca insanı etkileyen metabolik bir hastalıktır. Hastalık yetersiz insülin üretimi veya insülin direncinden kaynaklanan bozulmuş glukoz homeostazisinin neden olduğu hiperglisemi ile karakterizedir. Yemek sonrası kan şekeri seviyesini düşürmek amacıyla klinikte kullanılan α-glukosidaz inhibitörü ilaçlar bulunmaktadır. Ancak bu ilaçların yan etkileri olduğundan daha az yan etkili ve yüksek etkinliği olan yeni α-glukosidaz inhibitörlerinin keşfedilmesine ihtiyaç duyulmaktadır. Şeker hastalığıyla mücadelede doğal kaynaklı ürünlerin kullanımına olan ilgi giderek artmaktadır. Bu nedenle doğal kaynaklı α-*

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glukosidaz inhibitörlerinin enzimi inhibe etme potansiyelleri hesaplamalı yöntemlerle araştırılmıştır.

Gereç ve Yöntem: *Seçilmiş doğal kaynaklı α-glukosidaz inhibitörlerinin bağlanma potansiyeli, moleküler doking yoluyla araştırılmıştır. Daha sonra, en yüksek bağlanma potansiyeline sahip komplekslerin stabilitesi moleküler dinamik (MD) simülasyonu yoluyla değerlendirilmiştir.*

Sonuç ve Tartışma: *Moleküler doking çalışması bileşik **2**'nin standart ilaç olan akarbozdan daha iyi bağlanma potansiyeline sahip olduğunu göstermiştir. Bileşik 7 ise standart ilaca benzer bağlanma potansiyeline sahipti. Ayrıca, test edilen bileşiklerin hepsi enzime karşı makul bağlanma potansiyeli sergilemelerine rağmen standart ilaçtan daha zayıf bağlandığı görülmüştür. MD simülasyonu da bileşik 2 ve 7'nin standart ilaca benzer stabiliteye sahip kompleksler verdiğini göstermiştir. Hesaplama yöntemlerin sonuçları araştırılan doğal kaynaklı inhibitörlerin enzime bağlanma yeteneğine sahip olduğunu ve stabil kompleksler oluşturduğunu ortaya çıkarmıştır. Bu nedenle bu bileşikler klinik kullanım için potansiyel α-glukosidaz inhibitörleri olabilir. Bu yüzden en yüksek bağlanma potansiyeline sahip bileşikler üzerinde daha fazla in vitro araştırma yapılması tavsiye edilir.*

Anahtar Kelimeler: *Diyabet, doğal inhibitörler, α-glukosidaz, MD simülasyon, moleküler doking*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized mainly by hyperglycemia [1]. According to the International Diabetes Federation Diabetes figures, DM affected 537 million people in 2021 and also the ninth cause of death. The projection made estimated that the number of affected people will rise to 643 million by 2030 and it is expected to be the seventh cause of death worldwide by then [2]. Insulin plays a crucial role in regulating blood glucose level. It takes part in glucose uptake and transport, glycogen, fatty acid, and protein synthesis. Insufficient production of insulin or resistance to insulin impairs the glucose homeostasis that leads to hyperglycemia eventually [3]. Chronic hyperglycemia can lead to long-term complications like nerve damage, cardiovascular disease, and kidney failure [4].

There are three categories of DM: Type 1 DM, type 2 DM, and gestational DM. Among these categories, type 2 DM comprises approximately %90-95 of the cases [5]. The type 2 category is manifested mainly by insulin resistance and to some extent insulin secretion impairment [6]. The insensitivity of the related targets to insulin can lead to a decreased glucose uptake in the skeletal muscle cells and an increased glucose formation in the liver [6]. As a result, most of the drugs that are used to treat type 2 DM decrease glucose absorption, hinder hepatic gluconeogenesis, and slow down renal glucose reabsorption [7]. These types of drugs played a prominent role in attenuating blood glucose level [8]. However, there are various side effects of such drugs. Hence, there is a need for antidiabetic drugs with high efficacy and low side effects [9].

Alpha-glucosidase inhibitors are typical examples that delay intestinal glucose absorption and thus decrease postprandial blood glucose level (Figure 1) [10]. Acarbose, miglitol, and voglibose are α-glucosidase inhibitors that are available in the pharmaceutical market. The molecular structure of these drugs is similar to carbohydrates (Figure 2). As a result, these drugs can bind to the carbohydrate binding site of α-glucosidase. The resulting complexes have higher affinity than the carbohydrate complex. Consequently, a delay in carbohydrate digestion and absorption that leads to a decrease in postprandial hyperglycemia is observed. However, these drugs have adverse effects like flatulence, severe stomach discomfort, and allergic responses [11]. Therefore, there is need of new α-glucosidase inhibitors with high potency and less adverse effects.

There have been efforts to develop bioactive compounds to alleviate diabetic conditions together with the efforts to synthesize new active compounds. The interest in the use of natural products to prevent and treat type 2 DM has been increasing. Natural products have been utilized to prevent and treat various medical disorders, including DM, throughout history [2]. For instance, one of the approved antidiabetic drugs, metformin, was originally isolated from *Galega officinalis* [12]. In the light of reported findings, potential α-glucosidase inhibitor compounds from natural origin were selected and their inhibiting potential was investigated through computational methods. For this end, akebonoic acid (1), alaternin (2), morusin (3), mulberrofuran K (4), procyanidin A2 (5), psoralidin (6), rhaponticin (7),

and taxumariene F (8) compounds were selected based on the literature review performed to dig out the potency of natural α-glucosidase inhibitors (Figure 3) [9].

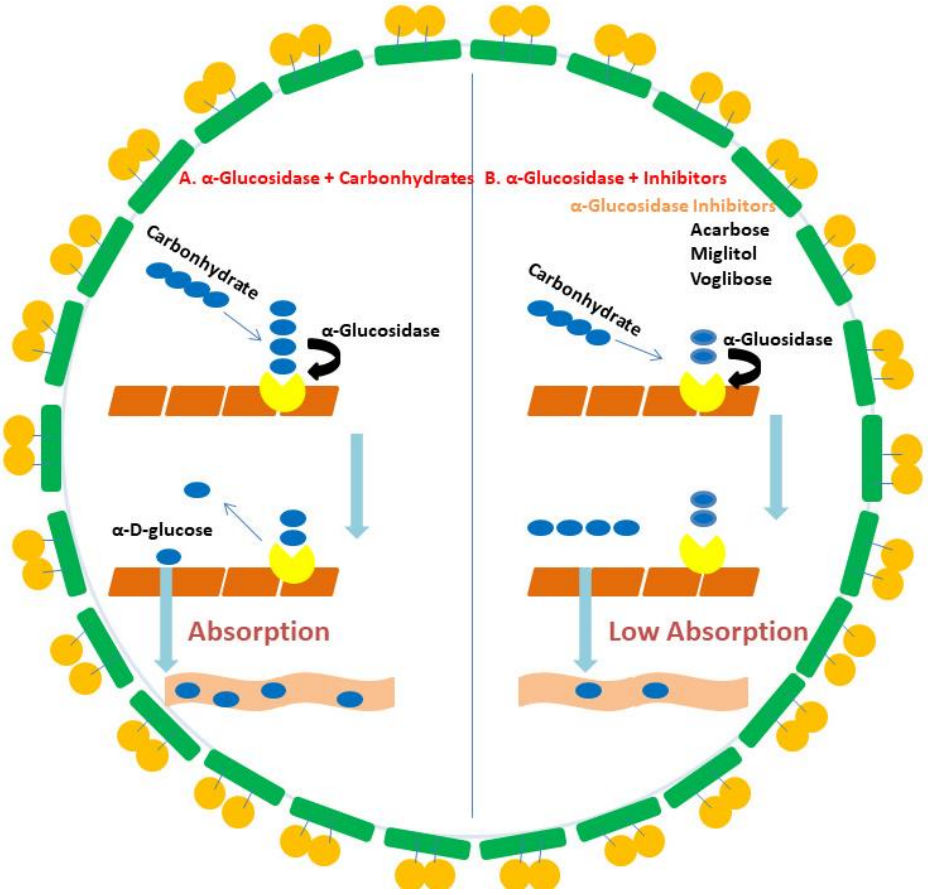

Figure 1. α-Glucosidase inhibition mechanism and its effect

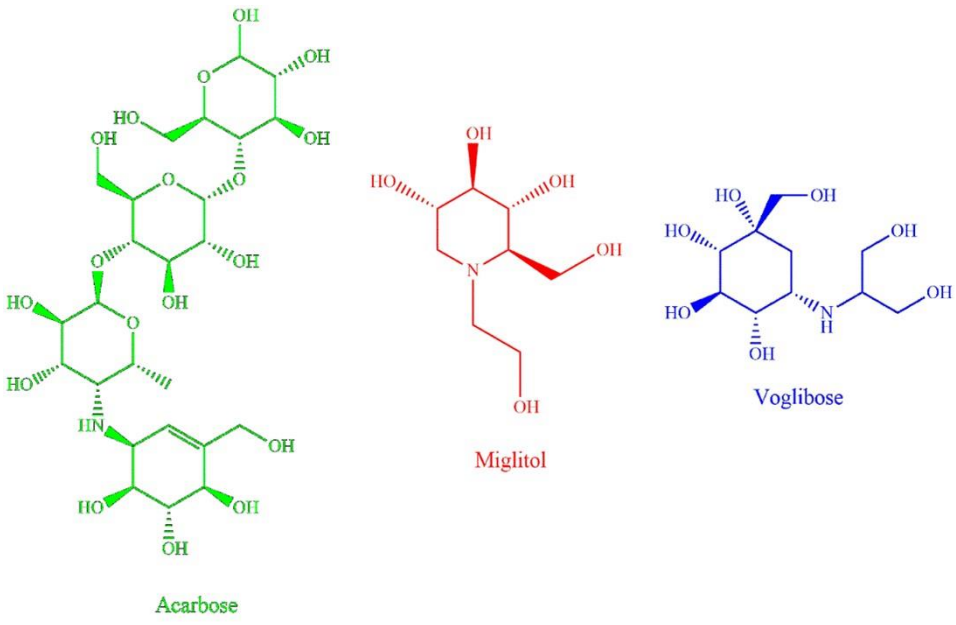

Figure 2. α-Glucosidase inhibitors that are clinically available

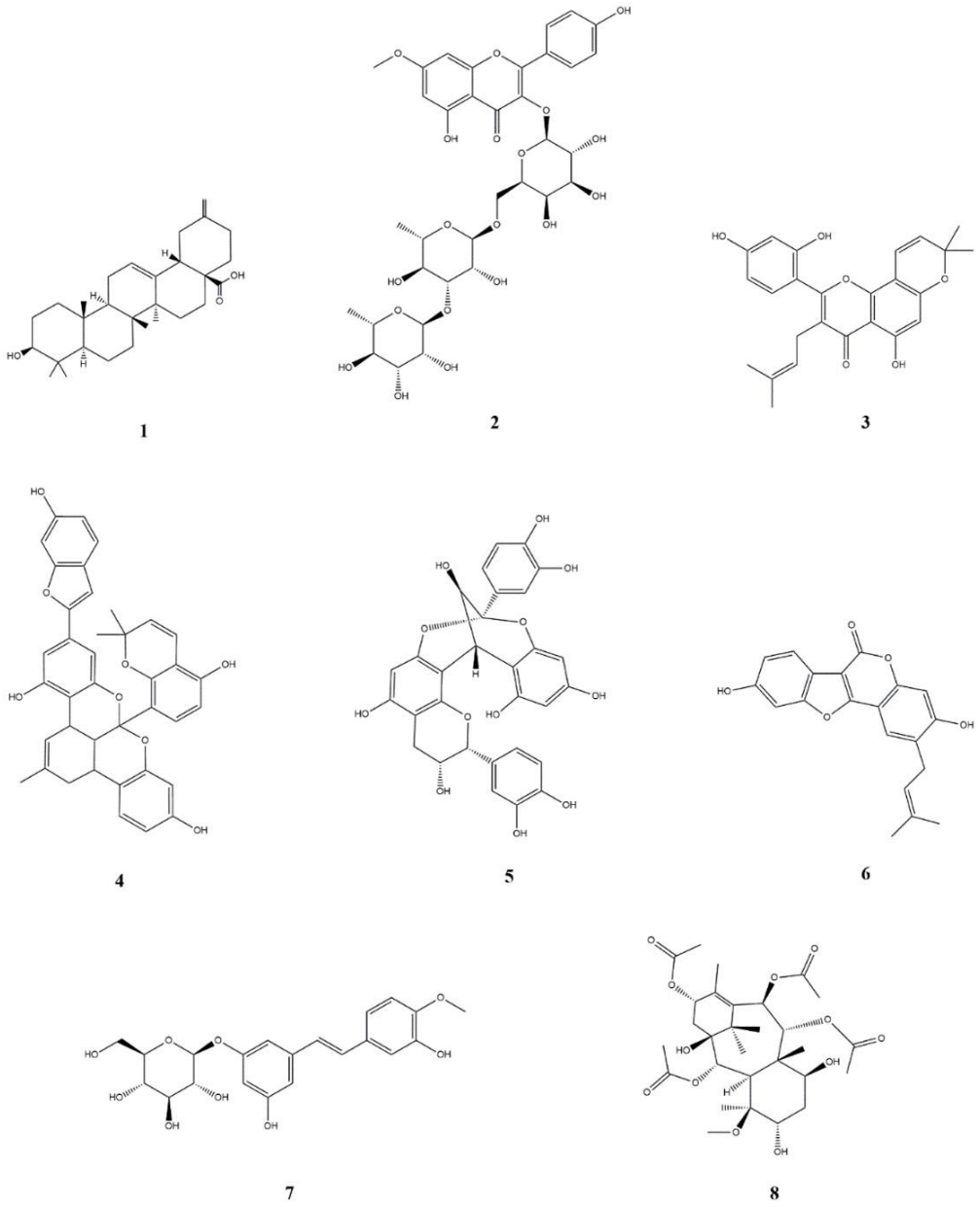

Figure 3. Natural α-glucosidase inhibitor compounds

Computational methods have been applied to minimize the cost and time required for drug design and discovery process [13]. Among such methods, molecular docking is employed to elucidate the mechanism of binding for drug candidate molecules [14,15]. Then, molecular dynamics (MD) simulation is undertaken to measure the stability level of the resulting target-compound complexes obtained from the docking.

In this study, natural α-glucosidase inhibitors were selected. The potential of these compounds to bind to α-glucosidase was investigated through computational methods. Molecular docking of these compounds to the enzyme crystal structure showed that the compounds had good binding potential, compounds **2** and **7** having the highest binding potential. The complexes of compounds **2** and **7** with the enzyme procured from the docking were tested for their stability via MD simulation. The two compounds had stability similar to the standard drug, acarbose. Compound **7** gave better stability profile according to some parameters.

MATERIAL AND METHOD

Molecular Docking

Molecular docking of the most active natural α-glucosidase inhibitors was done on crystal structure retrieved from the protein data bank (PDB). The crystal structure had a resolution of 1.55 Å and had a ligand complexed with it (PDB code: 5ZCE) [16]. The molecular docking was done with AutoDock Vina as described in previous studies [17,18].

MD Simulation

MD simulation of the natural α-glucosidase inhibitors with the highest binding potential to the enzyme was performed by using the respective complexes retrieved from the docking. MD simulations were done by using GROMACS (GROningen MAchine for Chemical Simulations) package as described in previous studies [19,20]. Then, root mean square deviation (RMSD), root mean square fluctuation (RMSF), Rg (radius of gyration), and ligand hydrogen bond plots were drawn through qtgrace and analyzed accordingly.

RESULT AND DISCUSSION

Molecular Docking

The interaction of the selected natural α-glucosidase inhibitor compounds to the crystal structure was investigated through molecular docking. Before docking the natural compounds to the crystal structure, the process was validated by redocking the ligand complexed in the crystal structure, alpha-maltotetraose. The bound ligand interacted to the structure very well. The ligand interacted to the structure with ten conventional hydrogen bonds (Asp60(2), His203, Gln256(2), Asp327(2), Asp382(2), Arg411) and two carbon hydrogen bonds (Phe163, Asp327) with the enzyme (Figure 4). The previous experimental study revealed similar conventional hydrogen bonding points detected in this study except with Asp382. The number of hydrogen bonds reported by the experimental study and detected in this study were similar [16]. Hence, the findings in the docking of the bound ligand to the crystal structure in this study were found to be similar to the previous experimental findings.

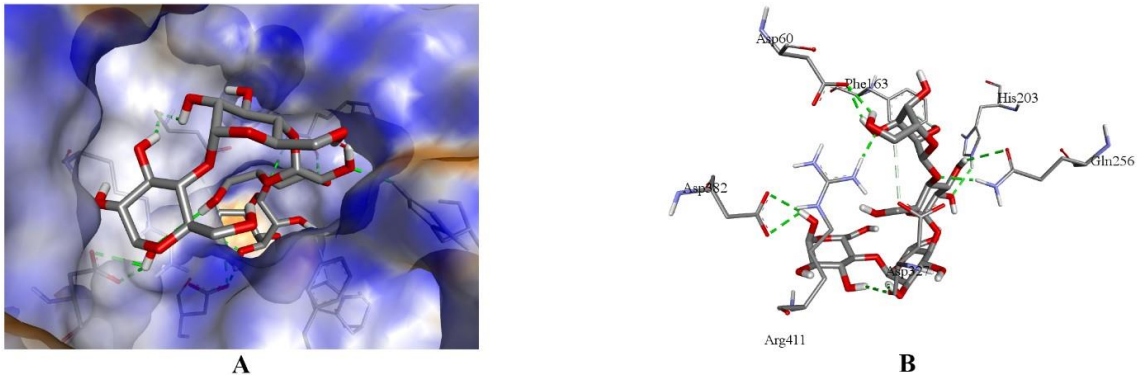

Figure 4. Binding mode of alpha-maltotetraose: A) its binding pose in the binding site, B) its 3D binding

In addition to docking to the bound ligand, the docking process was checked by docking the reference drug, acarbose, with the PDB structure. Acarbose interacted with the structure very well through seven conventional hydrogen bonds (Ile143(2), Asn258(3), Phe282, Gly286) and a carbon-hydrogen bond (Gly384) (Table 1, Figure 5). This has implicated that the natural compounds could also exhibit a reasonable interaction that fit their activity with the structure. The interaction residues of acarbose had also similarities with the crystallographic analysis ones. In this regard, the hydrogen bonding interactions with Asn258 and Phe282 were observed in the previous experimental study and

the docking study [16]. The standard drug had an interaction that fit its activity *in vitro* with the enzyme but less than with that of the complexed ligand inside the structure utilized. The standard ligands had an interaction that justified their activity with the enzyme. In addition to this, the complexed ligand had a high level of interaction similarity to the experimental structure analysis. The overall results implied that the docking process would result in a reliable interaction profile. Together with this, the stability of the complexes obtained from the docking was assessed through MD simulation.

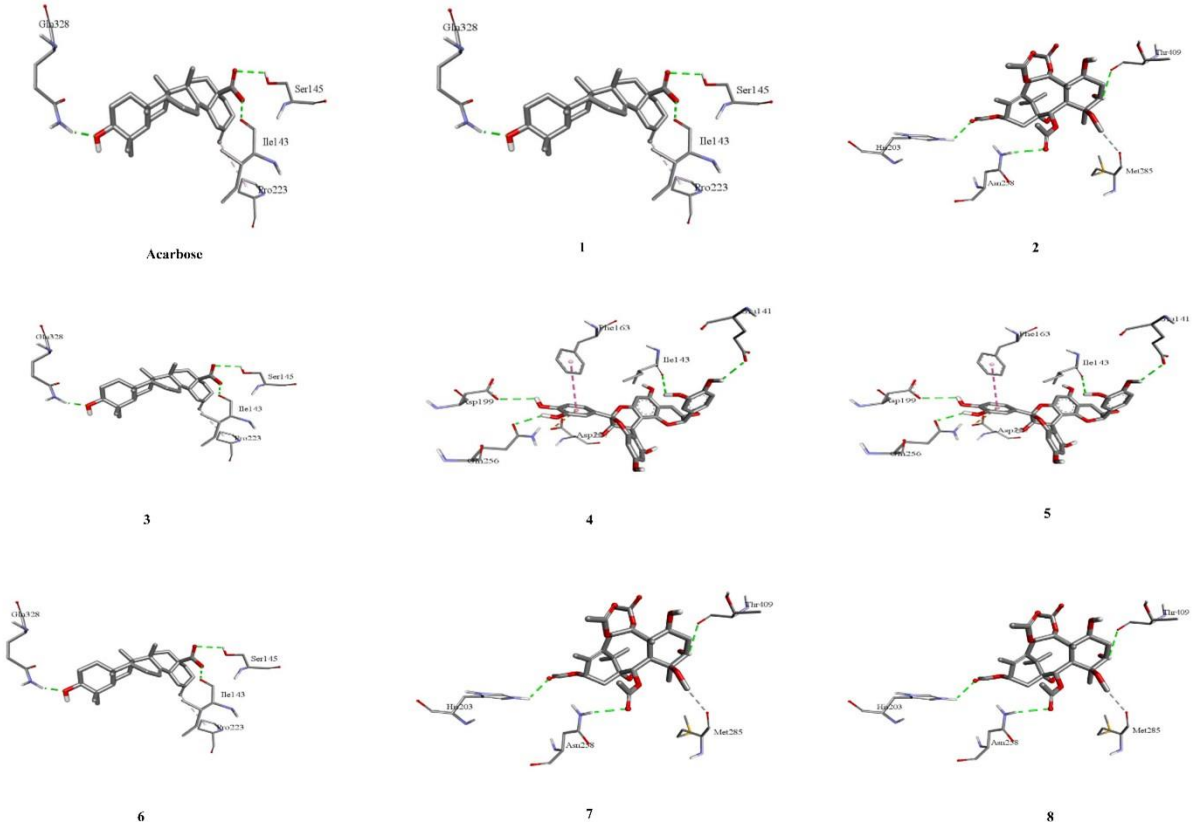

Figure 5. Binding profile of the natural inhibitors with the α-glucosidase crystal structure. In the figure color representation is; green-conventional hydrogen bonds, pale pink-alkyl/pi-alkyl, very pale green-carbon hydrogen bond, magenta-pi-sigma, yellow-pi-ion, and pink-pi-ion

The natural α-glucosidase inhibitors had interactions with the crystal structure. They interacted with at least three conventional hydrogen bonds and more other types of interactions. A previous crystallographic study revealed that interactions at Asp60, Tyr63, His103, Arg197, Asp199, His203, Gln256, Asn258, Phe282, Met285, His326, Asp327, Gln328, and Arg411 residues were important in the binding of ligands to the enzyme and stabilizing them inside the binding site [16]. In this study, compounds **2** and **7** exhibited the highest binding to the enzyme. Compound **2** had better binding than the standard drug, acarbose, as it formed more non-hydrogen bonds with the enzyme. Compound **7** had less number of conventional hydrogen bonds in relative to the reference drug but had more other types of interactions (Figure 5, Table 1). In addition to this, both of them had lower binding energy than the reference drug that implicated a better binding affinity for them (Table 1). The compounds with better binding potential had similar binding profile with the previous experimental study. Compound **2** had common binding at His203, Asn258, Phe282, and Arg411 amino acid residues. Similarly, compound **7** had common binding at Phe163, Asp199, His203, Gln256, Asn258, Phe282, and Asp327 residues (Table 1, Figure 5). All of its interaction residues except at Ala200 were similar to the previous experimental interaction revealed [16]. Therefore, it had high level of interaction residue similarity with the previous experimental findings. Furthermore, all the natural α-glucosidase inhibitors had at least one common

interaction residue with the experimental residue ones. This has implicated that the computational study findings were similar to the previous experimental finding [16]. The computational study demonstrated compounds **2** and **7** could bind to the enzyme and thus inhibit it at a comparable even better strength level than the reference drug. This premise was further assessed via MD simulation.

Table 1. Binding points of the ligands with the crystal structure

Ligands	Binding energy (kcal/mol)	Conventional hydrogen bonding residues	Other interaction residues
1	-8.4	Ile143, Ser145, Gln328	Pro223[a]
2	-9.0	Ile143(2), Ser145, Asn258, Gly384, Thr409, Arg411	Ile143(2)[a], Ile143[b], His203[a], Phe282[a]
3	-8.5	Asp327, Gln328, Asp382(2)	Phe163[a], Gly384[b], Met385[a], Tyr388[c]
4	-10.1	Ile143, Gln256, Phe282	Glu141[d], Ile143[a], Pro223(2)[a]
5	-9.4	Glu141, Ile143, Asp199, Gln256	Phe163[e], Asp327[d]
6	-9.4	Asn258, Thr409, Arg411	Tyr63[c], Phe163[c], Phe282(2)[e], Arg411[a]
7	-8.1	His203, Gln256, Asn258(2), Phe282, Asp327	Phe163[a], Asp199[b], Ala200[a], Asp327[d]
8	-7.1	His203, Asn258, Thr409	Met285[b]
Acarbose	-7.6	Ile143(2), Asn258(3), Phe282, Gly286	Gly384[b]

[a]Alkyl/pi-alkyl, [b]carbon-hydrogen bond, [c]pi-sigma, [d]pi-ion, [e]pi-pi

MD Simulation

The stability of the binding of acarbose, 2, and 7 to the enzyme was assessed through MD simulation. The MD simulation revealed that the three compounds had complexes with moderate stability as the plots flipped at some points. There was no significant difference in the stability of complexes of these compounds (Figure 6). As RMSD is used to measure fluctuation of a structure during the simulation period, the RMSD plots of the compounds with the highest binding potential and the standard drug were drawn [21]. At 29 ns, compound 2 exhibited high fluctuation that might be a sign of high movement for the compound inside the binding site. Thereafter, it got some level of stability but at a higher RMSD value. Similarly, acarbose had fluctuation at 42 ns and sustained relative stability then at a higher value. Compound **7** also had high fluctuation at 67 ns and then attained relative stability (Figure 6).

The RMSF plots of the compound containing complexes were drawn to evaluate changes in the enzyme structure amino acid residues [21]. RMSF value of complexes bearing the compounds was similar. Significant RMSF fluctuations were observed in 200-233, 283-300, and 370-413 residue intervals for the three complexes (Figure 6). Rg plots of the compound containing complexes were drawn to understand the effect of compound binding on the overall secondary structure of the enzyme [22]. Rg values of the complexes were similar to each other. Especially in the first 27 ns, they depicted similar Rg value. Thereafter, compound 7 had the lowest Rg value up to 70 ns that implied the highest compactness for it. After 70 ns, the complexes had varying Rg values, acarbose having the highest Rg value in this time interval (Figure 6). The role of intermolecular hydrogen bonding between the crystal structure and compounds was assessed by drawing ligand hydrogen bonds during the simulation period [22]. Acarbose had various number of hydrogen bonds during the simulation period. Though it formed up to seven hydrogen bonds, the dominant number of hydrogen bonds were two and three hydrogen bonds. Similarly, compound 2 had various number of hydrogen bonds up to seven during the simulation period. However, two and three hydrogen bonds were observed predominantly for it. Compound 7 also formed up to six hydrogen bonds with predominantly two and three hydrogen bonds during the simulation period (Figure 6). The maximum number of hydrogen bonds in the molecular docking for the compounds were met by the MD simulation. Together with this, the predominant number of

hydrogen bonds obtained from the MD simulation was less than that of the molecular docking. In short, the MD simulation revealed that compounds 2 and 7 gave complexes with similar stability to the standard drug.

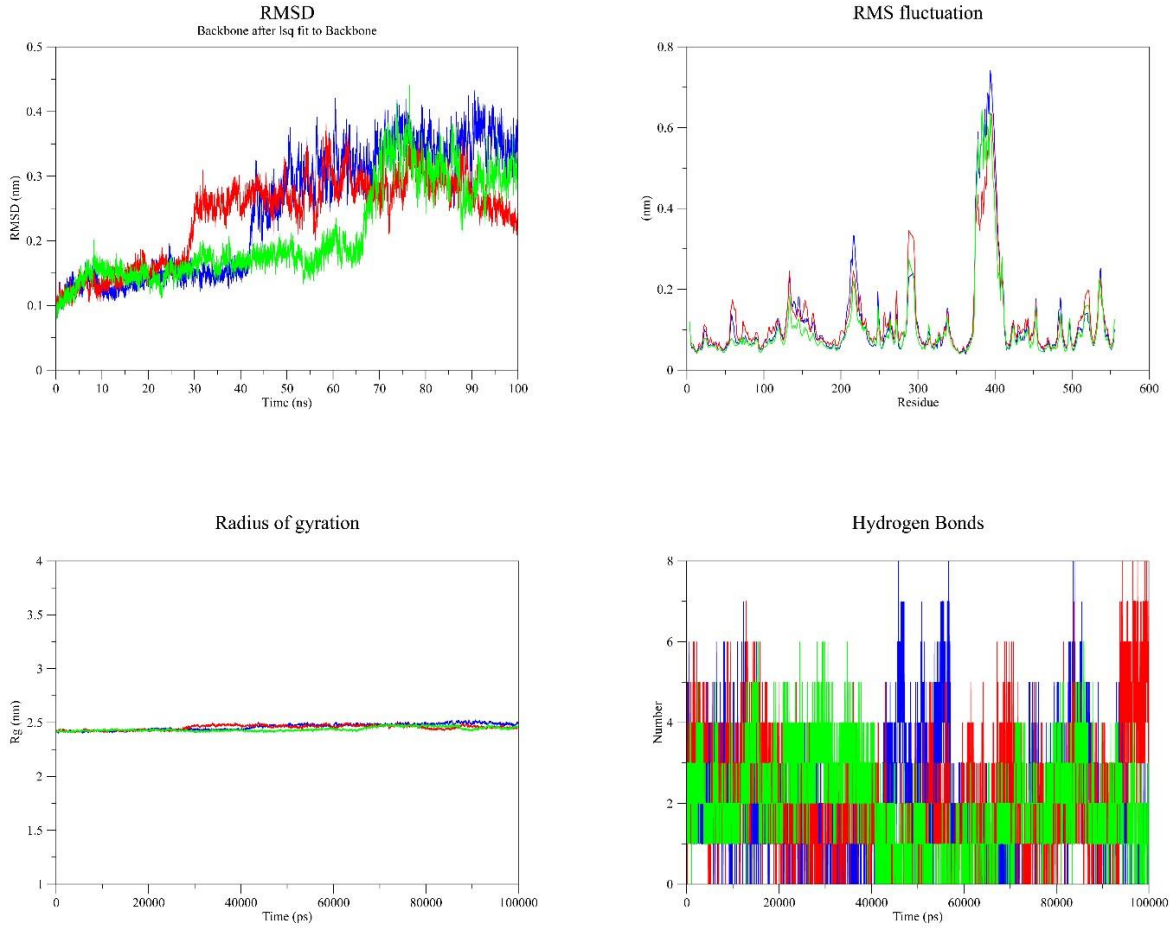

Figure 6. RMSD, RMSF, Rg, and number ligand hydrogen bonds plots obtained from the MD simulation (5ZCE-Acarbose in blue, 5ZCE-**2** in red, 5ZCE-**7** in green)

The potential of natural compounds in fighting DM is getting attention. For this, end, potential natural α-glucosidase inhibitors that could be used in DM treatment were selected by exploring the literature available. The potential of these compounds to inhibit α-glucosidase was investigated through molecular docking and MD simulation. For this end, molecular docking of the selected compounds towards the crystal structure of α-glucosidase was performed. The docking results demonstrated that the compounds had a reasonable interaction potential with the enzyme. Especially, compounds 2 and 7 had interactions similar to the standard drug. Compound 2 interacted better than the standard drug and compound 7 interacted at a comparable level. In general, the complexes procured from the docking were stable with some unexpected movements at a point. Compound 7 had relatively better stability in some time intervals in relative to compound 2 and the standard drug. In short, compounds 2 and 7 could bind to the enzyme and form a stable complex. Therefore, they have the potential to inhibit the enzyme.

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AUTHOR CONTRIBUTIONS

Concept: M.T.M.; Design: M.T.M., N.A., A.K., E.T.; Control: M.T.M.; Sources: M.T.M., N.A., A.K., E.T.; Materials: M.T.M., N.A., A.K., E.T.; Data Collection and/or Processing: N.A., A.K., E.T.; Analysis and/or Interpretation: M.T.M.; Literature Review: M.T.M., N.A., A.K., E.T.; Manuscript Writing: M.T.M.; Critical Review: M.T.M., N.A., A.K., E.T.; 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

PİYASADAN TEMİN EDİLEN NİOLİ UÇUCU YAĞI ÖRNEKLERİNİN FARMAKOPE ANALİZİ

PHARMACOPOEIA ANALYSIS OF NIOLI ESSENTIAL OIL PURCHASED FROM THE MARKET

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ÖZ

Amaç: *Halk arasında doğal kaynakların güvenli olarak kabul edilmesi ve sentetik içerikli ürünlerden uzaklaşma isteği, uçucu yağ kullanımını son zamanlarda arttırmıştır. Fakat uçucu yağlar, içerisinde çok sayıda bileşik barındırdığından standardizasyon konusunda sıkıntılar yaşanmaktadır. Bu sebeple uçucu yağların kalitesini ve terapötik amaçla kullanılabilirliğini değerlendirmek için monograflardan yararlanılmaktadır. Yapılan çeşitli çalışmalar sonucunda nioli uçucu yağının; üst solunum yolu ve üriner sistem enfeksiyonlarına, cilt rahatsızlıklarına karşı kullanılabileceği ve antioksidan, antienflamatuvar, antiseptik, antifungal, antibakteriyel, antihelmintik, insektisidal, larvisidal ve akarisidal etkinliği tespit edilmiştir. Halk arasında ise soğuk algınlığı, influenza, öksürük, sinüzit, farenjit, rinit, romatizmal rahatsızlıklarda ve üriner sistem enfeksiyonlarında kullanılmaktadır. Bu etkinlikleri ve halk arasındaki kullanımından yola çıkılarak, piyasada bulunan bazı nioli uçucu yağ örneklerinin Türk Farmakopesi 2017'ye uygunluğunun değerlendirilmesi hedeflenmiştir.*

Gereç ve Yöntem: *Çalışma kapsamında; 5 farklı markadan temin edilen nioli uçucu yağı örneklerinin Türk Farmakopesi'nde (2017) yer alan monografa göre analiz edilmiştir. Analizde, bağıl yoğunluk, kırılım imleci, optik çevirme değerleri tespit edilmiş; organoleptik kontrol, ince tabaka kromatografisi ve gaz kromatografisi analizleri yürütülmüştür.*

Sonuç ve Tartışma: *Çalışma kapsamında yapılan farmakope analizi sonucunda, temin edilen beş nioli uçucu yağ numunesinden sadece bir tanesi farmakope standardına uygun olduğu bulunmuştur. Diğer nioli uçucu yağ numuneleri, farmakopede istenilen şartları karşılamamıştır.*

Anahtar Kelimeler: *Aromaterapi, farmakope analizi, nioli uçucu yağı, Türk Farmakopesi*

ABSTRACT

Objective: *The public's considerstion of natural resources as safe and the tendency not to use synthetic component-containing products have increased the use of essential oils recently. However, since essential oils contain a vast number of compounds, there are difficulties in standardization. For this reason, monographs are used to evaluate the quality of the essential oils and their use for therapeutic purposes. As a result of various studies, it has been shown that nioli essential oil can be used against upper respiratory tract and urinary tract infections and skin disorders and has antioxidant, anti-inflammatory, antiseptic, antifungal, antibacterial, anthelmintic, insecticidal, larvicidal and acaricidal activities. It is traditionally used against cold, influenza, cough, sinusitis, pharyngitis, rhinitis, rheumatic disorders and urinary system infections. Due to these activities and*

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its traditional use; it is aimed to evaluate the compliance of some nioli essential oil samples available on the market with the Turkish Pharmacopoeia 2017.
Material and Method: *In the content of this study; nioli essential oil samples of 5 different brands are analyzed according to the monograph given in Turkish Pharmacopoeia (2017). In the analysis; relative density, refractive index, optical rotation values were determined; organoleptic control, thin layer chromatography and gas chromatography analyzes were carried out.*
Result and Discussion: *As the result of the pharmacopoeia analysis conducted within the scope of the study, only one of the five nioli essential oil samples provided was found to comply with the pharmacopoeia standard. Other nioli essential oil samples did not meet the required conditions in the pharmacopoeia.*
Keywords: *Aromatherapy, nioli essential oil, pharmacopoeia analysis, Turkish Pharmacopoeia*

GİRİŞ

Uçucu yağlar, oda sıcaklığında kolaylıkla buharlaşabilen, kuvvetli kokuya sahip, çok sayıda uçucu bileşeni içeriğinde barındıran karışımlardır. Uçucu yağların halk arasında gerek fizyolojik etkileri, gerekse kokuları dolayısıyla kullanımı çok eski zamanlara uzanmaktadır. Bu kullanım alanları arasında antiseptik etkisi ile, parfüm, mumyalama ve gıdaların saklanması gibi çeşitli amaçların yanı sıra; ağrı kesici, sakinleştirici, spazm giderici, iltihap giderici, lokal anestezik, antimikrobiyal etkileri dolayısıyla kullanıldığına dair kayıtlar da mevcuttur [1].

İnsanların doğal kaynaklı ürünlere yönelimi ve sentetik kökenli ürünlerin istenmeyen etkilerine dair endişenin artması nedeniyle, uçucu yağların gıda, kozmetik ve sağlık sektörlerinde kullanımı önemli ölçüde artmıştır. İçeriklerinde bulunan çok çeşitli bileşikler ve doğal olmaları dolayısıyla halk arasında güvenli olarak kabul görmektedirler. Bununla birlikte gösterdikleri farklı fizyolojik etkiler nedeniyle; üreticiler de pek çok ürün içeriğine uçucu yağları ilave etmeye başlamıştır [2].

Günümüzde bilinen üç binden fazla uçucu yağın yaklaşık 300 tanesi çeşitli sanayi kollarında kullanılmaktadır; bu kullanım alanları arasında sağlık, gıda ve kozmetik sektörleri ilk sıralarda gelmektedir. İlaç ve kozmetik formülasyonlarında hem fizyolojik etkileri nedeniyle hem de koku ve aroma verici olarak; gıda sektöründe ise aroma verici ve koruyucu olarak kullanımları örnek olarak verilebilir. Aynı zamanda günlük hayatta ve aromaterapi uygulamaları kapsamında; inhalasyon, masaj, banyo vb. şekillerde doğrudan kullanımı da mevcuttur [3,4]. Son yıllarda aromaterapi uygulamalarının da yaygınlaşmasıyla, halk arasında çoğunlukla inhalasyon ve lokal uygulama şeklinde kullanılan uçucu yağlar, pek çok rahatsızlığın giderilmesi amacıyla dahilen de kullanılmaktadır. Özellikle stres ve gerginliği azaltmak, uyku kalitesini arttırmak amacıyla difüzörler aracılığıyla veya sprey şeklinde; nezle, grip, sinüzit, bronşit gibi solunum sistemi rahatsızlıklarının tedavisinde destekleyici olarak inhaler şeklinde; sindirim sistemi rahatsızlıklarında, ağrıların giderilmesi amacıyla dahilen; cilt sorunlarının tedavisinde ve hatta doğumun kolaylaştırılması amacıyla topikal olarak yaygın şekilde kullanılmakta olan uçucu yağların kalitesi, halk sağlığı bakımından büyük önem taşımaktadır [5,6]. Yapılan çalışmalar; uçucu yağların antimikrobiyal, antioksidan, antiparazitik, insektisidal, antienflamatuvar, analjezik, sedatif, yara iyi edici, antihipertansif etkileri gibi çok çeşitli fizyolojik etkiler gösterebileceğini ortaya koymuştur [7].

Uçucu yağların içeriği; kullanılan elde ediliş yöntemi, saklama koşulları, bitkinin yetiştiği bölgedeki toprak ve iklim koşulları gibi parametrelerden önemli ölçüde etkilenmektedir [1]. Ayrıca uçucu yağların düşük verimle elde edilen yüksek maliyetli ürünler olması nedeniyle, bazı üretici veya satıcıların, kazanılan karı arttırmak amacıyla, uçucu yağlarda tağşişat yaptıkları da bilinmektedir [8]. Tağşişat amacıyla genellikle bitkisel veya mineral yağlar kullanılmaktadır. Seyreltme sonucunda uçucu yağın rengi değişmekte ve kokusu keskinliğini kaybetmektedir. Yapılan piyasa araştırmaları sonucunda tağşiş için; gazyağı, hindistan cevizi yağı, bademyağı polietilen glikol, triasetin, trietil sitrat veya benzil alkol, etil alkol kullanılabildiği tespit edilmiştir. Bunun yanı sıra doğal uçucu yağ olduğu iddia edilen ürünlere sentetik kaynaklı uçucu bileşiklerin ilave edilebildiği de bilinmektedir [9].

Dünya ticaret hacminde, uçucu yağların kapladığı alan 2010 ile 2015 yılları arasında yaklaşık %200 oranında büyüme göstermiştir [10]. Hızla artan bu talep; daha uygun fiyatlı yağlar veya sentetik bileşiklerin ilavesi ile tağşiş edilmiş, ancak tamamen doğal olduğu iddia edilen bazı ürünlerin piyasada yer bulması sonucunu doğurmuştur. Bu durum, uçucu yağdan beklenen etkinin görülmemesine; daha da

önemlisi, içeriği belli olmayan bu ürünlerin kullanan kişilerde istenmeyen etkilerin ortaya çıkmasına neden olabilmektedir [8]. Uçucu yağlar piyasaya sunulurken; kaynak bitkinin botanik adı, menşei, yetiştirme yöntemi, uçucu yağ eldesi için damıtma yapılan bitki bölümü ve ana bileşenleri belirtilmelidir ve ürün satın alınırken bunlara dikkat edilmelidir [11]. Halk arasında ve tedaviye destek olarak bu kadar yaygın kullanılan bu ürünlerin kalitesi ve güvenilirliği son derece önemlidir. Bu bağlamda, çeşitli farmakope ve monograflar, belirli standartların oluşturulabilmesi için ihtiyaç duyulan kaynaklardır [1].

Nioli uçucu yağının elde edildiği *Melaleuca* L. türlerinin dahil olduğu Myrtaceae familyası bitkileri, taşıdıkları uçucu yağlar dolayısıyla kullanımları bakımından öne çıkmaktadır [12]. Terapötik amaçlı kullanılan veya bu tip formülasyonlara yardımcı madde olarak dahil edilen ürünler için ülkemizde referans kaynak olan Türk Farmakopesi 2017'de verilen monografa göre; nioli uçucu yağı *Melaleuca quinquenervia* (Cav.) S.T. Blake'in genç yapraklı dallarından buhar distilasyonuyla elde edilir [13]. Piyasada bulunan nioli yağlarının önemli bir kısmı ise *Melaleuca viridiflora* Sol. ex Gaertn türünden elde edilen uçucu yağdır. *M. quinquenervia*'dan elde edilen ve piyasada bulunan uçucu yağ, sineol kemotipi olarak bilinen yağdır ve içeriğinde majör olarak bulunan 1,8-sineol, yağın yaklaşık %55'ini oluşturur [2,14]. Komisyon E monograflarında, droğun içermesi gereken sineol oranı ise %40-60 olarak belirtilmiştir [15]. Bunun yanı sıra; linalol (%23,9), viridiflorol (%40-45), nerolidol (%75,2-92) kemotiplerine de rastlamak mümkündür [14,16]. Yapılan fitokimyasal çalışmalar sonucunda; *M. quinquenervia* yapraklarından elde edilen uçucu yağın içinde kırktan fazla bileşik tespit edilmiş olup majör olan 1,8-sineol yanında, viridiflorol, p-simen, γ-terpinen, α-pinen, α-terpineol, terpinolen, limonen, ledol, β-pinen, karyofilen, β-terpinil asetat, karveol ve longifolen de farklı numunelerde nispeten yüksek miktarlarda tespit edilen bileşikler arasında bulunmuştur [4,17-22].

Komisyon E monograflarında, nioli yağının üst solunum yolu enfeksiyonlarında kullanımı kayıtlıdır. Kullanım dozu için ise; dahilen 0.2-2 g/gün, burun damlası şeklinde bitkisel yağ içinde %2-5'lik karışım, haricen uygulamada ise yağ içinde %10-30 uçucu yağ içeren karışım şeklinde önerilmektedir [15]. Bunun yanı sıra; çeşitli kaynaklarda, nioli uçucu yağının halk arasında soğuk algınlığı, influenza, öksürük, sinüzit, farenjit, rinit, romatizmal rahatsızlıklar, cilt rahatsızlıkları ve nevraljiye karşı kullanılımı belirtilmektedir [17,23]. Halk arasında kullanıma dair yapılan bir çalışmada, nioli yağının vajina florasının bozulması nedeniyle sık rastlanan *Candida* enfeksiyonunlarına karşı, kullanılan tampona damlatmak suretiyle kullanımı olduğu tespit edilmiştir [24].

Yapılan çalışmalar sonucunda; nioli uçucu yağının antioksidan, antienflamatuvar, antiseptik, antifungal, antibakteriyel, antihelmintik, insektisidal, larvisidal ve akarisidal etkinlik gösterdiği tespit edilmiştir [12,18,19,21,22,25-29].

Uçucu yağların gıdalar ile birlikte kullanımına dair çalışmalar doğrultusunda, nioli uçucu yağının antifungal etki gösterdiği tespit edilmiş ve bu doğrultuda yağın; ekmek, havuç, patates, peynir gibi uzun süre muhafaza edilen gıdalarda raf ömrü süresince mikrobiyal kontaminasyona karşı koruyucu olarak kullanılabileceği gösterilmiştir. Benzeri çalışmalar; gıdaların saklanması sırasında mikotoksin içeriği ve mikrobiyal kontaminasyon gibi nedenlerle kullanılamaz hale gelmesinin engellenmesinde nioli uçucu yağının kullanım potansiyelini ortaya koymuştur [4,20,30,31]. Nioli uçucu yağının sadece gıda patojenlerine değil, insanlar üzerinde etkili pek çok suşa karşı etkinliğinin değerlendirildiği çalışmalar mevcuttur. Yağın etkili bulunduğu suşlar arasında antibiyotiğe dirençli hastane enfeksiyonları *Candida* türleri de bulunmakta olup, sonuçlar bu tip enfeksiyonların önlenmesi ve tedavisinde nioli uçucu yağının önemli bir potansiyele sahip olduğunu göstermektedir [32].

Nioli uçucu yağının dahilen kullanımında gastrik ülsere karşı etkinliğinin değerlendirildiği bir çalışmada, ratlar üzerinde, büyük ölçüde antioksidan ve antienflamatuvar etkinliğe atfedilen pozitif sonuçlar elde edilmiştir [23].

Nioli uçucu yağı, bazı ilaç formulasyonlarının içeriğine de katılmaktadır. Özellikle östradiol içeren transdermal ve kütanöz ilaç taşıyıcı sistemlerde, penetrasyonu arttırıcı ajan olarak kullanımı üzerinde çalışmalar bulunmaktadır. Bu çalışmalardan birinde de, nanoemülsiyon formunda hazırlanan nioli uçucu yağının akne tedavisinde kullanım potansiyeli olduğu gösterilmiştir [2,33]. Nioli yağı içeren farklı yara filmlerinin etkinliğinin değerlendirildiği bir çalışmada, formülasyon içine dahil edilen yağın yara iyileştirici ve antibakteriyel etkisi saptanmış, doğada çözünür film formülasyonlarında etkili bileşen olarak nioli yağının kullanılabileceği gösterilmiştir [34]. Kozmetik sanayinde de önemli bir kullanımı olan nioli yağının, hücrelerde antitirozinaz ve antimelanojenik aktivitesinin değerlendirildiği bir

çalışmanın bulguları; bu yağın cilt rengini açma amacıyla kullanılan preparatların içeriğine dahil edilebileceğini göstermiştir. Aynı çalışmada uçucu yağın antioksidan kapasitesi de test edilmiş ve bu etkisinin de söz konusu hedefe katkı sağladığı belirtilmiştir [35].

Üriner sistem enfeksiyonu olan, kısmi felçli hastalarla yürütülen klinik bir çalışmada; nioli yağı damlatılarak hazırlanan yıkama çözeltisi kullanılmış ve enfeksiyonda önemli ölçüde iyileşme gözlenmiştir. Bu etki uçucu yağın antienflamatuvar ve antimikrobiyal etkinliğine atfedilmiş olup; uçucu yağ uygulamasının antibiyotik tedavisinin etkinliğini arttırarak antibiyotik kullanım süresini kısalttığı tespit edilmiştir [36].

Nioli uçucu yağının gıda sanayi ve tarım alanında farklı zararlılara karşı kullanım potansiyelinin değerlendirildiği çalışmalar ise, uçucu yağın fumigant olarak kullanımının mümkün olduğunu ortaya koymuştur [25,26]. Sivrisinek ile mücadelede nioli yağının kullanım potansiyelinin değerlendirildiği çalışmalarda; yağın hem larvalara hem de ergin sineklere karşı etkinlik gösterdiği tespit edilerek sivrisinek ile mücadelede kullanım potansiyeli belirtilmiştir [37,38].

Toksisite çalışmaları; nioli uçucu yağının ana bileşeni olan 1,8-sineolün karaciğerde CYP2B1 ve CYP3A2 enzimlerini uyardığını, ancak bu etkinin terapötik dozun çok üzerinde olduğu için bileşiğin kullanımına dair bir uyarı gerektirmediğini göstermiştir. Nadir vakalarda bileşiğin, deri iritasyonuna neden olabileceği bildirilmiştir. 9 aylık ile 3 yaş arasındaki bebek ve çocuklarla yapılan bir çalışmada, 1,8-sineol ve nioli uçucu yağının inhalasyon yoluyla, mukoza iritasyonu, taşikardi, nefes darlığı, bulantı, kusma, kaslarda güçsüzlük, vertigo gibi toksik etkilere neden olabileceği gözlenmiştir. 1,8-sineol içeriği yüksek olan diğer uçucu yağlar gibi, nioli uçucu yağının da, merkezi sinir sistemi ve solunum sistemi sorunlarına yol açma riskinden dolayı bebeklerde ve küçük çocularda kullanımı önerilmemektedir [14]. Fibroblast hücreleri üzerinde yapılan sitotoksisite çalışmaları ise, nioli uçucu yağının düşük dermal toksisite riski taşıdığını ve haricen güvenle kullanılabileceğini göstermiştir [21]. Nioli yağı uygulamasında melanoma hücrelerinin canlılığının değerlendirildiği bir çalışmada ise, uçucu yağın sitotoksik etki göstermediği tespit edilmiştir [36]. Topikal kullanımda, uçucu yağın bebek ve küçük çocukların yüz bölgesine, özellikle de burun çevresine sürülmemesi gerektiği belirtilmiştir [15]. Çalışmalarda fetal toksisite görülen dozun günlük kullanım dozunun çok üzerinde olması ve içeriğindeki bileşiklerin de bu bağlamda güvenli bulunması dolayısıyla, nioli uçucu yağının hamilelikte kullanımı riskli görülmemiştir [14]. Ancak hamilelerde inhalasyon yoluyla kullanılan nioli uçucu yağının hormonlar üzerine etkisinin değerlendirildiği bir çalışmada; yağın progesteron ve plasental laktojen salımını arttırdığı; dolayısıyla hormonlar üzerindeki etkisi nedeniyle dikkatli kullanılması gerektiği bildirilmiştir. Aynı çalışmada nioli uçucu yağının plasental toksisitesi de değerlendirilmiş ve bu bağlamda yağın güvenli olduğu tespit edilmiştir [5]. Bununla birlikte, karaciğer rahatsızlığı, gastrointestinal kanal ve safra yolları iltihabı olan kişilerin nioli yağını dahilen kullanması önerilmemektedir [15]. Nioli uçucu yağı, diğer uçucu yağlarda olduğu gibi doğrudan cilt üzerine uygulanmamalıdır. Oluşabilecek alerjik reaksiyonları öngörmek için cildin küçük bir yerine uygulanarak 1 saat gözlemlenmelidir. Göze temasından kaçınılıp temas olması durumunda, göz bol suyla yıkanmalıdır [39].

GEREÇ VE YÖNTEM

Çalışma Materyali

Nioli uçucu yağı numuneleri üzerinde yapılan farmakope analizi için çalışma kapsamında beş farklı markadan numune temin edilmiştir. Çalışma sonucunu etkilememesi amacıyla her markaya harf kodu verilmiştir. Çalışma kapsamında değerlendirilen numunelere A, B, C, D, E kodu verilmiştir. Analiz sonuçları markalar göz önüne alınmadan yorumlanmıştır.

Türk Farmakopesi 2017'de nioli uçucu yağının *Melaleuca quinquenervia* bitkisinden elde edilen türü kayıtlıdır fakat piyasada yaygın olarak *M. viridiflora* türünden elde edilen uçucu yağlar bulunmaktadır. Bu sebeple çalışmamızda *M. viridiflora* türünden elde edilen uçucu yağlar temin edilebilmiştir.

Nioli uçucu yağ numunelerinin farmakope analizi, Türk Farmakopesi 2017 (TF 2017) referans alınarak farmakopede belirtilen monografiara göre yapılmıştır. TF 2017 monografiarına göre Nioli uçucu yağı için istenilen testler;

-Organoleptik Kontrol
-İnce Tabaka Kromatografisi
-Bağıl Yoğunluk
-Kırılım İmleci
-Optik Çevirme
-Gaz Kromatografisi olarak verilmiştir [13].

Organoleptik Kontrol

TF 2017'ye göre organoleptik kontrol testinde uçucu yağın renksiz veya soluk sarı renkli görünüp aromatik sineol kokulu olması beklenmektedir. Tüm numuneler bu bağlamda değerlendirilmiştir.

İnce Tabaka Kromatografisi

Nioli uçucu yağı için TF 2017'de belirtilen monografa göre, 100 µl numune 2 ml toluen içinde çözülüp 10 ml'ye tamamlanarak test çözeltisi hazırlanmış, bu çözeltiden ve 25 µl trans-nerolidol ve 50 µl sineol, toluene içinde çözülüp 5 ml'ye tamamlanarak hazırlanan şahit çözeltisi ile ince tabaka kromatografisi (İTK) uygulaması yapılmıştır. Farmakopeye göre test çözeltisinin kromatogramında ilk önce starta yakın noktada viyole-kahverengi bir leke ve yoğun bir viyole-kahverengi leke gözükmelidir. Daha sonra 1,8-sineolün oluşturduğu yoğun bir viyole-kahverengi leke görülmelidir. Bu lekenin üstünde de sırasıyla mor ve hafif gri bir leke gözükmelidir. Farmakopeye göre şahit çözeltisinde, test çözeltisi ile aynı yerde 1,8-sineolü temsilen viyole-kahverengi bir leke ve bu lekenin altında trans-nerolidolü temsilen koyu viyole renkte leke gözükmelidir. Farmakopede, test çözeltisi ile elde edilen kromatogramda başka lekelerin de bulunabileceği belirtilmiş ve ince tabaka kromatografisinin deneysel prosedürü TF 2017'de 2.2.27 kısmında belirtilmiştir.

Çalışmamızda yapılan ince tabaka kromatografisi için; standart olarak trans-nerolidol ve 1,8-sineol, plak olarak ise İTK Silika Jel F254 kaplı alüminyum plak kullanılmıştır. Mobil faz olarak kullanılan etil asetat:toluen (5:95 h/h) ile doyurulmuş tankta, plak üzerinde 9 cm'lik sürüklenme sağlanmıştır. Plak en son anisaldehit sülfürik asit reaktifi ile muamele edilerek 3 dakika boyunca 100-105 °C'de ısıtıldıktan sonra gün ışığında incelenmiştir.

Bağıl Yoğunluk

Bir bileşiğin bağıl yoğunluğu; t_1 sıcaklığında belirli hacminin kütlesinin, t_2 sıcaklığında aynı hacimdeki suyun kütlesine oranıdır. Farmakopede bağıl yoğunluk testinin deneysel prosedürü ve formülü 2.2.5 kısmında yer almaktadır. Nioli uçucu yağı monografında bağıl yoğunluk değerinin bulunması gereken aralık 0,904-0,925 olarak verilmiştir. Çalışmada, numunelerin bağıl yoğunluğu piknometre yardımıyla ölçülmüştür.

Kırılım İmleci

Numunelerin kırılım imlecini ölçerken refraktometre kullanılmıştır. Nioli uçucu yağı monografında kırılım imleci değerinin bulunması gereken aralık 1,463-1,472 olarak verilmiştir. Farmakopede bağıl kırılım imleci testine dair deneysel prosedür 2.2.6 kısmında yer almaktadır.

Optik Çevirme

Optik çevirme testi yapılırken polarimetre cihazı ve 10 ml hacimli (10 cm uzunluğunda) polarimetre tüpü kullanılmıştır. Farmakopede optik çevirme testine ait deneysel prosedür ve formül 2.2.18 kısmında yer almaktadır. Nioli uçucu yağı monografında optik çevirme değerinin bulunması gereken aralık -4°-+1° olarak verilmiştir.

Gaz Kromatografisi

Gaz kromatografisi analizi hariç yapılan banko üzeri testler tamamlandıktan sonra farmakope standartlarına en yüksek uyumluluk gösteren numune ile GC-MS analizi yapılmış ve numunenin içeriği analiz edilmiştir. Nioli uçucu yağı için monografta verilen bileşenler ve % miktarları ve numunenin analiz sonuçları ile birlikte Tablo 1'de yer almaktadır.

Yapılan analiz için Agilent 7890B GC System kullanılmıştır. Kolon olarak, Agilent HP-Innowax

(60 m x 0,25 mm iç çap x 0,25 µm film kalınlığı) ve dedektör olarak ise Alev İyonlaşma Dedektörü (FID) kullanılmıştır. Enjeksiyon ünitesi ve kolon sıcaklığı 250°C olacak şekilde ayarlanmıştır. Sıcaklık programı ise 60°C (10 dk), 4°C/dk. 220°C (10 dk) 1°C/dk 240°C olmak üzere toplam 80 dakikadır. Taşıyıcı gaz olarak 0.7 ml/dk akışta helyum gazı kullanılmıştır.

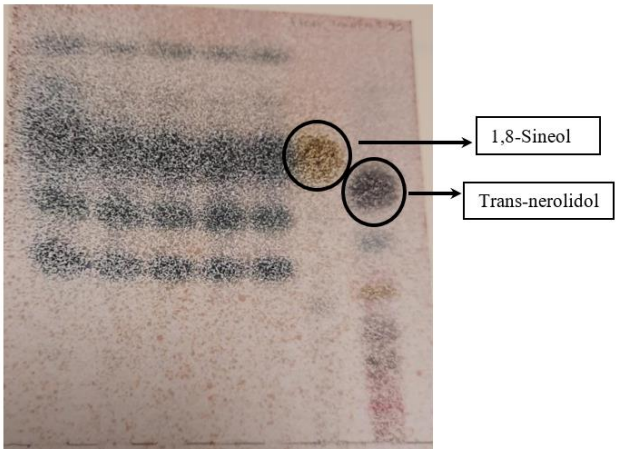
SONUÇ VE TARTIŞMA

Organoleptik Kontrol

İncelenen nioli uçucu yağ numunelerinin tümünün, TF 2017 ile uyumlu renk ve kokuya sahip olduğu tespit edilmiştir.

İnce Tabaka Kromatografisi

Sırasıyla uygulanan 5 uçucu yağ numunesi ile farmakopede referans olarak verilen 1,8-sineol ve trans-nerolidol maddelerinin İTK kromatogramı Şekil 1'de görülmektedir. Nioli uçucu yağı numunelerine ait kromatogramların hepsinde, standart olarak kullanılan 1,8-sineol ile aynı R_f değerinde leke gözlenmiştir ve nioli uçucu yağ numunelerinin hiçbirinde,trans-nerolidol ile aynı R_f değerinde leke görülmemiştir. Aynı zamanda nioli uçucu yağ numunerinde farmakopede belirtildiği gibi viyole-kahverengi bir leke ve yoğun bir viyole-kahverengi leke, mor ve gri leke gözlemlenmiştir. Çalışma kapsamında yapılan ince tabaka kromatografisi uygulamasına göre incelediğimiz nioli uçucu yağlarının farmakopeye uygun olduğu söylenebilir.



Şekil 1. Nioli uçucu yağı İTK kromatogramı

Bağıl Yoğunluk

Çalışma kapsamında bağıl yoğunluğu ölçülen numuneler arasında, elde edilen değeri farmakopede belirtilen 0,904-0,925 aralığında bulunan sadece A kodlu nioli uçucu yağı olmuştur. B, C, D, E kodlu nioli uçucu yağları referans aralığı dışında kalmıştır. Numuneler için hesaplanan bağıl yoğunluluk değerleri Tablo 1'de verilmiştir.

Tablo 1. Nioli Uçucu yağların bağıl yoğunluk testi sonucunun verileri

Marka Kodu	Bağıl Yoğunluk
A	0.91207
B	0.95230
C	0.93305
D	0.89120
E	0.86569

Kırılım İmleci

Çalışma kapsamında kırılım imleci ölçülen tüm numuneler için elde edilen sonuçlar, farmakopede belirtilen 1,463-1,472 referans aralığında bulunmuştur. Numuneler için ölçülen kırılım imleci değerleri Tablo 2'de verilmiştir.

Tablo 2. Nioli Uçucu yağ numunelerinin kırılım imleci değerleri

Marka Kodu	Kırılım İmleci
A	1.467
B	1.465
C	1.465
D	1.466
E	1.465

Optik Çevirme

Çalışma kapsamında optik çevirme dereceleri ölçülen tüm nioli uçucu yağ numuneleri için elde edilen sonuçlar farmakopede belirtilen -4° - +1° referans aralığında bulunmuştur. Numuneler için hesaplanan optik çevirme değerleri, Tablo 3'de verilmiştir.

Tablo 3. Nioli Uçucu yağları için hesaplanan optik çevirme dereceleri

Marka Kodu	Bağıl Yoğunluk	Polarimetrede okunan değer	Optik Çevirme
A	0.91207	-1.9525	-2.1407
B	0.95230	-1.5980	-1.6780
C	0.93305	-1.7940	-1.9222
D	0.89120	-1.8905	-2.1212
E	0.86569	-1.9995	-2.3097

Gaz Kromatografisi

Farmakopeye göre, nioli uçucu yağ numunelerinin içeriğinde bulunması gereken madde miktarları ve gaz kromatografisi (GC-MS) analizi sonucunda tespit edilen değerler Tablo 4'de verilmiştir.

Tablo 4. Nioli uçucu yağının GC-MS analizi için, TF 2017'de verilen limitler ve analizi yapılan numunenin içeriği

Bileşik	Analiz Sonucu Tespit Edilen Bağıl Yüzde (%)*	Farmakopede Verilen Bağıl Yüzde (%)
α-pinen	6.9	5.0-15.0
β-pinen	1.9	1.0-4.0
β-mirsen	0.8	
Limonen	6.9	5.0-10.0
1,8-sineol	57.4	45.0-65.0
γ-terpinen	1.1	
p-simen	1.3	0.05-4.0
Benzaldehit	-	0.05-0.5
α-terpinolen	0.6	
β-karyofillen	2.6	
α-terpineol	7.9	3.0-8.0
α-terpinil asetat	1.0	
Alloaromadendren	0.5	
Trans-nerolidol	0.5	0.05-1.5
Ledol	0.7	
Viridiflorol	4.3	2.5-9.0

*Analizde miktarı %0.5 ve üzerindeki bileşikler tespit edilmiştir.

Aromaterapi uygulamalarının son yıllarda yaygınlaşması ile birlikte uçucu yağ satışı yapan çok sayıda firma piyasada yer almaya başlamıştır. Fakat piyasada yer alan uçucu yağların kalitesi ve bileşimi farklılık göstermektedir. Piyasadaki bu çeşitlilik terapötik etki amacıyla kullanılmak istenilen uçucu yağlarda sorun yaratabilmektedir. Terapötik etki için kullanılmak istenilen uçucu yağların farmakope standardında olması gerekmektedir. Aksi takdirde bu ürünlerden beklenen etkinin görülmemesi ve daha da önemlisi, bu ürünlerin insan fizyolojisine zarar verme riski doğmaktadır. Uçucu yağların, ürün güvenliği için kalite kontrolleri son derece gereklidir [40]. Uçucu yağların kalitesi, güvenliği ve etkilerini değerlendirmek amacıyla Türk Farmakopesi 2017 (Avrupa Farmakopesi Adaptasyonu), Avrupa Farmakopesi, ESCOP Monografları, Komisyon E Monografları gibi kaynaklardan yararlanılmaktadır [41].

Nioli uçucu yağı üzerinde yapılan biyolojik aktivite testlerine göre; yağın antibiyotiğe dirençli hastane enfeksiyonlarına sebep olan *Candida* türleri üzerinde etki göstermesi, ratlar üzerinde dahilen kullanımda, antioksidan ve antienflamatuvar etkinliklik göstermesi ve nioli uçucu yağı içeren yıkama çözeltilerinin üriner sistem enfeksiyonlarında iyileşme göstermesi sebebiyle nioli uçucu yağının aromaterapideki yeri önemlidir [23,32,36].

Bu çalışmada elde edilen sonuçlar, bizlere piyasadaki durumun ciddiyetini göstermektedir. Halk sağlığı için terapötik amaçla kullanılmak istenilen yağların farmakope analizleri ile kontrol edilmesi, uçucu yağları kullanan bireylerin kullanım ve temin konusunda bilinçlendirilmesi gerekmektedir. Uçucu yağlar, sıcaklık ve ışıktan etkilenebilen, özel koşullarda saklanması gereken ürünlerdir. Ürünlerin uygun olmayan koşullarda saklanması da uçucu yağ içeriğinde değişikliklere neden olabilmektedir. Uçucu yağlar, koyu renkli cam şişelerde serin ve ışık görmeyen yerlerde saklanmalıdır [42]

Çalışma sonucunda; A kodlu nioli uçucu yağı için ince tabaka kromatografisi, bağıl yoğunluk, kırılım imleci, optik çevirme testleri sonucunda elde edilen değerlerin farmakope sınırı içinde kaldığı görülmüştür. Çalışma kapsamında yürütülen GC analizinde, numune içinde %0,5 ve üzerindeki oranlarda bulunan bileşiklerin miktarı tespit edilebildiğinden, farmakopede limitleri %0,05-1,5 oranında belirtilen benzaldehit bileşiğine dair yorum yapılamamıştır. Diğer bileşikler için GC analizinin sonucuna göre farmakopeye uygunluğu tespit edilmiştir. Tüm bu testler ışığında A kodlu nioli uçucu yağının farmakopeye uygun olduğu söylenebilmektedir.

B, C, D, E numuneleri ile elde edilen İTK kromatogramında, farmakopede istenilen lekeler gözlemlenmiştir. Bağıl yoğunluk testine göre; B, C, D, E kodlu numuneler referans aralığı dışında kalmıştır. Kırılım imleci ve optik çevirme testlerine göre tüm numuneler referans değeri aralığında kalmıştır. Ancak bağıl yoğunluğun piknometreyle ölçüldüğü, ve düşük hacimlerle çalışıldığı için sonuçlarda sapmanın fazla olabileceği göz önünde bulundurulmalıdır. Dolayısıyla, bağıl yoğunluk kullanılarak hesaplanan spesifik optik çevirme değerindeki sapmalara bu etkenin sebep olma ihtimali de değerlendirilmelidir. Tüm testler baz alındığında farmakopede verilen değerlere göre A kodlu nioli uçucu yağı farmakopeye göre uygun bulunmuştur.

Çalışma kapsamında elde edilen sonuçlar, küçük bir örneklem dahilinde uçucu yağ piyasasının durumunu gözler önüne sermektedir. Terapötik etki beklenerek kullanılan uçucu yağların farmakopeye uygunluğu, insan sağlığı bakımından büyük önem arz etmekte olup, piyasada bulunan çok sayıdaki ürünün analizi ile etkinlik ve güvenilirliğinin tespiti; bunun yanı sıra halkın uçucu yağların kullanımına dair bilinçlendirilmesi bu alandaki suistimallerin önüne geçmek için önemli adımlar olarak görülmektedir.

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ÖZGÜN MAKALE / ORIGINAL ARTICLE

BAZI BENZOKSAZOL-2(3*H*)-ON/BENZOTİYAZOL-2(3*H*)-ON TÜREVLERİNİN SENTEZİ VE ALZHEİMER HASTALIĞINA KARŞI ETKİLERİNİN İNCELENMESİ

SYNTHESIS OF SOME BENZOXAZOLE-2(3H)-ONE/BENZOTHIAZOLE-2(3H)-ONE DERIVATIVES AND INVESTIGATION OF THEIR EFFECTS AGAINST ALZHEIMER'S DISEASE

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ÖZ

Amaç: *Bu çalışmada benzoksazolon/benzotiyazolon halkası taşıyan asetamit türevi 28 yeni bileşik sentez edilmiş ve Alzheimer hastalığına karşı etkileri in vitro olarak test edilmiştir.*
Gereç ve Yöntem: *Sonuç bileşiklerin sentezinde kullanılan amin türevleri (5 ve 6), öncelikle ticari olarak mevcut 1,3-benzoksazol-2(3H)-on ve 1,3-benzotiyazol-2(3H)-on halkalarının metillenmesi, nitrik asit ile nitrolanması ve ardından kalay klorürle indirgenmesi ile sentez edilmiştir. Daha sonra, amin türevi 5 ve 6'nın bromoasetil bromür ile açilasyonundan hazırlanan ara ürünlerin (7 ve 8) uygun amin türevleri ile tepkimesinden sonuç bileşikler (9a-n ve 10a-n) elde edilmiştir. Sentezlenen bileşiklerin kimyasal yapıları spektroskopik yöntemler, HRMS ve elementel analiz ile aydınlatılmıştır. Tüm sonuç bileşiklerin modifiye Ellman yöntemiyle kolinesteraz inhibitör aktiviteleri belirlendikten sonra DPPH ve ORAC yöntemiyle antioksidan aktiviteleri ölçülmüştür. Son olarak sonuç bileşiklerin metal şelatör özellikleri tayin edilmiştir.*
Sonuç ve Tartışma: *Yeni 2-sübstitüe-N-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol/1,3-benzotiyazol-6-il)asetamit türevi bileşikler sentez edilmiştir. Kolinesteraz inhibitör etkileri ve yapı aktivite ilişkileri belirlenmiştir. Asetilkolinesteraz (AKE) inhibisyonu için bileşik 10g'nin (IC_{50}: 52.90 µM), butirilkolinesteraz BKE inhibisyonu için bileşik 10h'nin (IC_{50}: 51.03 µM) en yüksek aktiviteye sahip olduğu bulunmuştur. ORAC testi ile yapılan antioksidan aktivite tayininde ise yan zincirinde fenilpiperazin türevleri taşıyan bileşiklerin referans trolokstan daha yüksek antioksidan aktivite gösterdikleri bulunmuştur. Ayrıca sonuç bileşiklerin metal şelatör etkileri incelendiğinde büyük çoğunluğunun metal şelatör özellik taşıdığı belirlenmiştir.*
Anahtar Kelimeler: *Alzheimer hastalığı, benzoksazolon, benzotiyazolon, kolinesteraz inhibisyonu, metal şelasyonu*

ABSTRACT

Objective: *In this study, 28 new acetamide derivatives bearing benzoxazole/benzothiazole rings were synthesized and their effects against Alzheimer's disease were tested in vitro.*
Material and Method: *Amine derivatives (5 and 6) used in the synthesis of final compounds were synthesized by the methylation of commercially available 1,3-benzoxazol-2(3H)-one or 1,3-benzothiazol-2(3H)-one rings with dimethyl sulfate followed by nitration with nitric acid and then*

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reduction with tin chloride. In order to synthesize acetamide derivatives, 5 and 6 was first acylated with bromoacetyl bromide to obtain intermediates 7 and 8. Then, these compounds was reacted with appropriate secondary amines to get the title compounds (9a-n and 10a-n). The structure of the synthesized compounds was elucidated by spectroscopic methods, HRMS and elemental analysis. After the cholinesterase inhibitor activities of all title compounds were determined by the modified Ellman method, their antioxidant activities were measured by the DPPH and ORAC method. Finally, the metal chelator properties of the title compounds were determined.

Result and Discussion: *New 2-substituted-N-(3-methyl-2-oxo-2,3-dihydro-1,3-benzoxazol/1,3-benzothiazol-6-yl)acetamide derivative compounds were synthesized. Their cholinesterase inhibitory effects and structure activity relationships were determined. Compound 10g (IC_{50}: 52.90 µM) was identified as the most potent acetylcholinesterase (AChE) inhibitor, and also compound 10h (IC_{50}: 51.03 µM) was determined as the most active derivative for the butyrylcholinesterase (BChE). In the ORAC antioxidant activity test, compounds carrying phenylpiperazine derivatives in their side chains were found to show 3-5 times higher antioxidant activity than the reference trolox. Additionally, the metal chelator activity results of the title compounds showed that the majority of them had metal chelator properties.*

Keywords: *Alzheimer's disease, benzoxazolone, benzothiazolone, cholinesteraze inhibition, metal-chelating*

GİRİŞ

Hastanın hafızasının, karar verme yetisinin, öğrenme yeteneğinin ve yaşamsal aktivitelerinin zamanla azaldığı ilerleyici nörodejeneratif bir hastalık olan Alzheimer Hastalığı (AH), 65 yaş üstü nüfusun önemli kısmını etkileyen bir sağlık sorunudur [1].

Uzun yıllardır üzerinde gayretle çalışılmasına rağmen hastalığın nedeni tam olarak belirlenememiştir. Bu nedenle hastalığın tedavisi araştırılırken kolinerjik eksiklik, hücre dışı amiloid beta (Aβ) plaklar, hücre içi nörofibriler yumaklar (NFY), nöroenflamasyon, oksidatif stres ve metal dishomeostazisi gibi önemi çeşitli çalışmalarla kanıtlanmış patofizyolojik bulgulardan yola çıkılmaktadır [2,3]. AH'nin tedavisi amacıyla patofizyolojik bulgulardan faydalanılarak geliştirilen hipotezlerden üzerinde en çok çalışılanı kolinerjik hipotezdir. Güncel olarak tedavide kullanılan dört bileşikten üçü; donepezil, galantamin ve rivastigmin kolinerjik hipotez temel alınarak geliştirilmiştir. Dördüncü bileşik ise glutamat seviyesindeki artışı düzenleyen *N*-metil-D-aspartat antagonisti memantindir. Ancak bu dört bileşik sadece hastalığın semptomlarını hafifletmeye yönelik etki gösterdiğinden yeni keşifler hastalığın tedavisi için çok büyük önem arz etmektedir [4,5]. Son yıllarda AH tedavisinde etkili olabilecek moleküllerin geliştirilmesinde çoklu hedefe yönelik ligand (Multiple Target Directed Ligand, MTDL) tasarımı benimsenmiş durumdadır. Bu yaklaşımla hastalığın karmaşık ve çok faktörlü yapısıyla mücadele etmek için hastalığın iki veya daha fazla patolojik hedefi ile etkileşebilecek moleküllerin geliştirilmesinin tedavide etkili olacağı savunulmaktadır [6,7].

Kolinerjik hipotez, merkezi sinir siteminde öğrenmeden ve hafızadan sorumlu bir nörotransmitter olan asetilkolinin azalan seviyesinin, asetilkolinesteraz (AKE) ve butirilkolinesteraz (BKE) adlı iki kolinerjik enzimin inhibisyonu yoluyla arttırılmasının tedavide yararlı olacağını öne sürmektedir [8]. AKE ve BKE, asetilkolini hidroliz ederek kolinerjik iletimi sonlandıran serin hidrolaz ailesine ait enzimlerdir. Her iki enzim de asetilkolini hidroliz etmelerine rağmen aktif bölgelerindeki aminoasit dizilimleri dolayısıyla seçicilikleri ve kinetikleri farklılık göstermektedir. AKE enzim aktif bölgesinde 14 aromatik aminoasit taşırken BKE 8 adet taşımaktadır. Bu durum BKE enzim aktif bölgesinin AKE'den daha büyük olması ile sonuçlanmaktadır. Aktif bölge aminoasitleri farklılık göstermesine rağmen her iki enzim de katalitik aktif bölge (KAB) ve periferal anyonik bölge (PAB) adı verilen iki ana bölüm bulundurmaktadır. KAB, silindir şeklindeki aktif bölgenin dibinde yer alan ve temel katalitik işlevi gerçekleştiren bölümken PAB aktif cebin girişinde yer alır ve ligandların yönlendirilmesinden sorumludur [9-11].

Metal iyonlarının organizmaların metabolik süreçlerinde yeri doldurulamaz fizyolojik bir rol oynadığı bilinmektedir. Ancak son yıllarda yapılan çalışmalar, Aβ plaklarda Cu(II), Fe(II) ve Zn(II) gibi metal iyonlarının fazlasının bulunduğunu göstermiştir. Ayrıca Alzheimer hastalarının beyinlerindeki Aβ plaklarda bulunan Cu(II), Fe(II), Zn(II) gibi metal iyonlarının konsantrasyonunun normal beyinlerdeki

metal iyonu konsantrasyonundan daha yüksek olduğu tespit edilmiştir. Bu metal iyonları Aβ agregasyonunu indükleyerek serbest radikallerin üretimi yoluyla Aβ toksisitesini körükler ve Aβ peptidlerinin oluşumunu arttırır [12,13]. Bu bilgilere ek olarak beyinde yüksek seviyedeki redoks aktif metal iyonları, oksidatif stresin ana nedeni olan reaktif oksijen türlerinin aşırı üretimine katkıda bulunabilir [14]. Bu nedenle metal şelasyonu ve antioksidan maddeler de AH tedavisi için önemli bir terapötik strateji olabilir.

Yukarıdaki literatür bilgilerinden yola çıkarak biz de çalışmamızda hastalığın farklı patolojik hedefleri ile etkileşmesini beklediğimiz asetamit yapısı taşıyan yirmi sekiz yeni bileşik sentez ettik. Çalışmamızda kolinesteraz enzimlerinin enzim aktif bölgesinde yer alan PAB ile etkileşmesini planladığımız elektronca zengin benzoksazolon ve benzotiyazolon halkalarını kullandık. Benzoksazolon/benzotiyazolon halka sistemleri ile ilgili olarak bugüne kadar grubumuz da dahil olmak üzere pek çok çalışma yapılmış ve farklı biyolojik aktiviteler gösterdiği belirlenmiştir [15-22]. Ayrıca günümüzde kullanılmakta bazı olan ilaçlarda bu halka sistemlerini taşımaktadır. Benzoksazolon/benzotiyazolon halka sistemlerinin yanında kolinesteraz inhibitör etki gösteren moleküllerin yapısında yer alan tersiyer amin türevleri de yapıya dahil edilmiştir. Sentezlenen tüm bileşiklerin önce kolinesteraz inhibitor etkileri daha sonra da antioksidan ve metal şelatör etkileri değerlendirilmiştir.

GEREÇ VE YÖNTEM

Kimyasal Çalışmalar

Kullanılan tüm kimyasal malzemeler Merck'ten satın alınmıştır. Kimyasal tepkimeler 254 nm UV ışığı altında silika jel plakalar kullanılarak ince tabaka kromatografisi ile takip edilmiş ve doğrulanmıştır. Sonuç bileşiklerin NMR spektrumları Ankara Üniversitesi Eczacılık Fakültesi Merkez Laboratuvarındaki Varian Mercury 400 MHz Digital FT-NMR spektrofotometresinden kaydedilmiştir. Bileşiklerin saflığı, Gazi Üniversitesi Eczacılık Fakültesi Merkez Laboratuvarındaki ultraperformanslı yüksek basınçlı sıvı kromatografisi (UPLC) ile birleştirilmiş yüksek çözünürlüklü kütle spektrometresinde (HRMS), elektron sprey iyonizasyon (ESI) yöntemi kullanılarak tayin edilmiştir. Bileşiklerin erime noktalarının belirlenmesinde Stuart SMP50 erime noktası cihazı kullanılmıştır. Sonuç bileşiklerin kimyasal yapıları, DMSO-d_6 içindeki çözeltilerinden yararlanılarak ^{1}H-NMR ve ^{13}C-NMR spektrumları ile aydınlatılmıştır.

3-Metil-1,3-benzoksazol-2(3*H*)-on (1): 5 g (0.037 mol) benzoksazolon, 50 ml 1M NaOH çözeltisi içerisinde çözüldü. Üzerine 3.5 ml (0.037 mol) dimetilsülfat ilave edildi ve 30 dakika oda sıcaklığında karıştırıldı. Süre sonunda balon içeriği suya boşaltıldı çöken ürün süzüldü, kurutuldu ve kristalizasyon yapılmaksızın bir sonraki basamak için kullanıldı. Verim: 4.38 g (%80). Erime noktası: 84˚C'dir [19,20].

3-Metil-1,3-benzotiyazol-2(3*H*)-on (2): 2 g (0.013 mol) benzotiyazolon, 20 ml 1M NaOH çözeltisi ve 1.48 ml (0.016 mol) dimetilsülfat kullanılarak yukarıdaki yönteme göre sentez edilmiştir. Verim:1.98 g (%90). Erime noktası: 76˚C'dir [19,20].

3-Metil-6-nitro-1,3-benzoksazol-2(3*H*)-on (3): 15 ml %65'lik nitrik asit, yağ banyosunda 40˚C'ye kadar ısıtıldı ve balon içi sıcaklığın 40-50°C'de olması sağlanarak 3 g (0.02 mol) 3-metil-1,3-benzoksazol-2(3H)-on porsiyonlar halinde ilave edildi. İlave tamamlandıktan sonra yarım saat karıştırıldı. Süre sonunda balon içeriği buzlu suya boşaltılıp 15 dakika daha karıştırıldı ve çöken ürün süzüldü. Kristalizasyon yapılmaksızın bir sonraki basamak için kullanıldı. Verim: 3.32 g (%85). Erime noktası:182˚C'dir [19,20].

3-Metil-6-nitro-1,3-benzotiyazol-2(3*H*)-on (4): 10 ml %65'lik nitrik asit ve 2 g (0.012 mol) 3-metil-1,3-benzotiyazol-2(*3H*)-on kullanılarak yukarıdaki yönteme göre yapıldı. Verim:2.31 g (%91). Erime noktası: 164˚C'dir [19,20].

6-Amino-3-metil-1,3-benzoksazol-2(3*H*)-on (5): 2 g (0.01 mol) 3-metil-6-nitro-1,3-benzoksazol-2(3*H*)-on iki boyunlu balona alındı üzerine 40 ml etanol ve 8 ml 6 N HCI çözeltisinden ilave edilerek geri çeviren soğutucu altında ısıtıldı. Kaynamaya başladıktan sonra 11.8 g (0.05 mol) $SnCl_2.2H_2O$ porsiyonlar halinde 30 dakikada ilave edildi. Süre sonunda etanol uçuruldu ve balon içeriği sodyum hidroksit çözeltisi ile pH 10-11 arasına getirildi. Ardından diklorometan ile ekstraksiyon yapılarak

organik faz alındı ve kuruluğa kadar uçurularak ürün elde edildi. Elde edilen ürün kristalizasyon yapılmaksızın bir sonraki basamak için kullanıldı. Verim: 1.5 g (%87). Erime noktası: 154°C'dir [19,20].

6-Amino-3-metil-1,3-benzotiyazol-2(3*H*)-on (6):2 g (0.009 mol) 3-metil-6-nitro-1,3-benzotiyazol-2(3*H*)-on, 40 ml etanol, 8 ml 6 N HCI ve 10.74 g (0.048 mol) kalay klorür kullanılarak yukarıdaki yönteme göre yapıldı. Verim: 1.54 g (%90). Erime noktası:189°C'dir [19,20].

2-Bromo-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (7): 500 mg (0.003 mol) 6-amino-3-metil-1,3-benzoksazol-2(3*H*)-on 6 ml DMF içerisinde çözüldü. Çözeltiye 37 mg (0.0003 mol) 4-dimetilaminopiridin (DMAP) ve 0.31 ml (0.0022 mol) trietilamin (TEA) ilave edilerek 10 dakika karıştırıldı. Daha sonra buz banyosuna alınarak 0.31 ml (0.0036 mol) bromoasetil bromür ilave edildi ve 1 saat oda sıcaklığında karıştırıldı. Süre sonunda balon içeriği buzlu suya boşaltılıp bir süre daha karıştırıldı. Çöken madde vakumdan süzüldü, kurutuldu ve kristalizasyon yapılmaksızın bir sonraki basamak için kullanıldı. Verim: 620 mg (%66)'dır. Erime noktası: 234°C (dekompoze)'dir. $C_{10}H_9BrN_2O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 284.9875, bulunan: 284.9868.

2-Bromo-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (8): 500 mg (0.0028 mol) 6-amino-3-metil-1,3-benzotiyazol-2(3*H*)-on, 8 ml DMF, 34 mg (0.00028 mol) 4-dimetilaminopiridin (DMAP), 0.29 ml (0.002 mol) trietilamin (TEA) ve 0.29 ml (0.0033 mol) bromoasetil bromür kullanılarak yukarıdaki yönteme göre sentez edildi. Verim: 689 mg (%82)'dır. Erime noktası: 212°C(dekompoze)'dir. $C_{10}H_9BrN_2O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 300.9646, bulunan: 300.9640.

2-Sübstitüe-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit Türevlerinin Genel Sentez Yöntemi (9a-9n)

285 mg (0.001 mol) 2-bromo-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit 6 ml DMF içerisinde çözüldü. Bu çözeltiye 346 mg (0.0025 mol) potasyum karbonat ve 0.002 mol uygun amin türevi ilave edilerek 1 saat oda sıcaklığında karıştırıldı. Süre sonunda balon içeriği buzlu suya boşaltıldı. Çöken katı vakumdan süzüldü, kurutuldu ve uygun çözücüden kristallendirildi.

N-(3-Metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)-2-(4-fenilpiperazin-1-il)asetamit (9a): Etanolden kristallendirildi. 304 mg elde edildi, verim %83'tür. Erime noktası: 214.2℃. ^{1}H NMR (DMSO-d_6) δ: 9.83 (s, 1H, N-H), 7.77 (d, 1H, *J*=1.9 Hz, H^7), 7.42 (dd, 1H, *J*=8.4 Hz, *J*=1.9 Hz, H^5), 7.21 (t, 2H, *J*=7.4 Hz, $H^{3'}$, $H^{5'}$), 7.18 (d, 1H, *J*=8.4 Hz, H^4), 6.94 (d, 2H, *J*=7.4 Hz, $H^{2'}$, $H^{6'}$), 6.77 (t, 1H, *J*=7.4 Hz, $H^{4'}$), 3.31 (s, 3H, N-CH_3), 3.21 (t, 4H, *J*=4.8 Hz, H^3, H^5-piperazin), 3.19 (s, 2H, -CH_2), 2.67 (t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.06, 154.10, 150.96, 141.67, 133.69, 128.85, 127.45, 118.74, 115.35, 115.06, 108.64, 101.94, 61.62, 52.66, 48.01, 27.97. $C_{20}H_{22}N_40_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 367.1765, bulunan: 367.1763. $C_{20}H_{22}N_40_3$ için elementel analiz: C, 65.56; H, 6.05; N,15.29. Bulunan: C, 65.60; H, 6.12; N, 15.30.

2-[4-(4-Florofenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9b): Etanolden kristallendirildi. 342 mg elde edildi, verim %89'dur. Erime noktası: 211.4℃.^{1}H NMR (DMSO-d_6) δ: 9.83 (s, 1H, N-H), 7.77 (d, 1H, *J*=2.0 Hz, H^7), 7.42 (dd, 1H, *J*=8.3 Hz, *J*=2.0 Hz, H^5), 7.18 (d, 1H, *J*=8.3 Hz, H^4), 7.06-7.05 (m, 2H, $H^{3'}$, $H^{5'}$), 6.97-6.93 (m, 2H, $H^{2'}$, $H^{6'}$), 3.31 (s, 3H, N-CH_3), 3.19 (s, 2H, -CH_2), 3.15 (t, 4H, *J*=4.8 Hz, H^3, H^5-piperazin), 2.67(t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.06, 155.93 (d, *J*=234.0 Hz), 154.10, 147.88, 141.68, 133.69, 127.46, 117.08 (d, *J*=6.8 Hz), 115.18 (d, *J*=23.7 Hz), 115.08, 108.65, 101.95, 61.57, 52.65, 48.80, 27.98. $C_{20}H_{21}FN_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 385.1670, bulunan: 385.1671. $C_{20}H_{21}FN_4O_3$ için elementel analiz: C, 62.49; H, 5.51; N, 14.57. Bulunan: C, 62.12; H, 5.60; N, 14.59.

2-[4-(4-Klorofenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9c): Etanolden kristallendirildi. 340 mg elde edildi, verim %85'dir. Erime noktası: 204.4℃. ^{1}H NMR (DMSO-d_6) δ: 9.80 (s, 1H, N-H), 7.76 (d, 1H, *J*=2.0 Hz, H^7), 7.41 (dd, 1H, *J*=8.4 Hz, *J*=2.0 Hz, H^5), 7.22 (d, 2H, *J*=9.0 Hz, $H^{3'}$, $H^{5'}$), 7.17 (d, 1H, *J*=8.4 Hz, H^4), 6.94 (d, 2H, *J*=9.0 Hz, $H^{2'}$, $H^{6'}$), 3.27 (s, 3H, N-CH_3), 3.21 (t, 4H, *J*=4.8 Hz, H^3, H^5-piperazin), 3.18 (s, 2H, -CH_2), 2.66 (t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.96, 154.03, 149.70, 141.64, 133.62, 128.48, 127.42, 122.18, 116,72, 115.04, 108.55, 101.93, 61.46, 52.41, 47.81, 27.91. $C_{20}H_{21}ClN_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 401.1375, bulunan: 401.1382. $C_{20}H_{21}ClN_4O_3$ için elementel analiz: C, 59.92; H,

5.28; N, 13.98. Bulunan: C, 59.52; H, 5.29; N, 14.14.

2-[4-(4-Metilfenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9d): Etanolden kristallendirildi. 342 mg elde edildi, verim %90'dır. Erime noktası: 225.5℃. ^{1}H NMR (DMSO-d_6) δ: 9.83 (s, 1H, N-H), 7.77 (d, 1H, *J*=2.0 Hz, H^{7}), 7.42 (dd, 1H, *J*=8.7 Hz, *J*=2.0 Hz, H^{5}), 7.18 (d, 1H, *J*=8.7 Hz, H^{4}), 7.02 (d, 2H, *J*=8.6 Hz, $H^{3'}$, $H^{5'}$), 6.84 (d, 2H, *J*=8.6 Hz, $H^{2'}$, $H^{6'}$), 3.32 (s, 3H, N-CH_3), 3.18 (s, 2H, -CH_2), 3.14 (t, 4H, *J*=5.0 Hz, H^{3}, H^{5}-piperazin), 2.65 (t, 4H, *J*=5.0 Hz, H^{2}, H^{6}-piperazin), 2.19 (s, 3H, fenil-CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.09, 154.12, 148.91, 141.68, 133.71, 129.32, 127.55, 127.45, 115.64, 115.07, 108.67, 101.96, 61.68, 52.73, 48.51, 28.00, 20.01. $C_{21}H_{24}N_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 381.1921, bulunan: 381.1921. $C_{21}H_{24}N_4O_3$ için elementel analiz: C, 66.30; H, 6.36; N, 14.73. Bulunan: C, 66.34; H, 6.36; N, 14.81.

2-[4-(4-Metoksifenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9e): Butanolden kristallendirildi. 333 mg elde edildi, verim %84'tür. Erime noktası: 225℃. ^{1}H NMR (DMSO-d_6) δ: 9.83 (s, 1H, N-H), 7.77 (d, 1H, *J*=1.9 Hz, H^{7}), 7.42 (dd, 1H, *J*=8.7 Hz, *J*=1.9 Hz, H^{5}), 7.18 (d, 1H, *J*=8.7 Hz, H^{4}), 6.89 (d, 2H, *J*=9.0 Hz, $H^{3'}$, $H^{5'}$), 6.81 (d, 2H, *J*=9.0 Hz, $H^{2'}$, $H^{6'}$), 3.68 (s, 3H, O-CH_3), 3.32 (s, 3H, N-CH_3), 3.18 (s, 2H, -CH_2), 3.08 (t, 4H, *J*=4.8 Hz, H^{3}, H^{5}-piperazin), 2.66 (t, 4H, *J*=4.8 Hz, H^{2}, H^{6}-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.11, 154.12, 152.85, 145.36, 141.68, 133.71, 127.46, 117.34, 115.06, 114.21, 108.67, 101.95, 61.68, 55.13, 52.82, 49.42, 28.00. $C_{21}H_{24}N_4O_4$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 397.1870, bulunan: 397.1880. $C_{21}H_{24}N_4O_4$ için elementel analiz: C, 63.62; H, 6.10; N, 14.13. Bulunan: C, 63.66; H, 6.14; N, 14.18.

2-[4-(4-Triflorometilfenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9f): Etanolden kristallendirildi. 356 mg elde edildi, verim %82'dir. Erime noktası: 217.6℃. ^{1}H NMR (DMSO-d_6) δ: 9.86 (s, 1H, N-H), 7.77 (d, 1H, *J*=1.9 Hz, H^{7}), 7.50 (d, 2H, *J*=8.8 Hz, $H^{3'}$, $H^{5'}$), 7.42 (dd, 1H, *J*=8.4 Hz, *J*=1.9 Hz, H^{5}), 7.19 (d, 1H, *J*=8.4 Hz, H^{4}), 7.07 (d, 2H, *J*=8.8 Hz, $H^{2'}$, $H^{6'}$), 3.36 (t, 4H, *J*=5.0 Hz, H^{3}, H^{5}-piperazin), 3.32 (s, 3H, N-CH_3), 3.20 (s, 2H, -CH_2), 2.67 (t, 4H, *J*=5.0 Hz, H^{2}, H^{6}-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.98, 154.07, 153.15, 141.63, 133.64, 127.42, 126.07 (q, *J*=3.9 Hz), 124.91 (q, *J*=268.6 Hz), 117.71 (q, *J*=31.7 Hz), 115.04, 114.09, 108.62, 101.92, 61.43, 52.27, 46.79, 27.94. $C_{21}H_{21}F_3N_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 435.1639, bulunan: 435.1644. $C_{21}H_{21}F_3N_4O_3$ için elementel analiz: C, 58.06; H, 4.87;N, 12.90. Bulunan: C, 57.81; H, 4.56; N, 12.91.

2-(4-Benzilpiperazin-1-il)-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9g): Metanolden kristallendirildi. 350 mg elde edildi, verim %92'dir. Erime noktası: 208.4℃. ^{1}H NMR (DMSO-d_6) δ: 9.76 (s, 1H, N-H), 7.75 (d, 1H, *J*=1.6 Hz, H^{7}), 7.39 (dd, 1H, *J*=8.4 Hz, *J*=1.6 Hz, H^{5}), 7.34-7.22 (m, 5H, fenil protonları), 7.17 (d, 1H, *J*=8.4 Hz, H^{4}), 3.48 (s, 2H, -CH_2-C_6H_5), 3.11 (s, 2H, -CH_2-), 3.32 (s, 3H, N-CH_3), 2.53 (bt, 4H, H^{2}, H^{6}-piperazin), 2.44 (bt, 4H, H^{3}, H^{5}-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.08, 154.04, 141.61, 138.13, 133.63, 128.69, 128.05, 127.36, 126.79, 114.94, 108.59, 101.84, 61.96, 61.68, 52.73, 52.32, 27.92. $C_{21}H_{24}N_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 381.1921, bulunan: 381.1926. $C_{21}H_{24}N_4O_3$ için elementel analiz: C, 66.30; H, 6.36; N, 14.73. Bulunan: C, 66.13; H, 6.51; N, 14.73.

2-{4-[(4-Florofenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9h): Etanolden kristallendirildi. 358 mg elde edildi, verim %90'dır. Erime noktası: 202.9℃. ^{1}H NMR (DMSO-d_6) δ: 9.73 (s, 1H, N-H), 7.72 (d, 1H, *J*=2.0 Hz, H^{7}), 7.36 (dd, 1H, *J*=8.8 Hz, *J*=2.0 Hz, H^{5}), 7.32-7.28 (m, 2H, $H^{2'}$, $H^{6'}$), 7.16-7.09 (m, 3H, $H^{3'}$, $H^{5'}$, H^{4}), 3.44 (s, 2H, -CH_2- C_6H_5), 3.29 (s, 3H, N-CH_3), 2.54-2.41 (m, 8H, piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.09, 161.14, 154.05, 141.62, 134.30 (d, *J*=3.0 Hz), 133.64, 130.50 (d, *J*=8.3 Hz), 127.37, 114.95, 114.77 (d, *J*=21.4), 108.60, 101.85, 61.67, 61.07, 52.70, 52.21, 27.94. $C_{21}H_{23}FN_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 399.1832, bulunan: 399.1825. $C_{21}H_{23}FN_4O_3$ için elementel analiz: C, 63.30; H, 5.82; N, 14.06. Bulunan: C, 63.31; H, 5.99; N, 14.06.

2-{4-[(4-Klorofenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9i): Etanolden kristallendirildi. 377 mg elde edildi, verim %91'dir. Erime noktası: 283.9℃. ^{1}H NMR (DMSO-d_6) δ: 9.71 (s, 1H, N-H), 7.73 (d, 1H, *J*=2.0 Hz, H^{7}), 7.39-7.32 (m, 5H, H^{5}, fenil protonları), 7.16 (d, 1H, *J*=8.4 Hz, H^{4}), 3.47 (s, 2H, -CH_2- C_6H_5), 3.31 (s, 3H, N-CH_3), 3.11 (s, 2H, -CH_2), 2.53-2.43 (m, 8H, piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.11, 154.08, 141.66, 137.28, 133.67, 131.34, 130.48, 128.06, 127.41, 115.00, 108.62, 101.90, 61.68, 61.03, 52.74, 52.28, 27.96.

$C_{21}H_{23}ClN_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 415.1537, bulunan: 415.1530. $C_{21}H_{23}ClN_4O_3$ için elementel analiz: C, 60.79; H, 5.59; N, 13.50. Bulunan: C, 60.54; H, 5.67; N, 13.63.

2-{4-[(4-Metoksifenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9*J*): Butanolden kristallendirildi. 357 mg elde edildi, verim %87'dir. Erime noktası: 199.4℃. ^{1}H NMR (DMSO-d_6) δ: 9.68 (s, 1H, N-H), 7.71 (d, 1H, *J*=1.7 Hz, H^7), 7.36 (dd, 1H, *J*=8.5 Hz, *J*=1.7 Hz, H^5), 7.17 (d, 2H, *J*=8.4 Hz, $H^{2'}$, $H^{6'}$), 7.14 (d, 1H, *J*=8.5 Hz, H^4), 6.85 (d, 2H, *J*=8.4 Hz, $H^{3'}$, $H^{5'}$), 3.73 (s, 3H, O-CH_3), 3.40 (s, 2H,-CH_2- C_6H_5), 3.31 (s, 3H, N- CH_3), 3.10 (s, 2H, -CH_2), 2.51-2.41(m, 8H, piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.12, 158.19, 154.08, 141.66, 133.67, 129.94, 127.41, 15.00, 113.46, 108.62, 101.89, 61.73, 61.40, 54.92, 52.78, 52.23, 27.96. $C_{22}H_{26}N_4O_4$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 411.2032, bulunan: 411.2034. $C_{22}H_{26}N_4O_4$ için elementel analiz: C, 64.37; H, 6.38; N, 13.65. Bulunan: C, 64.47; H, 6.45; N=13.69.

2-{4-[(4-Triflorometilfenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9k): Butanolden kristallendirildi. 390 mg elde edildi, verim %87'dir. Erime noktası: 186.3℃. ^{1}H NMR (DMSO-d_6) δ: 9.73 (s, 1H, N-H), 7.74 (d, 1H, *J*=1.6 Hz, H^7), 7.67 (d, 2H, *J*=8.4 Hz, $H^{3'}$, $H^{5'}$), 7.53 (d, 2H, *J*=8.4 Hz, $H^{2'}$, $H^{6'}$), 7.36 (dd, 1H, *J*=8.5 Hz, *J*=1.6 Hz, H^5), 7.17 (d, 1H, *J*=8.5 Hz, H^4), 3.58 (s, 2H, -CH_2-C_6H_5), 3.28 (s, 3H, N-CH_3), 3.12 (s, 2H, -CH_2), 2.55-2.47 (m, 8H, piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.14, 154.11, 143.34, 141.68, 133.70, 129.32, 127.55 (q, *J*=31.4 Hz), 127.44, 125.01 (q, *J*=3.9 Hz), 124.33 (q, *J*=270.0 Hz), 115.02, 108.67, 101.92, 61.69, 61.26, 52.75, 52.39, 27.99. $C_{22}H_{23}F_3N_4O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 449.1801, bulunan: 449.1802. $C_{22}H_{23}F_3N_4O_3$ için elementel analiz: C, 58.92; H, 5.17; N, 12.71. Bulunan: C, 58.75; H, 5.12; N, 12.61.

2-(4-Fenilpiperidin-1-il)-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9l): 2-propanolden kristallendirildi. 325 mg elde edildi, verim %89'tür. Erime noktası: 181℃. ^{1}H NMR (DMSO-d_6) δ: 9.78 (s, 1H, N-H), 7.77 (d, 1H, *J*=1.9 Hz, H^7), 7.43 (dd, 1H, *J*=8.4 Hz, *J*=1.9 Hz, H^5), 7.30-7.15 (m, 6H, H^4, fenil protonları), 3.30 (s, 3H, N-CH_3), 3.12 (s, 2H, -CH_2), 2.95 (d, 2H, *J*=11.2 Hz, H^{2e}, H^{6e}-piperidin), 2.48 (m, 1H, H^4-piperidin, DMSO ile birlikte), 2.25 (td, 2H, *J*=11.2 Hz, *J*=2.8 Hz, H^{2a}, H^{6a}-piperidin), 1.81-1.70 (m, 4H, H^3, H^5-piperidin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.41, 154.06, 146.13, 141.64, 133.66, 128.22, 127.38, 126.64, 125.91, 115.02, 108.60, 101.89, 62.06, 53.79, 41.25, 32.81, 27.94. $C_{21}H_{23}N_3O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 366.1818, bulunan: 366.1816. $C_{21}H_{23}N_3O_3$ için elementel analiz: C, 69.02; H, 6.34; N, 11.50. Bulunan: C, 69.09; H, 6.33; N, 11.58.

2-(4-Benzilpiperidin-1-il)-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9m): Metanolden kristallendirildi. 315 mg elde edildi, verim %83'tür. Erime noktası: 172.8℃. ^{1}H NMR (DMSO-d_6) δ: 9.69 (s, 1H, N-H), 7.74 (d, 1H, *J*=1.7 Hz, H^7), 7.39 (dd, 1H, *J*=8.1 Hz, *J*=1.7 Hz, H^5), 7.26 (t, 2H, *J*=7.6 Hz, $H^{3'}$, $H^{5'}$), 7.17-7.14 (m, 4H, $H^{2'}$, $H^{4'}$, $H^{6'}$, H^4), 3.30 (s, 3H, N-CH_3), 3.05 (s, 2H, -CH_2), 2.82 (d, 2H, *J*=11.6 Hz, H^{2e}, H^{6e}-piperidin), 2.51-2.48 (m, 2H, -CH_2-C_6H_5, DMSO ile birlikte), 2.05 (t, 4H, *J*=10.8 Hz, H^{2a}, H^{6a}-piperidin), 1.54-1.29 (m, 5H, H^3, H^4, H^5-piperidin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.41, 154.05, 141.63, 140.24, 133.64, 128.86, 128.03, 127.36, 125.64, 114.96, 108.58, 101.85, 62.12, 53.34, 42.27, 36.84, 31.48, 27.93. $C_{22}H_{25}N_3O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 380.1967, bulunan: 380.1970. $C_{22}H_{25}N_3O_3$ için elementel analiz: C, 69.64; H, 6.64; N, 11.07. Bulunan: C, 69.29; H, 6.62; N, 11.18.

2-[Benzil(metil)amino]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit (9n): 2-propanolden kristallendirildi. 270 mg elde edildi, verim %83'tür. Erime noktası: 160.3℃. ^{1}H NMR (DMSO-d_6) δ: 9.77 (s, 1H, N-H), 7.75 (d, 1H, *J*=2 Hz, H^7), 7.42-7.23 (m, 6H, H^5, fenil protonları), 7.17 (d, 1H, *J*=8.4 Hz, H^4), 3.64 (s, 2H, -CH_2- C_6H_5), 3.32 (s, 3H, N-CH_3), 3.18 (s, 2H, -CH_2-), 2.27 (s, 3H, N_2-CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.66, 154.09, 141.66, 138.17, 133.71, 128.88, 128.14, 127.40, 127.03, 115.02, 108.62, 101.92, 60.96, 60.51, 42.16, 27.96. $C_{18}H_{19}N_3O_3$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 326.1505, bulunan: 326.1500. $C_{18}H_{19}N_3O_3$ için elementel analiz: C, 66.45; H, 5.89; N, 12.91. Bulunan: C, 66.06; H, 5.93; N, 12.86.

2-Sübstitüe-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit Türevlerinin Genel Sentez Yöntemi (10a-10n)

301 mg (0.001 mol) 2-bromo-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit, 6 ml DMF, 346 mg (0.0025 mol) potasyum karbonat ve 0.002 mol uygun amin türevi kullanılarak 2-

sübstitüe-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit türevlerinin genel sentez yöntemine göre hazırlandı.

N-(3-Metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)-2-(4-fenilpiperazin-1-il)asetamit (10a): Butanolden kristallendirildi. 351 mg elde edildi, verim %92'dir. Erime noktası: 217.9℃. ^{1}H NMR (DMSO-d_6) δ: 9.84 (s, 1H, N-H), 7.99 (d, 1H, *J*=2.0 Hz, H^7), 7.58 (dd, 1H, *J*=8.7 Hz, *J*=2.0 Hz, H^5), 7.25 (d, 1H, *J*=8.7 Hz, H^4), 7.21 (t, 2H, *J*=7.5 Hz, $H^{3'}$, $H^{5'}$), 6.93 (d, 2H, *J*=7.5 Hz, $H^{2'}$, $H^{6'}$), 6.77 (t, 1H, *J*=7.5 Hz, $H^{4'}$), 3.38 (s, 3H, N-CH_3), 3.21-3.19 (m, 6H, H^3, H^5-piperazin, -CH_2-), 2.67 (t, 4H, *J*=5.0 Hz, H^2, H^6-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.41, 168.12, 150.98, 134.33, 133.54, 128.89, 121.41, 118.78, 118.52, 115.37, 113.90, 111.21, 61.61, 52.68, 48.07, 28.98. $C_{20}H_{22}N_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 383.1542, bulunan: 383.1541. $C_{20}H_{22}N_4O_2S$ için elementel analiz: C, 62.80; H, 5.80; N, 14.65; S, 8.38. Bulunan: C, 63.10; H, 6.09; N, 14.56; S, 8.41.

2-[4-(4-Florofenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10b): Metanolden kristallendirildi. 352 mg elde edildi, verim %88'dir. Erime noktası: 189.9℃. ^{1}H NMR (DMSO-d_6) δ: 9.84 (s, 1H, N-H), 7.99 (d, 1H, *J*=2.0 Hz, H^7), 7.58 (dd, 1H, *J*=8.5 Hz, *J*=2.0 Hz, H^5), 7.25 (d, 1H, *J*=8.5 Hz, H^4), 7.06-7.02 (m, 2H, $H^{3'}$, $H^{5'}$), 6.96-6.93 (m, 2H, $H^{2'}$, $H^{6'}$), 3.38 (s, 3H, N-CH_3), 3.19 (s, 2H, -CH_2-), 3.15 (t, 4H, *J*=4.8 Hz, H^3, H^5-piperazin), 2.67(t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.42, 168.12, 155.96 (d, *J*=233.9 Hz), 147.89 (d, *J*=2.3 Hz), 134.33, 133.55, 121.41, 118.51, 117.10 (d, *J*=7.6Hz), 115.22 (d, *J*=21.3 Hz), 113.89, 111.22, 61.56, 52.67, 48.84, 28.89. $C_{20}H_{21}FN_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 401.1448, bulunan: 401.1456. $C_{20}H_{21}FN_4O_2S$ için elementel analiz: C, 59.98; H, 5.29; N, 13.99; S, 8.01. Bulunan: C, 60.11; H, 5.44; N, 13.91; S, 8.02.

2-[4-(4-Klorofenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10c): Butanolden kristallendirildi. 379 mg elde edildi, verim %91'dir. Erime noktası: 213.4℃. ^{1}H NMR (DMSO-d_6) δ: 9.84 (s, 1H, N-H), 7.99 (d, 1H, *J*=2.0 Hz, H^7), 7.58 (dd, 1H, *J*=8.5 Hz, *J*=2.0 Hz, H^5), 7.25 (d, 1H, *J*=8.5 Hz, H^4), 7.22 (d, 2H, *J*=9.0 Hz, $H^{3'}$, $H^{5'}$), 6.94 (d, 2H, *J*=9.0 Hz, $H^{2'}$, $H^{6'}$), 3.38 (s, 3H, N-CH_3), 3.22-3.19 (m, 6H, H^3, H^5-piperazin, -CH_2-), 2.66 (t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.41, 168.09, 149.78, 134.33, 133.55, 128.58, 122.27, 121.41, 118.54, 116.81, 113.91, 111.22, 61.53, 52.49, 47.88, 28.99. $C_{20}H_{21}ClN_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 417.1152, bulunan: 417.1149. $C_{20}H_{21}ClN_4O_2S$ için elementel analiz: C, 57.62; H, 5.08; N, 13.44; S, 7.69. Bulunan: C, 57.70; H, 5.15; N, 13.34; S, 7.65.

2-[4-(4-Metilfenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10d): Etanolden kristallendirildi. 356 mg elde edildi, verim %90'dır. Erime noktası: 194.5℃. ^{1}H NMR (DMSO-d_6) δ: 9.83 (s, 1H, N-H), 7.99 (d, 1H, *J*=2.0 Hz, H^7), 7.58 (dd, 1H, *J*=8.7 Hz, *J*=2.0 Hz, H^5), 7.25 (d, 1H, *J*=8.7 Hz, H^4), 7.01 (d, 2H, *J*=8.4 Hz, $H^{3'}$, $H^{5'}$), 6.83 (d, 2H, *J*=8.4 Hz, $H^{2'}$, $H^{6'}$), 3.38 (s, 3H, N-CH_3), 3.18 (s, 2H, -CH_2-), 3.14 (t, 4H, *J*=4.8 Hz, H^3, H^5-piperazin), 2.66 (t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin), 2.19 (s, 3H, fenil-CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.41, 168.13, 148.91, 134.33, 133.54, 129.33, 127.57, 121.40, 118.52, 115.65, 113.89, 111.21, 61.64, 52.72, 48.54, 28.98, 20.00. $C_{21}H_{24}N_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 397.1698, bulunan: 397.1702. $C_{21}H_{24}N_4O_2S$ için elementel analiz: C, 63.61; H, 6.10; N, 14.13; S, 8.09. Bulunan: C, 63.6; H, 6.12; N, 14.05; S, 8.08.

2-[4-(4-Metoksifenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10e): 2-propanolden kristallendirildi. 370 mg elde edildi, verim %90'dır. Erime noktası: 183.3℃. ^{1}H NMR (DMSO-d_6) δ: 9.83 (s, 1H, N-H), 7.99 (d, 1H, *J*=2.0 Hz, H^7), 7.58 (dd, 1H, *J*=8.4 Hz, *J*=2.0 Hz, H^5), 7.25 (d, 1H, *J*=8.4 Hz, H^4), 6.89 (d, 2H, *J*=9.2 Hz, $H^{3'}$, $H^{5'}$), 6.81 (d, 2H, *J*=9.2 Hz, $H^{2'}$, $H^{6'}$), 3.68 (s, 3H, O-CH_3), 3.38 (s, 3H, N-CH_3), 3.18 (s, 2H, -CH_2-), 3.08 (t, 4H, *J*=4.8 Hz, H^3, H^5-piperazin), 2.66 (t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.48, 168.20, 152.90, 145.41, 134.36, 133.59, 121.46, 118.57, 117.40, 114.25, 113.94, 111.26, 61.67, 55.18, 52.85, 49.49, 29.02. $C_{21}H_{24}N_4O_3S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 413.1647, bulunan: 413.1651. $C_{21}H_{24}N_4O_3S$ için elementel analiz: C, 61.14; H, 5.86; N, 13.58; S, 7.77. Bulunan: C, 61.13; H, 6.05; N, 13.53; S, 7.81.

2-[4-(4-Triflorometilfenil)piperazin-1-il]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10f): 2-propanolden kristallendirildi. 410 mg elde edildi, verim %91'dir. Erime noktası: 194.6℃. ^{1}H NMR (DMSO-d_6) δ: 9.86 (s, 1H, N-H), 7.99 (d, 1H, *J*=1.7 Hz, H^7), 7.58 (dd, 1H, *J*=8.4 Hz, *J*=1.7 Hz, H^5), 7.49 (d, 2H, *J*=9.0 Hz, $H^{3'}$, $H^{5'}$), 7.25 (d, 1H, *J*=8.4 Hz, H^4), 7.06 (d, 2H, *J*=9.0 Hz, $H^{2'}$,

$H^{6'}$), 3.38-3.34 (m, 8H, N-CH_3, H^3, H^5-piperazin, DMSO H_2O), 3.20 (s, 2H, -CH_2-), 2.67 (t, 4H, *J*=4.8 Hz, H^2, H^6-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.43, 168.09, 153.22, 134.34, 133.57, 126.14 (q, *J*=3.8 Hz), 124.98 (q, *J*=268.5 Hz), 121.42, 118.56, 117.81 (q, *J*=32.0 Hz), 114.16, 113.94, 111.21, 61.48, 52.34, 46.88, 28.99. $C_{21}H_{21}F_3N_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 451.1416, bulunan: 451.1415. $C_{21}H_{21}F_3N_4O_2S$ için elementel analiz: C, 55.99; H, 4.70; N, 12.44; S, 7.12. Bulunan: C, 56.11; H, 4.67; N, 12.40; S, 7.08.

2-(4-Benzilpiperazin-1-il)-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10g): 2-propanolden kristallendirildi. 329 mg elde edildi, verim %83'tür. Erime noktası: 136.5℃. ^{1}H NMR (DMSO-d_6) δ: 9.76 (s, 1H, N-H), 7.96 (d, 1H, *J*=2.0 Hz, H^7), 7.55 (dd, 1H, *J*=9.0 Hz, *J*=2.0 Hz, H^5), 7.34-7.22 (m, 6H, fenil protonları, H^4), 3.47 (s, 2H, -CH_2-C_6H_5), 3.37 (s, 3H, N-CH_3), 3.11 (s, 2H, -CH_2-), 2.53-2.49 (m, 4H, H^2, H^6-piperazin), 2.46-2.43 (m, 4H, H^3, H^5-piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.41, 168.19, 138.19, 134.32, 133.52, 128.79, 128.13, 126.87, 121.40, 118.48, 113.86, 111.21, 62.05, 61.72, 52.79, 52.41, 28.98. $C_{21}H_{24}N_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 397.1698, bulunan: 397.1696. $C_{21}H_{24}N_4O_2S$ için elementel analiz: C, 63.61; H, 6.10; N, 14.13; S=8.09. Bulunan: C, 63.32; H, 6.06; N, 14.05; S, 8.06.

2-{4-[(4-Florofenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10h): 2-propanolden kristallendirildi. 352 mg elde edildi, verim %85'dir. Erime noktası: 183.8℃. ^{1}H NMR (DMSO-d_6) δ: 9.75 (s, 1H, N-H), 7.96 (d, 1H, *J*=2.0 Hz, H^7), 7.55 (dd, 1H, *J*=8.8 Hz, *J*=2.0 Hz, H^5), 7.33-7.30 (m, 2H, $H^{2'}$, $H^{6'}$), 7.24 (d, 1H, *J*=8.8 Hz, H^4), 7.15-7.10 (m, 2H, $H^{3'}$, $H^{5'}$), 3.45 (s, 2H, -CH_2-C_6H_5), 3.37 (s, 3H, N-CH_3), 3.11 (s, 2H, -CH_2-), 2.54-2.41 (m, 8H, piperazin). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 168.42, 168.18, 161.22 (d, *J*=240.8 Hz), 134.36, 134.32, 133.51, 130.58 (d, *J*=7.6 Hz), 121.40, 118.47, 114.84 (d, *J*=21.3 Hz), 113.86, 111.21, 61.69, 61.08, 52.77, 52.29, 28.98. $C_{21}H_{23}FN_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 415.1604, bulunan: 415.1602. $C_{21}H_{23}FN_4O_2S$ için elemental analiz: C, 60.85; H, 5.59; N, 13.52; S, 7.74. Bulunan: C, 61.18; H, 5.55; N, 13.39; S, 7.74.

2-{4-[(4-Klorofenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10i): Metanolden kristallendirildi. 391 mg elde edildi, verim %91'dir. Erime noktası: 182.2℃. ^{1}H NMR (DMSO-d_6) δ: 9.76 (s, 1H, N-H), 7.96 (d, 1H, *J*=2.0 Hz, H^7), 7.55 (dd, 1H, *J*=8.8 Hz, *J*=2.0 Hz, H^5), 7.36 (d, 2H, *J*=8.3 Hz, $H^{2'}$, $H^{6'}$), 7.31 (d, 2H, *J*=8.3 Hz, $H^{3'}$, $H^{5'}$), 7.24 (d, 1H, *J*=8.8 Hz, H^4), 3.46 (s, 2H, -CH_2-C_6H_5), 3.37 (s, 3H, N-CH_3), 3.11 (s, 2H, -CH_2-), 2.52-2.43 (m, 8H, piperazin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.42, 168.17, 137.29, 134.32, 133.52, 131.38, 130.53, 128.11, 121.40, 118.48, 113.86, 111.21, 61.69, 61.06, 52.76, 52.33, 28.98. $C_{21}H_{23}ClN_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 431.1309, bulunan: 431.1306. . $C_{21}H_{23}ClN_4O_2S$ için elementel analiz: C, 58.53; H, 5.38; N, 13.00; S, 7.44. Bulunan: C, 58.93; H, 5.65; N, 13.03; S, 7.47.

2-{4-[(4-Metoksifenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10j): 2-propanolden kristallendirildi. 358 mg elde edildi, verim %84'tür. Erime noktası: 147.5℃. ^{1}H NMR (DMSO-d_6) δ: 9.75 (s, 1H, N-H), 7.96 (d, 1H, *J*=2.0 Hz, H^7), 7.55 (dd, 1H, *J*=8.7 Hz, *J*=2.0 Hz, H^5), 7.24 (d, 1H, *J*=8.7 Hz, H^4), 7.19 (d, 2H, *J*=8.8 Hz, $H^{2'}$, $H^{6'}$), 6.87 (d, 2H, *J*=8.8 Hz, $H^{3'}$, $H^{5'}$), 3.73 (s, 3H, O-CH_3), 3.39 (s, 2H,-CH_2-C_6H_5), 3.37 (s, 3H, N- CH_3), 3.10 (s, 2H, -CH_2-), 2.54-2.41(m, 8H, piperazin). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 168.42, 168.10, 158.23, 134.32, 133.52, 129.99, 129.97, 121.40, 118.47, 113.86, 113.49, 111.21, 61.74, 61.45, 54.95, 52.81, 52.29, 28.98. $C_{22}H_{26}N_4O_3S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 427.1804, bulunan: 427.1803. $C_{22}H_{26}N_4O_3S$ için elementel analiz: C, 61.95; H, 6.14; N, 13.14; S, 7.52. Bulunan: C, 61.81; H, 6.15; N, 13.03; S, 7.50.

2-{4-[(4-Triflorometilfenil)metil]piperazin-1-il}-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10k): 2-propanolden kristallendirildi. 372 mg elde edildi, verim %80'dir. Erime noktası: 181.7℃. ^{1}H NMR (DMSO-d_6) δ: 9.76 (s, 1H, N-H), 7.96 (d, 1H, *J*=2.0 Hz, H^7), 7.67 (d, 2H, *J*=8.4 Hz, $H^{3'}$, $H^{5'}$), 7.56-7.52 (m, 3H, $H^{2'}$, $H^{6'}$, H^5), 7.24 (d, 1H, *J*=8.4 Hz, H^4), 3.57 (s, 2H, -CH_2-C_6H_5), 3.37 (s, 3H, N-CH_3), 3.12 (s, 2H, -CH_2-), 2.54-2.46 (m, 8H, piperazin). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 168.39, 168.15, 143.33, 134.31, 133.51, 129.31, 127.55 (q, *J*=31.5 Hz), 125.00 (q, *J*=3.8 Hz), 124.32 (q, *J*=270.5 Hz), 121.39, 118.45, 113.84, 111.19, 61.66, 61.26, 52.74, 52.40, 28.96. $C_{22}H_{23}F_3N_4O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 465.1572, bulunan: 465.1575. $C_{22}H_{23}F_3N_4O_2S$ için elementel analiz: C, 56.89; H, 4.99; N, 12.06; S, 6.90. Bulunan: C, 56.79; H, 4.97; N, 11.97; S, 6.90.

2-(4-Fenilpiperidin-1-il)-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10l): 2-propanolden kristallendirildi. 350 mg elde edildi, verim %92'dir. Erime noktası: 173.3℃. ^{1}H NMR

(DMSO-d_6) δ: 9.80 (s, 1H, N-H), 8.00 (d, 1H, *J*=1.9 Hz, H^7), 7.61 (dd, 1H, *J*=8.6 Hz, *J*=1.9 Hz, H^5), 7.32-7.16 (m, 6H, H^4, fenil protonları), 3.38 (s, 3H, N-CH_3), 3.15 (s, 2H, -CH_2-), 2.93 (d, 2H, *J*=11.2 Hz, H^{2e}, H^{6e}-piperidin), 2.50 (m, 1H, H^4-piperidin, DMSO ile birlikte), 2.27 (td, 2H, *J*=11.2 Hz, *J*=2.6 Hz, H^{2a}, H^{6a}-piperidin), 1.86-1.70 (m, 4H, H^3, H^5-piperidin). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.52, 168.43, 146.21, 134.35, 133.55, 128.31, 126.72, 126.00, 121.42, 118.56, 113.93, 111.23, 62.10, 53.86, 41.32, 32.91, 29.01. $C_{21}H_{23}N_3O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 382.1589, bulunan: 382.1587. $C_{21}H_{23}N_3O_2S$ için elementel analiz: C, 66.12; H, 6.08; N, 11.01; S, 8.41. Bulunan: C, 66.24; H, 5.94; N, 11.03; S, 8.39.

2-(4-Benzilpiperidin-1-il)-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10m)*:* Etanolden kristallendirildi. 363 mg elde edildi, verim %92'dir. Erime noktası: 151.6℃. ^{1}H NMR (DMSO-d_6) δ: 9.72 (s, 1H, N-H), 7.97 (d, 1H, *J*=2 Hz, H^7), 7.57 (dd, 1H, *J*=8.4 Hz, *J*=2.0 Hz, H^5), 7.28-7.23 (m, 3H, *J*=7.6 Hz, $H^{3'}$, $H^{5'}$, H^4), 7.18-7.14 (m, 3H, $H^{2'}$, $H^{4'}$, $H^{6'}$), 3.37 (s, 3H, N-CH_3), 3.06 (s, 2H, -CH_2), 2.83 (d, 2H, *J*=11.1 Hz, H^{2e}, H^{6e}-piperidin), 2.52-2.50 (m, 2H, -CH_2-C_6H_5, DMSO ile birlikte), 2.06 (t, 4H, *J*=11.1 Hz, H^{2a}, H^{6a}-piperidin), 1.53-1.30 (m, 5H, H^3, H^4, H^5-piperidin). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 168.52, 168.42, 140.31, 134.32, 133.52, 128.94, 128.11, 125.72, 121.39, 118.48, 113.85, 111.21, 62.16, 53.40, 42.36, 36.92, 31.58, 28.98. $C_{22}H_{25}N_3O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 396.1746, bulunan: 396.1750. $C_{22}H_{25}N_3O_2S$ için elementel analiz: C: 66.81, H, 6.37; N, 10.62; S, 8.11. Bulunan: C, 67.18; H, 6.39; N, 10.69; S, 8.13.

2-[Benzil(metil)amino]-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit (10n): Aseton-hekzan solvan sistemi ile kristallendirildi. 256 mg elde edildi, verim %75'dir. Erime noktası: 98.2℃. ^{1}H NMR (DMSO-d_6) δ: 9.78 (s, 1H, N-H), 7.98 (d, 1H, *J*=2.0 Hz, H^7), 7.56 (dd, 1H, *J*=8.8 Hz, *J*=2.0 Hz, H^5), 7.39 (d, 2H, *J*=7.5 Hz, $H^{2'}$, $H^{6'}$), 7.31 (t, 2H, *J*=7.5 Hz, $H^{3'}$, $H^{5'}$), 7.25 (t, 1H, *J*=7.5 Hz, $H^{4'}$), 7.24 (d, 1H, *J*=8.8 Hz, H^4), 3.65 (s, 2H, -CH_2-C_6H_5), 3.38 (s, 3H, N-CH_3), 3.19 (s, 2H, -CH_2-), 2.27 (s, 3H, N_2-CH_3). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 168.63, 168.33, 138.15, 134.29, 133.46, 128.84, 128.10, 126.99, 121.33, 118.45, 113.82, 111.11, 60.93, 60.45, 42.11, 28.90. $C_{18}H_{19}N_3O_2S$ için HRMS (m/z) $[M+H]^+$: hesaplanan: 342.1276, bulunan: 342.1275. $C_{18}H_{19}N_3O_2S$ için elementel analiz: C, 63.32; H, 5.61; N, 12.31; S, 9.39. Bulunan: C, 63.51; H, 5.32; N, 12.36; S, 9.37.

Biyolojik Aktivite

Kolinesteraz İnhibitör Aktivite Tayini

Sonuç bileşiklerin asetilkolinesteraz (AKE) ve butirilkolinesteraz (BKE) enzimlerine karşı inhibitör etkileri ilk olarak 100 µM konsantrasyonda, modifiye Ellman testi kullanılarak taranmış ve sonrasında %50'den fazla inhibisyon gösteren bileşiklerin farklı konsantrasyonları çalışılarak elde edilen doz-cevap eğrilerinden IC_{50} değerleri belirlenmiştir. Deney sonucunda oluşan sarı renk 412 nm'de Molecular Devices Versamax Tunable mikroplak okuyucusu kullanılarak ölçülmüştür. Asetilkolinesteraz enzimi (Electrophorus electricus, tipVI-S, 200-1,000 ünite/mg protein), Butirilkolinesteraz enzimi (At serumu, ≥900 ünite/mg protein), asetiltiyokolin iyodür (ATK), butiriltiyokolin iyodür (BTK), 5,5'-ditiyobis (2-nitrobenzoik asit) (DTNB), galantamin hidrobromür, donepezil hidroklorür monohidrat ve tris HCl Sigma Aldrich'ten satın alınmıştır. Çalışmada test edilecek bileşikler final hacimde 100 µM konsantrasyonlu olacak şekilde DMSO kullanılarak çözülmüştür. Ancak test çözeltilerinin hazırlanmasında AKE aktivitesini etkilememesi için DMSO miktarının final hacmin %1'ini aşmamasına dikkat edilmiştir. Aktivite çalışmasında 96 kuyucuklu mikroplak kullanılmıştır. Her kuyucukta final hacim 200 µl olacak şekilde; 168 µl 50 mM Tris HCl (pH 8.0) tampon çözeltisi, 10 µl 6.8 mM DTNB, 10 µl (0.4 U/ml) AKE ve 2 µl test çözeltisi ilave edilir. İlk üç kuyucuk kör olarak kullanılmak üzere AKE çözeltisi içermeyecek şekilde, sonraki 3 kuyucuk tam aktivite olarak kullanılmak üzere test çözeltisi yerine 2 µl solvan karışımı eklenerek hazırlanmıştır. Referans olarak kullanılmak üzere ise galantamin ve donepezil çözeltisi içeren 3'er kuyucuk daha hazırlanmıştır. Son olarak tüm kuyucuklara 10 µl ATK hızla ilave edilip mikroplak okuyucuya yerleştirilmiş, dakika bir tarama olacak şekilde, 20 dakika boyunca, 412 nm dalga boyunda kinetik ölçüm yapılmıştır. Sonuç bileşiklerin BKE inhibitor aktiviteleri 0.5 U/ml BKE ve substrat olarak 30 mM BTI kullanılarak aynı yöntemle belirlenmiştir. Bileşiklerin IC_{50} değerleri, GraphPad Prism (GraphPad Software, La Jolla California USA) programında, bileşik derişiminin logaritmasına karşı elde edilen %

inhibisyon verilerinin non-lineer regresyonu ile oluşturulan sigmoid doz-cevap grafikleri kullanılarak hesaplanmıştır [23,24].

Antioksidan Aktivite Tayini

Oksijen Radikali Absorbans Kapasitesi (ORAC-Fluorescein) Testi

Metotta 75 mM fosfat tamponu (pH 7.4) kullanılmıştır ve final çalışma hacmi 200 µl'dir. Test bileşiklerinin 10 mM (aseton ile hazırlanmış) stok çözeltileri %50 aseton-su karışımı ile hedef tarama konsantrasyonlarına seyreltilmiştir. Fluorescein (0.117 µM) ve AAPH çözeltileri (40 mM) fosfat tamponu ile hazırlanmıştır. 96 kuyucuklu siyah mikroplak kullanılmıştır ve her kuyucuğa 20 µl test bileşiği ve 120 µl fluorescein çözeltisi eklendikten sonra 37°C'de 15 dakika inkübe edilmiştir. Süre sonunda her kuyucuğa 60 µl of AAPH çözeltisi eklenmiş ve floresans değerleri (eksitasyon 485 nm, emisyon 535 nm) 37°C'de 90 dakika boyunca Molecular Devices SpectraMax i3x Multi-Mode Detection Platform cihazı ile takip edilmiştir. Standart Troloks kalibrasyon eğrisi oluşturmak üzere her plakta eş zamanlı olarak finalde 0.5–8 µM troloks içeren standart çözeltilerinin (minimum 9 farklı konsantrasyon değeri) floresans değerleri ölçülmüştür. Test bileşikleri (2 µM, 5 µM ve 10 µM) farklı konsantrasyonlarda taranarak uygun AUC (Area Under Curve) değeri elde edilen konsatrasyondaki alan değerlerinin, Troloks kalibrasyon grafiğindeki karşılığı hesaplanmış ve sonuçlar Troloks eşdeğerliği olarak sunulmuştur [25,26].

DPPH (1,1-Difenil-2-pikrilhidrazil) Antioksidan Aktivite Tayini

DPPH yöntemi antioksidan aktivite tayininde kullanılan spektrofotometrik bir yöntemdir. Öncelikle referans (gallik asit) ve test bileşikleri final konsantrasyonu 100 µM olacak şekilde etanol içerisinde çözüldü. Bu çözeltilerden 20 µl alınıp 180 µl 0.15 mM DPPH çözeltisi ile birleştirildi (DPPH de etanol ile çözülür). Oda sıcaklığında 30 dk inkübe edildikten sonra değişmeyen DPPH miktarı, 96 kuyucuklu mikroplaka ile SpectraMax i3x multi-Mode kullanılarak 517 nm'de ölçüldü. Elde edilen sonuçlar [(A kontrol - A numune)/A kontrol] x 100 formülüne yerleştirilerek test bileşiklerinin DPPH radikal temizleme aktivite yüzdesi belirlenmiştir. A kontrol, test numunesi yokluğunda elde edilen sonuç ve A numune, test bileşiğinin veya referansın varlığında elde edilen sonuçtur. Tüm numuneler için tekrar çalışılmış ve sonuçlar ortalama ± SD olarak hesaplanmıştır [25].

Metal Şelatör Etki Tayini

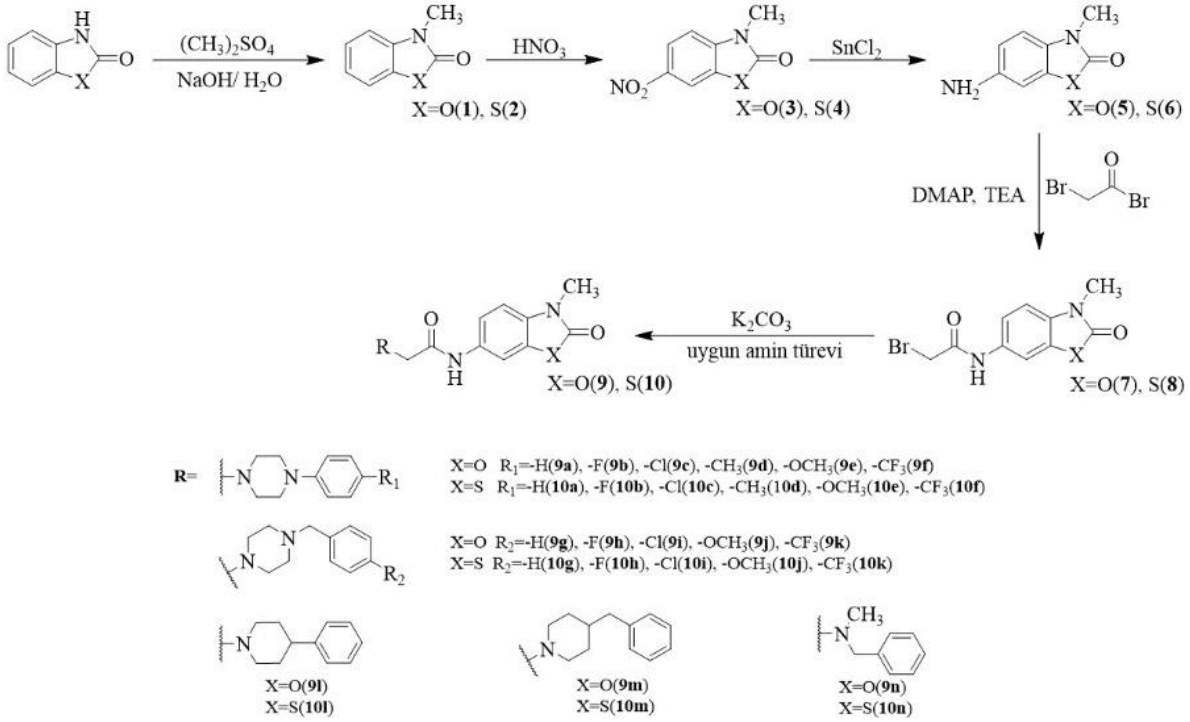
Etanol ile $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ ve $ZnCl_2$ için 400 µM'lık stok çözeltiler hazırlanmıştır. Test bileşiklerinin 10 mM'lık DMSO stok çözeltilerinden etanol ile dilüsyon yapılarak 400 µM'lık çalışma çözeltileri hazırlanmıştır. Sonrasında, 800 µl of etanol, 100 µl ilgili metal çözeltisi ve 100 µl test bileşiği karışımı 25°C'de 30 dakika inkübe edilmiştir. Süre sonunda çözeltilerin absorbans değerleri 230–500 nm aralığında, Molecular Devices SpectraMax i3x Multi-Mode Detection Platform cihazı ile ölçülmüştür. 100 µl test bileşik çözeltisi ve 900 µl etanol karışımı kontrol çözeltisi olarak kullanılmıştır. Ölçülen tüm absorbans değerleri ilgili kör çözeltisi değerleri ile düzeltildikten sonra elde edilen spektrumlar hesaplamalar için kullanılmıştır. Değerlendirme amacıyla bileşik-metal karışım çözeltisinin spektrumu, aynı bileşiğin kontrol çözeltisine ait spektrum ile kıyaslandığı, dalgaboyu (nm)/absorbans grafikleri hazırlanmıştır. Ayrıca sonuçların daha iyi değerlendirilebilmesi amacıyla kıyaslanan spektrumların fark/değişim grafikleri çizdirilmiştir [25-27].

SONUÇ VE TARTIŞMA

Çalışmamız kapsamında on dördü benzoksazolon, diğer on dördü benzotiyazolon yapısında toplam yirmi sekiz yeni bileşiğin sentezi yapıldı, yapıları kanıtlandı ve *in vitro* testler ile bileşiklerin kolinesteraz inhibitör aktiviteleri değerlendirildi.

Sentez çalışmalarında öncelikle halkaların 3. konumu bazik ortamda dimetilsülfat ile metillenmiştir. Daha sonra nitrik asit varlığında optimal sıcaklık belirlenerek 6. konumdan nitrolama tepkimesi yapılmıştır. Nitrolanmış türevler kalay klorür ile asit ortamda redüklendikten sonra elde edilen amin türevlerinden, bromoasetil bromür varlığında 2-bromo-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzoksazol-6-il)asetamit ve 2-bromo-*N*-(3-metil-2-okso-2,3-dihidro-1,3-benzotiyazol-6-il)asetamit

ara ürünleri sentezlenmiştir. Ara ürünlerin bazik ortamda uygun amin türevler ile verdiği yer değiştirme tepkimesi ile de sonuç bileşikler elde edilmiştir (Şekil 1).



Şekil 1. Sonuç bileşiklerin genel sentez şeması

Benzoksazolon türevi asetamit serisinin (9a-n) ^{1}H-NMR spektrumlarında amit protonu 9.86-9.68 ppm aralığında gözlenmiştir. Benzoksazolon halkasının 3. konumundaki azota bağlı metil grubu protonları 3.32-3.27 ppm aralığında 3H singlet olarak gözlenmiştir. Halkanın 4. konumundaki proton 7.22-7.14 ppm aralığında dublet, 5. konumundaki proton 7.43-7.36 ppm aralığında dubletin dubleti, 7. konumundaki proton ise 7.77-7.71 aralığında dublet olarak gözlenmiştir. Benzotiyazolon türevi asetamit serisinin (10a-n) ^{1}H-NMR spektrumlarında amit protonu 9.86-9.72 ppm aralığında gözlenmiştir. Benzotiyazolon halkasının 3. konumundaki azota bağlı metil protonları 3.38-3.37 ppm aralığında 3H singlet olarak gözlenmiştir. Halkanın 4. konumundaki proton 7.317.22 ppm aralığında dublet, 5. konumundaki proton 7.61-7.55 ppm aralığında dubletin dubleti, 7. konumundaki proton ise 8.00-7.96 aralığında dublet olarak gözlenmiştir.

Kolinesteraz inhibitor aktivite sonuçları genel olarak değerlendirildiğinde sentezlenen 28 bileşiğin AKE inhibitör aktivitelerinin, BKE inhibitör aktivitelerine göre daha iyi olduğu söylenebilir (Tablo 1). Bileşik 10g AKE inhibitör aktivitesi en yüksek bileşiktir ve IC_{50} değeri 52.90 µM bulunmuştur. Bileşik 10h ise en yüksek BKE inhibitör aktiviteye sahiptir, IC_{50} değeri 51.03 µM bulunmuştur. İki bileşik de benzotiyazolon çekirdeğine sahiptir. Yapı etki ilişkileri düşünüldüğünde benzotiyazolon çekirdeği taşıyan bileşikler genel olarak benzoksazolon çekirdeği taşıyanlara göre daha aktiftir. Yan zincire bağlı grupların inhibisyona etkisi incelendiğinde ise AKE inhibisyonu için benzilpiperazin yapısının, BKE inhibisyonu için de 4-florobenzilpiperazin yapısının uygun olduğu belirlenmiştir.

Sonuç bileşiklerin antioksidan özelliklerini değerlendirmek amacıyla DPPH ve ORAC antioksidan testleri uygulanmıştır. Bileşikler DPPH antioksidan testinde referans gallik asite göre düşük antioksidan profil sergilemişlerdir. Bununla birlikte canlı sistemlerde daha doğru sonuçlar verdiği bilinen ORAC antioksidan testinde daha iyi sonuçlar verdikleri görülmektedir. Özellikle yan zincirinde fenilpiperazin türevleri taşıyan bileşikler trolokstan 3-5 kat daha yüksek antioksidan etki göstermiştir.

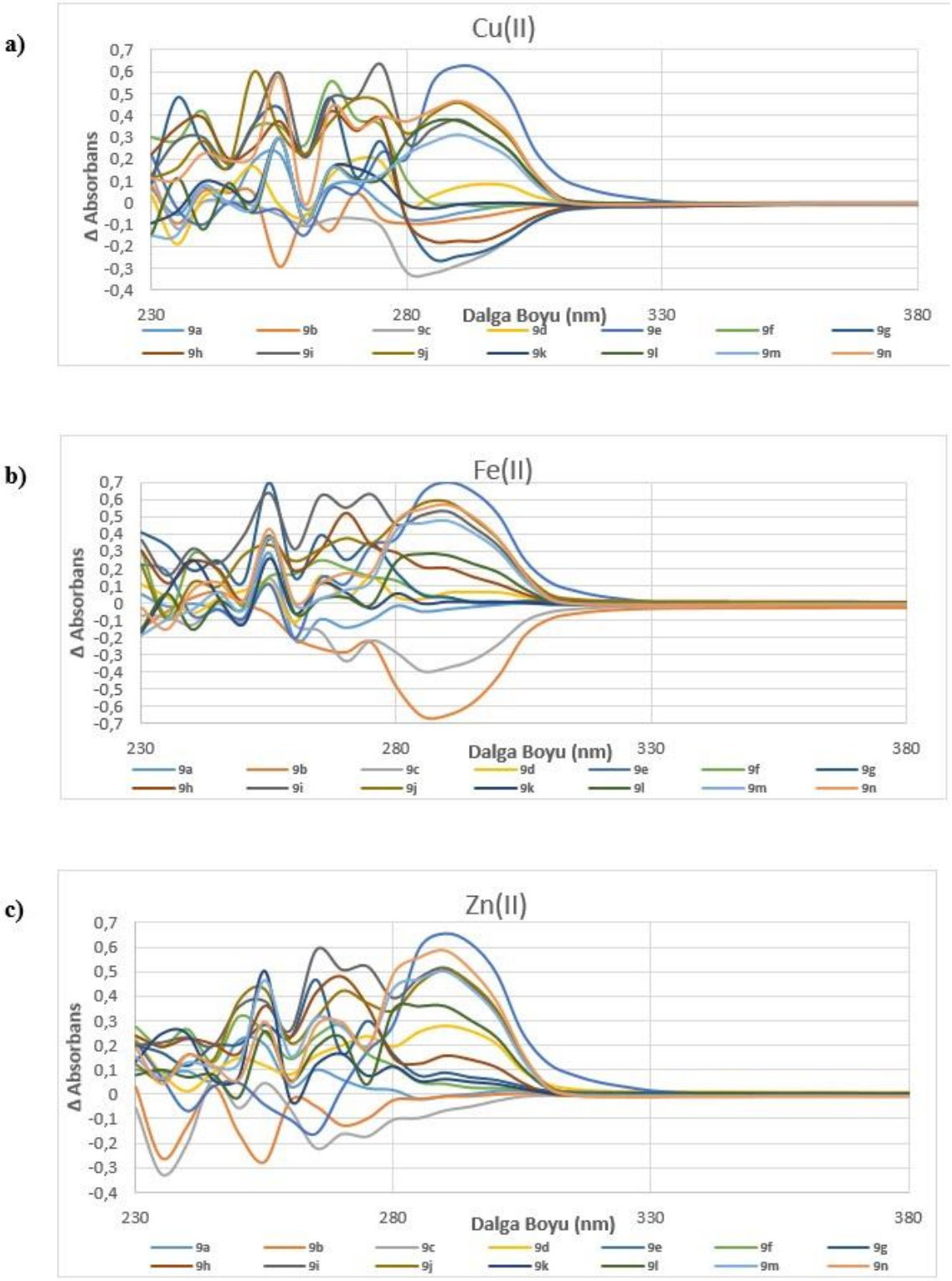
Kolinesteraz inhibitör aktiviteleri ve antioksidan özellikleri belirlenen bileşiklerin metal şelatör

etkileri incelendiğinde bileşiklerin çoğunun Cu(II), Fe(II) ve Zn(II) iyonları ile etkileştiği ve metal şelatör etki gösterdiği bulunmuştur.

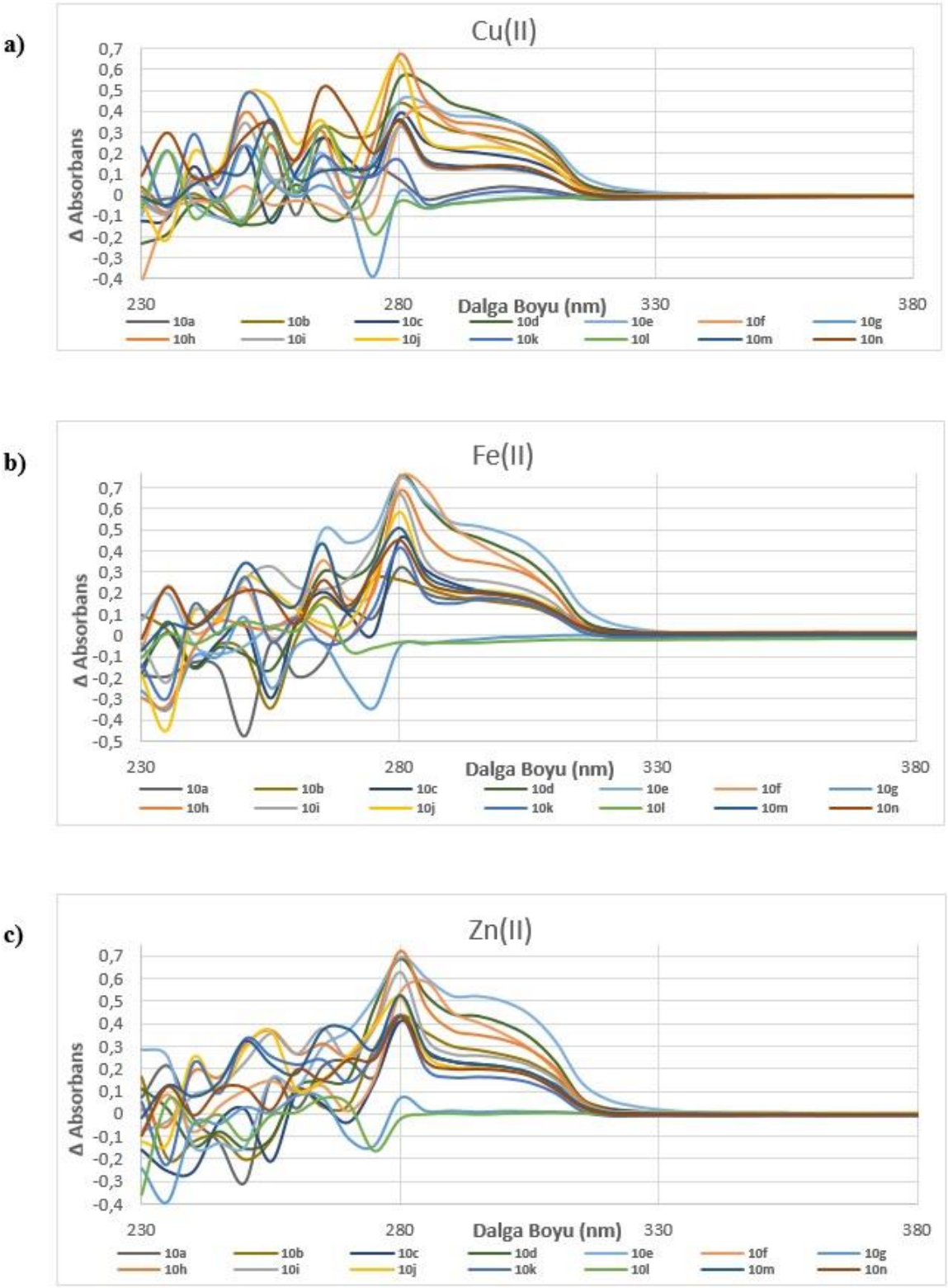
Tablo 1. Sentezlenen bileşiklerin kolinesteraz inhibitör aktiviteleri, DPPH ve ORAC sonuçları

Bileşik	*ee*AKE için % inhibisyon ± SD (100 µM)	AKE için IC_{50} (µM)	*eq*BKE için % inhibisyon ± SD (100 µM)	BKE için IC_{50} (µM)	DPPH** (100 µM)	ORAC***
9a	23 ± 2.9	-	<10	-	≤5	5.77 ± 0.25
9b	21 ± 3.2	-	<10	-	≤5	4.93 ± 0.24
9c	25 ± 3.0	-	<10	-	≤5	4.59 ± 0.07
9d	27 ± 3.5	-	<10	-	≤5	3.63 ± 0.42
9e	23 ± 1.2	-	<10	-	≤5	2.67 ± 0.25
9f	29 ± 3.0	-	<10	-	≤5	0.71 ± 0.10
9g	32 ± 3.1	-	<10	-	≤5	0.52 ± 0.05
9h	29 ± 2.2	-	<10	-	≤5	1.07 ± 0.15
9i	22 ± 3.2	-	<10	-	≤5	0.27 ± 0.01
9j	25 ± 1.5	-	<10	-	≤5	0.30 ± 0.02
9k	35 ± 3.2	-	<10	-	≤5	0.47 ± 0.08
9l	22 ± 2.9	-	<10	-	≤5	0.89 ± 0.13
9m	27 ± 3.1	-	<10	-	≤5	0.27 ± 0.04
9n	31 ± 3.4	-	<10	-	≤5	0.94 ± 0.16
10a	25 ± 2.7	-	<10	-	≤5	5.37 ± 0.38
10b	24 ± 2.4	-	<10	-	≤5	4.64 ± 0.10
10c	29 ± 3.3	-	<10	-	≤5	4.48 ± 0.03
10d	32 ± 1.4	-	<10	-	≤5	3.85 ± 0.07
10e	25 ± 3.3	-	<10	-	≤5	3.43 ± 0.21
10f	31 ± 3.5	-	<10	-	≤5	0.62 ± 0.02
10g	58 ± 1.1	52.90 ± 1.4	38 ± 2.7	-	≤5	0.68 ± 0.05
10h	45 ± 2.4	-	57 ± 1.5	51.03 ± 1.1	≤5	0.30 ± 0.06
10i	25 ± 3.2	-	<10	-	≤5	0.93 ± 0.31
10j	39 ± 2.4	-	<10	-	≤5	1.04 ± 0.08
10k	37 ± 3.4	-	<10	-	≤5	0.60 ± 0.22
10l	24 ± 3.2	-	<10	-	≤5	0.68 ± 0.22
10m	29 ± 2.6	-	<10	-	≤5	0.50 ± 0.04
10n	39 ± 4.2	-	62 ± 0.8	59.89 ± 2.7	≤5	0.55 ± 0.09
Donepezil	**100 ± 0.003**	**0.062 ± 0.02**	**67 ± 0.003**	**3.55 ± 0.07**	-	-
Gallik asit	-	-	-	-	**44.8 ± 1.0**	-

* (-): bileşik %50'nin altında enzim inhibisyonu gösterdiğinden IC_{50} değeri hesaplanmamıştır.
** Bileşiklerin 100 µM konsantrasyonda DPPH'yi azaltma yüzdesi (%) verilmiştir.
*** Bileşiklerin ORAC antioksidan testi sonuçları tabloda µmol Trolox eşdeğeri/µmol test bileşikleri olarak sunulmuştur.



Şekil 2. Benzoksazolon halkası taşıyan sonuç bileşikler (9a-9n) ile Cu(II) (a), Fe(II) (b), Zn(II) (c) iyonları ile komplekslerinin UV-vis spektrum farkları



Şekil 3. Benzotiyazolon halkası taşıyan sonuç bileşikler (10a-10n) ile Cu(II) (a), Fe(II) (b), Zn(II) (c) iyonları ile komplekslerinin UV-vis spektrum farkları

TEŞEKKÜR

Bu çalışma Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından 115S192 kodlu proje ile desteklenmiştir.

YAZAR KATKILARI

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ÇIKAR ÇATIŞMASI BEYANI

Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler.

ETİK KURUL ONAYI

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

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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE EFFECT OF *ESCHERICHIA COLI* BACTERIOPHAGE COCKTAIL ON BACTERIAL CONTAMINATION IN WATER

ESCHERICHIA COLI BAKTERİYOFAJ KOKTEYLİNİN SUDAKİ BAKTERİ KONTAMİNASYONU ÜZERİNE ETKİSİ

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ABSTRACT

Objective: *Wastewater and environmental water resources are treated to eliminate pathogenic microorganisms but contamination is still a significant problem. In particular, the presence of contamination with Escherichia coli (E. coli) is an important indicator of fecal contamination. Due to increasing antimicrobial resistance and failures of new antimicrobial processes, interest in bacteriophages in pathogen control has increased. Therefore, in our study, phage-based bacteria control in environmental waters was investigated as a natural solution.*
Material and Method: *In our study, E. coli and lytic bacteriophages specific to these E. coli were isolated from environmental water samples in Ankara. The lytic activities of the isolated phages were determined on environmental and clinical extended-spectrum β-lactamases E. coli isolates. Three phages with high lytic activity were selected, and the effectiveness of the single phage and their mixtures on E. coli contamination in water was tested.*
Result and Discussion: *As a result of the study, 17 E. coli strains were isolated from 30 environmental water samples. Lytic bacteriophages in 30 different plaque structures were also isolated from water samples. The isolated phages were found to have lytic activity in the range of 32-70% on the tested bacteria. The effectiveness of three selected phages and their cocktail on E. coli contamination in water was measured at 6th and 24th. As a result, it was observed that the cocktail application reduced the number of host bacteria in the water below detectable limits, also provided a 5-log reduction in non-host test bacteria and maintained its effect for 24 hours. When the results are evaluated, it is thought that cocktail phage application will be an effective method against E. coli contamination in water.*
Keywords: *Escherichia coli, phage application, phage cocktail, water contamination*

ÖZ

Amaç: *Atık su ve çevresel su kaynaklarında patojen mikroorganizmaları ortadan kaldırmak için arıtma yapılsa da bulaş hâlâ önemli bir sorundur. Özellikle Escherichia coli (E. coli) ile kontaminasyonun varlığı dışkı ile kontaminasyonun önemli bir göstergesidir. Artan antimikrobiyal direnç ve yeni antimikrobiyal geliştirme süreçlerindeki başarısızlıklar nedeniyle patojen kontrolünde bakteriyofajlara olan ilgi artmıştır. Bu nedenle çalışmamızda doğal bir çözüm önerisi olarak çevresel sularda faj bazlı bakteri kontrolü araştırılmıştır.*
Gereç ve Yöntem: *Çalışmamızda Ankara ili çevresel su örneklerinden E. coli ve bu bakterilere özgü litik bakteriyofajlar izole edilmiştir. İzole edilen fajların litik etkinlikleri çevresel ve klinik E.*

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coli izolatları üzerinde belirlenmiştir. Yüksek litik etkinliğe sahip 3 faj seçilerek, fajların tek tek ve karışımlarından hazırlanan kokteylinin sudaki E. coli kontaminasyonu üzerine etkinlikleri test edilmiştir.

Sonuç ve Tartışma: *Çalışma sonucunda 30 çevresel su örneğinden 17 E. coli suşu izole edilmiştir. Su örneklerinden aynı zamanda 30 farklı plak yapısında litik bakteriyofaj izolasyonu sağlanmıştır. İzole edilen fajların test edilen bakteriler üzerinde %32-70 aralığında litik etkinliğe sahip oldukları bulunmuştur. Seçilen üç fajın ve bunlardan hazırlanan kokteylin sudaki E. coli kontaminasyonu üzerine etkinliği 6 ve 24. saatte ölçülmüştür. Bunun sonucunda kokteyl uygulamasının sudaki konak bakteri sayısını tespit edilebilen sınırların altına düşürdüğü ayrıca konak olmayan test bakterilerinde 5 log azalma sağladığı ve etkisini 24 saat süresince de koruduğu görülmüştür. Sonuçlar değerlendirildiğinde kokteyl faj uygulamasının sudaki E. coli kontaminasyonuna karşı etkin bir yöntem olacağı düşünülmektedir.*

Anahtar Kelimeler: *Escherichia coli, faj kokteyli, faj uygulaması, su kontaminasyonu*

INTRODUCTION

Water that is safe for health and drinkable must have certain properties. These include the absence of pathogenic microorganisms, the absence of toxic or harmful substances within certain limits or at all, clarity, absence of saltiness, absence of offensive odors and tastes, not being hard enough to cause economic damage, and not being corrosive [1]. Quality water for human health is directly related to socio-economic development and the demand for safe drinking water is increasing. However, water resources are limited around the world, resulting in water inequality. To reduce this inequality, effective treatment and reuse of wastewater is important [2]. More than 80% of wastewater produced by society worldwide returns to the ecosystem without treatment or reuse, and as a result, 1.8 billion people use a drinking water source contaminated with feces. It causes approximately 842,000 deaths each year due to unsafe water and hygiene conditions. 663 million people around the world do not have access to reliable water sources [3].

Water-related infections occur as a result of contamination of water sources. Pathogens such as *Escherichia coli* (*E. coli)* are major causes of waterborne diseases, and poor sanitation and storage conditions contribute to the spread of these pathogens [4]. An increase in antibiotic-resistant bacteria may further complicate the problem of microbiological water pollution. *E. coli* is an indicator bacterium that gives clear evidence of fecal contamination. *E. coli* is the most notable example of fecal contaminant bacteria because of the variety of pathogenicity mechanisms and diseases it can cause [5].

These untreated water sources cause global diseases. Although antibacterial agents are widely used to kill microorganisms, they can lead to antibiotic resistance. This is an important public health problem and shows that more precise and efficient methods need to be developed for water pollution control [6]. Bacteriophages play an important role in controlling water pollution. They are preferred due to their short replication times and their lack of harm to non-pathogenic bacteria. Bacteriophages are used in many applications in environmental fields, from water purification systems to monitoring water resources. As a result, water quality is critical for human health and socio-economic development, and the need for new methods for water pollution control is increasing. One of these methods is research on bacteriophages [7-9].

One of the most successful therapeutic interventions in the history of medicine, antibiotics have played an important role in achieving medical breakthroughs such as fighting infections, organ transplants, and even cancer chemotherapy [10]. Antibiotics are used in many medical conditions. Therefore, it is thought that the decrease or loss of their effectiveness will cause a disaster. Unfortunately, we are rapidly entering a period called the "post-antibiotic era" [11]. Although bacteriophage therapy is not a new treatment approach, it is seen as a new hope for resistance to antimicrobials. Approximately a century ago, the first report on the effectiveness of bacteriophage therapy was reported. Bacteriophages or phages are small viruses ranging in size from 20 nm to 200 nm. Their proliferation and dissemination are particularly depend on the biosynthetic pathways of bacteria [12].

Phages play an important role in the ecosystem and were discovered independently by two scientists - Frederick Twort in 1915 and Felix d'Herelle in 1917. Temperature, nutrients, light and other

environmental factors affect the formation of new phages [13]. Their genetic material (DNA or RNA) is encapsulated with capsid proteins [14]. Phages have lytic or lysogenic life cycles. In the lytic cycle, host DNA is degraded and different proteins, such as capsid protein and lysis protein, are formed [15]. Phages are the most widespread viruses on the biosphere. They are easily found and isolated wherever bacteria are found [16]. The most important advantages of bacteriophages are that they are cheap and easy to obtain, as well as protecting the natural microbiota and being non-toxic. In addition, phages can go to where they are needed and multiply, regardless of the application method, and they act and show activity regardless of the antibiotic resistance in that area [17]. In order for phages to be used therapeutically, they must (1) preferably be lytic, (2) have a wide host range, and (3) be fully characterized without side effects. Considering these features, the development of therapeutic phages requires the coordinated work of multiple stakeholders [18]. Currently, great progress has been made in bacteriophage research. The potential application of phages as therapeutic agents in different hospitals, clinics and food industries in different parts of the world, especially in western countries, has increased due to the increase in antimicrobial resistance of different bacterial pathogens. In our study, *E. coli* and lytic phages specific to them was isolated from environmental water samples. We also investigated phage-based bacteria control in water contaminated with *E. coli*.

MATERIAL AND METHOD

Isolation of Bacteria

Water samples were taken into sterile bottles from 0.3-0.6 meters below the surface of Ankara environmental water resources (Cubuk Stream, Mogan Lake, Eymir Lake) and transported to the laboratory in an ice box and studied within 4 h. Water samples taken for bacterial isolation were added to tubes containing 3 ml of Tryptic Soy Broth (Merck, Germany) liquid medium and incubated at 37°C in an oven overnight. The next day, the samples were inoculated into the CHROMagar *E. coli* selective medium and the isolates giving blue colonies were selected as *E. coli* [19]. The isolated bacteria were stored in 20% glycerol at -20°C.

Isolation of Bacteriophages

For bacteriophage isolation, phage enrichment was first performed [20]. For this purpose, the environmental water samples taken were first centrifuged at 10000 rpm and passed through a 0.22 µm membrane filter. Water filtrates were incubated overnight with fresh bacterial cultures in x2 Luria Bertani (LB) broth (Merck, Germany) medium enriched with $CaCl_2$ and $MgSO_4$ at 37 °C for one-night. The following day, the suspension was centrifuged at 10000 rpm for 10 min. The double layer agar method was used to determine the presence of phages. Host *E. coli* bacteria in log phase were added to the phage suspension and waited for 10 min. Simultaneously, the soft agar (0.6% agar) was heated and cooled to 45 °C. 3750 µl of soft agar was added to this mixture and poured onto LB agar medium. After overnight incubation, the petri dishes were evaluated for the presence of phage plaques. A single plaque was isolated for purification in petri dishes showing bacteriophage plaque. After the phage plaques were selected with a Pasteur pipette, they were placed in tubes containing 4000 µl LB broth. Then 100 µl bacteria were added. Then, 4000 µl of LB broth was added to the tubes. This process was repeated at least five times until a uniform plaque was seen in the petri dish.

Determination of Host Ranges

Concentrations of isolated and purified bacteriophages were calculated as plaque-forming units (PFU/ml). The host range of phages was determined on a total of 37 bacteria using our own newly isolated *E. coli* isolates (n:17) and clinical *E. coli* isolates (n:20) from the culture collection of Ankara University, Department of Pharmaceutical Microbiology.

In test procedure, a suspension of 10^8 CFU/ml concentration of each test bacteria were prepared from fresh cultures and strips were sown on the agar plate, and 10 µl of 10^8 PFU/ml concentration of bacteriophage suspensions were dropped onto these areas. After one-night incubation, the inoculation areas were evaluated for bacterial growth [21].

Effect of Bacteriophages on Water Contamination

Water biocontrol with bacteriophages was carried out according to Kauppen et al. method with some modifications [22]. The environmental water sample was autoclaved prior to tests. Phage biocontrol tests were carried out with the Ea1, Ea3 used for host bacteria (used in phage production) test and Ea6, Ea7, and Ea8 used for non-host bacteria (outside the range of phage effect) test. Bacterial concentration of sterile environmental water samples was adjusted to McFarland 0.5 turbidity with fresh bacterial cultures mix.

Five aliquots of 10 ml of water sample with bacteria were collected in sterile tubes. The first sample is considered as a control (not added phage). The second, third and the fourth samples were inoculated respectively with 2 ml volume of F4, F14, and F23 phage suspension at titter of 10^8 PFU/ml. The last tubes were inoculated with 2 ml volume of phage cocktail (F4+F14+F23). The samples were incubated at 37°C for 24 h. Triplicate 100 ml samples were taken after 6 and 24 h. At the end of the period, samples were taken from each tube, diluted and 20 µl inoculated on LB agar. All petri dishes were incubated overnight at 37 °C. The following day, the number of viable bacteria in each petri dish was calculated. Phage biocontrol test without the host were also performed for each phage.

Statistical Analysis

Each experiment was repeated three times. The results were presented as mean values and standard deviation values of the mean. One-way Anova Kruskal Wallis test ($p < 0.05$; GraphPad Prism version 5) was used to determine statistically significant differences between the treatment and control groups.

RESULT AND DISCUSSION

E. coli is an important cause of urinary tract and gastrointestinal infections in humans and is an indicator of wastewater contamination of water, food and agricultural products [23]. Pathogens such as *E. coli, Staphylococcus aureus and Campylobacter jejuni* are generally detected in biological wastewater treatment systems [24]. These pathogens are adsorbed by activated sludge and, although they can be removed with more sludge, the presence of pathogens can often be detected in wastewater and pose potential health risks to consumers or environmental water supplies [25]. Therefore, it is necessary to remove as many pathogens as possible during the biological wastewater treatment process. Compared to physicochemical treatment methods, the use of pathogen-specific phage control systems may offer an effective solution [26,27].

In our study, 30 water samples were taken from Ankara province between April to October, 2023. From the water samples taken, 17 bacteria were isolated and purified in CHROMagar *E. coli* selective medium. Location and time information of the isolated bacteria are given in Table 1.

Table 1. Location and time information of the isolated bacteria

Water Number	*E. coli* isolates	Time (month)	Location
1	Ea1	April	Golbası (Mogan lake)
2	Ea2	April	Golbası (Mogan lake)
3	-	April	Golbası (Mogan lake)
4	-	April	Golbası (Mogan lake)
5	-	April	Golbası (Mogan lake)
6	Ea3	May	Cubuk Stream (Etimesgut)
7	Ea4	May	Cubuk Stream (Etimesgut)
8	Ea5	May	Cubuk Stream (Etimesgut)
9	-	May	Cubuk Stream (Etimesgut)
10	Ea6	May	Cubuk Stream (Akköprü)
11	Ea7	May	Çubuk Stream (Akköprü)
12	Ea8	May	Çubuk Stream (Akköprü)
13	-	June	Ankara University Faculty of Science Artificial Lake
14	Ea10	June	Ankara University Faculty of Science Artificial Lake

Table 1 (*continue*). Location and time information of the isolated bacteria

Water Number	*E. coli* isolates	Time (month)	Location
15	-	June	Ankara University Faculty of Science Artificial Lake
16	Ea9	June	Cubuk Stream (Gumusdere)
17	-	August	Cubuk Stream (Gumusdere)
18	-	August	Cubuk Stream (Gumusdere)
19	Ea11	August	Cubuk Stream (Gumusdere)
20	-	August	Cubuk Stream (Gumusdere)
21	Ea12	August	Cubuk Stream (Gumusdere)
22	-	August	Cubuk Stream (Gumusdere)
23	Ea13	August	Ankara University Faculty of Science Artificial Lake
24	Ea14	August	Ankara University Faculty of Science Artificial Lake
25	Ea15	August	Cubuk Stream (Gumusdere)
26	-	August	Cubuk Stream (Gumusdere)
27	Ea16	September	Cubuk Stream (Gumusdere)
28	-	September	Eymir lake
29	-	October	Eymir lake
30	Ea17	October	Eymir lake

For phage isolation, 4 water samples and 8 bacterial isolates (host bacteria) were used. In our study, 30 different plaques were selected from the water samples studied and purified, and their concentrations were calculated as plaque-forming units (PFU/ml). The host bacteria of the phages, the water samples they were isolated from, and their concentrations are given in Table 2. Some plaque images of isolated and uniformly purified phages are seen in Figure 1.

Adhesion of phage to bacteria depends on the relationship between host cell surface receptors and phage binding structures [26]. Phages are assumed to have a narrow host range by nature, which is one of the main issues limiting their use in therapy. However, studies report that some phages are effective on different serotypes of the tested host bacteria and even on different types of bacteria [28]. Yamaki et al. reported that the EscoHU1 phage they characterized was effective against different serotypes of *E. coli*, Salmonella, Citrobacter and Shigella and had a wide host range [29]. As a result of the host range test of 30 phages isolated in the study, it was found that the phages had lytic activity in the range of 32-70%. The table containing the host ranges of the phages is given in supplementary file. The host ranges of the phages isolated in our study were tested not only on environmental *E. coli* isolates but also on clinical extended-spectrum β-lactamases *E. coli* isolates. It has been observed that environmental phage isolates also show high lytic activity on clinical strains.

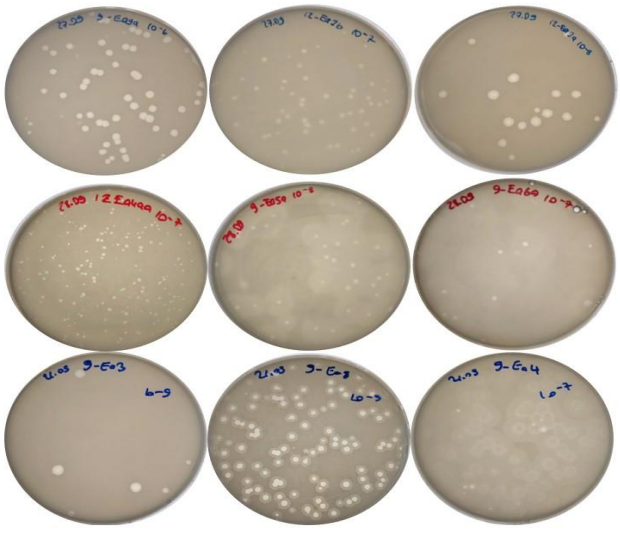

Figure 1. Different plaque images of isolated phages

Table 2. List of isolated bacteriophages

Phage No	Host bacteria	PFU/ml
F1	Ea4	$4.4x10^{9}$
F2	Ea4	$6.8x10^{8}$
F3	Ea1	$0.3x10^{8}$
F4	Ea1	$0.5x10^{8}$
F5	Ea2	$1.1x10^{3}$
F6	Ea2	$2.3x10^{5}$
F7	Ea2	$1.6x10^{10}$
F8	Ea3	$0.1x10^{1}$
F9	Ea3	$0.3x10^{8}$
F10	Ea3	$0.6x10^{6}$
F11	Ea3	$2.2x10^{8}$
F12	Ea5	$0.9x10^{8}$
F13	Ea2	$0.3x10^{8}$
F14	Ea3	$6.5x10^{9}$
F15	Ea4	$4.4x10^{9}$
F16	Ea5	$4.4x10^{10}$
F17	Ea6	$1.3x10^{9}$
F18	Ea7	$0.3x10^{6}$
F19	Ea8	$4.7x10^{10}$
F20	Ea9	$0.3x10^{8}$
F21	Ea1	$2.2x10^{12}$
F22	Ea2	$0.3x10^{8}$
F23	Ea3	$1.6x10^{10}$
F24	Ea3	$6.4x10^{9}$
F25	Ea4	$2.09x10^{10}$
F26	Ea4	$6.5x10^{9}$
F27	Ea4	$1.96x10^{10}$
F28	Ea5	$0.3x10^{8}$
F29	Ea6	$0.5x10^{8}$
F30	Ea7	$2.2x10^{12}$

In this study, the activities of F4, F14, and F23 phages, selected for their high lytic activities, on *E. coli* in water, alone and with a cocktail prepared from a mixture of three phages, were investigated. Two-time parameters (6 h and 24 h) were tested in the study. The bacterial concentration of the water sample was increased with the host bacteria Ea1 and Ea3 of the selected phages and the number of bacteria was calculated after phage treatment. At the same time, the test was repeated with environmental *E. coli* isolates (Ea6, Ea7, Ea8), which were not host bacteria of the phages and at least one phage was effective.

In the test protocol using host bacteria (Figure 2A), it was observed that *E. coli* was completely eliminated in the water in the samples treated with F4 and phage cocktail as a result of the first 6 h. Here, it is thought that the F4 phage's complete destruction of bacteria is due to its ability to completely lysis both bacteria. It is observed that F14 and F23 phages cause a 1-2 log decrease in bacterial concentration compared to the control, but cannot completely destroy the bacteria. In addition, it is seen that the effects of phages decrease over time and the effect almost completely disappears after 24 h. Since F4 and phage cocktail completely eliminated the bacteria in the first 6 h, no bacterial growth was observed in 24 h.

In the test protocol with non-host bacteria (Figure 2B), it was observed that all single phage applications showed low levels of effect at 6 and 24 h, as it is known that phages show low sensitivity to a single bacterium. However, it was found remarkable that in the sample where phage cocktail was applied, the cocktail reduced the bacterial density by ~5 log and this effect continued at the 24 h.

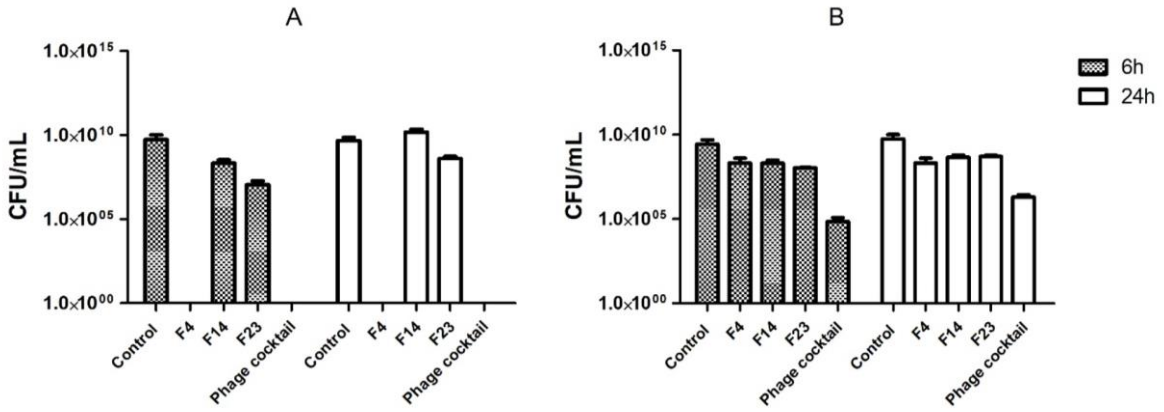

Figure 2. The effect of phages and phage cocktail on contaminated water with *E. coli* (**A.** Host bacteria, **B.** Non-host bacteria)

In literature, mixing phages and using them as cocktails provides advantages in limiting the formation of phage resistance and increasing the effective range of the phage [30]. Turki et al. tested single, double and triple use of phage on Salmonella in wastewater and reported that the cocktail consisting of three phages showed the best removal effect against bacteria [31]. Yu et al. reported that phage cocktails with a wide range of activity were more effective than phage cocktails with a narrow host range in suppressing multidrug-resistant *E. coli* NDM-1 in activated sludge systems [32].

Phage studies specific to the pathogens (*E. coli, Staphylococus aureus, Salmonella spp., Pseudomonas aeruginosa, Acinetobacter baumannii* and *Klebsiella pneumoniae)* in water can be found in the literature. Dhevagi and Anusuya reported that the addition of *E. coli* and Salmonella phages reduced the number of pathogens in sewage sludge [33]. Maal et al. reported that they reduced the coliform value of municipal sewage by 22-fold (from 2400 to 110) using the most probable number method after two hours of incubation with the coliphage mixture they isolated from [34].

In conclusion, the present study *E. coli*-specific lytic bacteriophages were isolated from environmental water samples. The isolated phages showed high lytic activity on environmental and clinical *E. coli* strains. The effectiveness of the cocktail prepared from 3 phages, selected due to their high activity, on *E. coli* contamination in water was tested. As a result, it was observed that the cocktail application reduced the number of host bacteria in the water below detectable limits and also provided a 5-log reduction in non-host test bacteria. It is thought that phage cocktail application will be an effective method against *E. coli* contamination in water.

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AUTHOR CONTRIBUTIONS

Concept: H.B.E., A.K.; Design: H.B.E.; Sources: H.B.E., A.K.; Materials: H.B.E., A.K.; Data Collection and/or Processing: H.B.E., A.K.; Analysis and/or Interpretation: H.B.E., A.K.; Manuscript Writing: H.B.E., A.K.; Critical Review: H.B.E.; 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

AKARYAKIT İSTASYONUNDA ÇALIŞANLARIN KİMYASALLARA MARUZİYETİ

CHEMICAL EXPOSURE OF FUEL STATION WORKERS

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ÖZ

Amaç: *Günümüzde sayıları her geçen gün artan kimyasal maddeler hem hayatımızı kolaylaştırmakta hem de olumsuz sağlık etkilerine neden olabileceği için endişe yaratmaktadır. Kimyasalların olası sağlık risklerini minimize ederek kullanmak için iyi yönetilmesi gerekmektedir. Kimyasallara akut maruziyetten ziyade kronik maruziyet daha tehlikeli sonuçlar doğurabilir. Kronik maruziyet mesleki maruziyette de görülmektedir. Sunulan derleme makalesinde akaryakıt istasyonlarında maruz kalınan kimyasallar ve özellikleri, bu kimyasallara mesleki maruziyetin sebep olacağı olası sağlık etkileri ve olumsuz sağlık etkilerini minimize etmek için gerekli önlemlerden bahsedilmiştir.*

Sonuç ve Tartışma: *Akaryakıt istasyonlarında özellikle benzin, motorin ve LPG gibi satış ürünleri bulunmaktadır ve bunlar çeşitli kimyasal maddeler içermektedir. Benzen, toluen, etilbenzen ve ksilen başlıca maruz kalınan maddelerdir. Benzen Uluslararası Kanser Araştırma Ajansı (IARC) tarafından Grup 1 "insan karsinojeni" ve etilbenzen Grup 2B "olası insan karsinojeni" olarak sınıflandırılmıştır. Akaryakıtta bulunan bu maddelere başta inhalasyon ve dermal yolla maruziyet söz konusudur. Regülasyonlarla belirlenen limit değerlere uyulduğu ve yapılan işe göre eldiven, maske ve iş kıyafeti gibi koruyucu önlemler alındığında olası sağlık riskleri azaltılabilir. Birçok çalışmada akaryakıt istasyonunda çalışan ve çalışmayan bireyler karşılaştırılarak özellikle korunma önlemi almayan bireylerde maruziyet grubunda ciddi sağlık sorunları gözlenmiştir. Bu nedenle koruyucu önlemlerin sıkı olarak uygulanması ve iş yeri hava ölçümleri yapılarak havadaki kimyasalların limit değerleri aşmadığının denetlenmesi gerekmektedir.*

Anahtar Kelimeler: *Benzen, benzin, etilbenzen, kanser, mesleki maruziyet*

ABSTRACT

Objective: *Chemical substances, the number of which is increasing day by day, both make our lives easier and cause concern as they may cause negative health effects. Chemicals must be managed well in order to use them by minimizing possible health risks. Chronic rather than acute exposure to chemicals can have more dangerous consequences. Chronic exposure is also seen in occupational exposure. In presented article, chemicals exposed at fuel stations and their properties, possible health effects of occupational exposure to these chemicals and necessary precautions to minimize negative health effects are mentioned.*

Result and Discussion: *There are sales products such as gasoline, diesel and LPG at gas stations and these contain various chemicals. Benzene, toluene, ethylbenzene and xylene are main exposures. Benzene has been classified as Group 1 "human carcinogen" and ethylbenzene Group 2B "possible human carcinogen" by the International Agency for Research on Cancer (IARC). Exposure to these*

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substances in fuel is primarily by inhalation and dermal exposure. Possible health risks can be reduced if limit values determined by regulations are followed and protective measures such as gloves, masks and work clothes are taken. In many studies, serious health problems were observed in the exposure group, especially in individuals who did not take protective measures by comparing the individuals working and not working at the gas station. For this reason, it is necessary to apply protective measures strictly and to check that the chemicals do not exceed the limit values by making workplace air measurements.
Keywords: *Benzene, cancer, ethylbenzene, gasoline, occupational exposure*

GİRİŞ

Günümüzde tüm dünyada on milyonun üzerinde farklı yapıda kimyasal maddeler bulunmaktadır. Bu kimyasallara her yıl binin üzerinde yeni kimyasal madde piyasaya sunulmaktadır. Kimyasallar günlük hayatımızı kolaylaştırdığı için birçok sektörde (sanayi, tarım, ilaç endüstrisi vs.) hatta yaşamımızın önemli parçası olan gıdalarda dahi kullanılmaktadır. Kimyasallar bir yandan hayatımızı kolaylaştırırken diğer taraftan da olası olumsuz sağlık etkilerine neden olması sebebiyle endişe yaratmaktadır. Ancak gelişen teknoloji ve yaşam koşulları çerçevesinde kullanılan kimyasal maddeleri çok iyi bir şekilde yöneterek onlardan gerekli olan faydayı sağlayıp olası olumsuz etkilerini de minimuma indirecek koruyucu ve önleyici önlemler almalıyız. Özellikle kimyasal maddelere kronik olarak maruziyetin söz konusu olduğu mesleki maruziyetin kontrol edilmesi işçi sağlığı açısından son derece önemlidir.

Akaryakıt istasyonları da diğer birçok sektörde olduğu gibi sağlık yönünden risk oluşturabilecek birçok kimyasal maddeyi bünyesinde bulundurmaktadır. Kronik maruziyetler akut maruziyetlere göre daha endişe verici maruziyet şeklidir, çünkü sürekli maruziyet düşük dozlarda dahi olsa çeşitli mekanizmalarla (akümülasyon, tersinir etkinin geri dönüştürülemez etkiye dönmesi ve yaşlanma süreci gibi) daha tehlikeli olmaktadır [1]. Kronik maruziyetin söz konusu olduğu mesleki maruziyette koruyucu önlemler ve mesleğe göre hava ölçümleri gibi hayati önlemlerin alınması elzemdir. Sunulan derlemede birçok kimyasalla maruz kalan akaryakıt istasyonunda çalışanlar ve maruz kaldıkları kimyasal maddeler ve olası sağlık etkileri üzerinde durulacaktır.

Akaryakıt istasyonunda çalışan pompa görevlileri farklı kimyasallara mesleki maruziyet nedeniyle sağlık açısından risk oluşturmaktadır. Akaryakıt istasyonu pompa görevlilerinin çeşitli kimyasallara maruziyeti akaryakıt doldurma esnasında ve taşıtlardan kaynaklı egzoz dumanına maruziyet sonucu meydana gelmektedir. Bu maruziyetler bazı kimyasal maddelerin toksik olması ve sağlığa zararlı etkileri açısından riskli olabileceği göstermektedir. Benzen başta olmak üzere akaryakıt istasyonunda solunan birçok kimyasalın karsinojenik ve genotoksik etkilerinin olduğu bilinmektedir. Dünya Sağlık Örgütü'ne (World Health Organisation; WHO) bağlı Uluslararası Kanser Araştırmaları Ajansı (International Agency for Research on Cancer; IARC) tarafından akaryakıt istasyonlarında olası maruz kalınan bazı kimyasallar yeterli hayvan ya da insan verilerine göre karsinojenesite açısından sınıflandırılmışlardır. Bunun dışında benzen ve diğer organik çözücüler akciğere toksik ve böbreklerde, karaciğerde ve merkezi sinir sistemi ve hematolojik sistemde hasarlara sebep olabilmektedir [2-6]. Bu tür organik çözücülerin her gün ve uzun süreli olarak solunması merkezi sinir sisteminde hasarlara, gözde tahrişe, kemik iliği hasarına ve deri lezyonlarına sebep olabilmektedir [7].

Akaryakıt istasyonlarında solunan başta benzen olmak üzere çeşitli kimyasalların ortamda bulunması gereken sınır değerleri çeşitli regülasyonlarla belirlenmiştir. Bu regülasyonlar ve referans limit değerler Türkiye'de Kimyasal Maddelerle Çalışmalarda Sağlık ve Güvenlik Tedbirleri Hakkındaki Yönetmelik [8] ve Kanserojen ve Mutajen Maddelerle Çalışılırken Alınacak Sağlık ve Güvenlik Tedbirleri Hakkındaki Yönetmelik [9] tarafından Zaman Ağırlıklı Ortalama (Time-Weighted Average; TWA-günde 8 saat haftada 40 saat boyunca ortalaması alınan havadaki toksik madde konsantrasyonlarıdır) sayısal değeri kullanarak belirlenmiştir.

Mesleki maruziyet limit refererans değerleri TWA olarak Amerika Birleşik Devletleri'nde (ABD) ise Mesleki Güvenlik ve Sağlık İdaresi (Occupational Safety and Health Administration; OSHA) [10] ve Ulusal İşçi Sağlığı ve Güvenliği [11] ve Amerikan Hükümeti Endüstriyel Hijyenistler Konferansı (American Conference of Governmental Industrial Hygienists; ACGIH) [12] tarafından düzenlendiği

görülmektedir. Bu kimyasalların solunan hava ortamında referans limit değerlerin altında bulunması sağlık üzerinde herhangi bir olumsuz etki oluşturmadığı öngörülmektedir.

Akaryakıt istasyonlarında maruz kalınan kimyasallar başta benzen olmak üzere toluen, ksilen, etilbenzen, hekzan, etanol, metanol, fenol, izopropil alkol, izobutil alkol, karbonmonoksit, karbondioksit, azot oksitler, kükürt dioksit, bütan olarak sıralanabilirler. Bu kimyasallar çok düşük dozda dahi birtakım sağlık problemlerine neden olabilirler. Bu kimyasallardan en sık maruz kalınanlardan benzen, toluen, etilbenzen ve ksilendir [4].

Akaryakıt istasyonunda çalışan işçiler için günümüzde benzine maruziyetinden korunmak için eldiven ve önlük (iş kıyafeti) dışında herhangi bir koruyucu ekipman bulunmamaktadır. Dolayısı ile benzin, mazot ve sıvılaştırılmış petrol gazı (liquefied petroleum gas; LPG) solunmasını önlemek için herhangi bir korunma aracı yer almamaktadır. Akaryakıt solunması kaynaklı maruziyetin henüz önlenemediği gerçeğini ortaya koymaktadır. Bu da akaryakıt kaynaklı kimyasal maruziyetin ne kadar önemli olduğunu göstermektedir. Akaryakıt sektöründe çalışan pompa görevlileri başta benzen olmak üzere diğer kimyasallara maruziyetinin genotoksisiteye ve kansere sebep olabileceği düşünülmektedir [2,3,5].

İşlenmiş olarak ham petrolden imal edilen benzin, mazot-motorin ve LPG ürünlerin satışı yapılmaktadır. Dolayısı ile akaryakıt istasyonunda çalışan pompa görevlileri en çok bu üç temel ürünün satışı esnasında bu maddelere inhalasyon olmak üzere oral ve dermal yol ile maruz kalmaktadırlar.

Ham Petrolden İmal Edilen Başlıca Satış Ürünleri

Benzin

Motor yakıtı olan benzin, ham petrolden, kaynama noktaları 30-200°C olan hidrokarbonların ayrılmasıyla elde edilmektedir. Ülkemizde 1 Ocak 2006 tarihinden itibaren benzin içeriğinde kurşun bulunması yasaklanmıştır. İçeriğinde aromatikler, benzen, etilbenzen, toluen, ksilen, olefinler, metanol, etanol, izopropil alkol, tersiyer bütil alkol, izobütil alkol, eterler gibi kimyasallar bulunmaktadır. Benzin düşük moleküler ağırlıklı genellikle 3-11 arasında karbon numaralı parafinik, naftenik, olefinik ve aromatik bileşiklerin karışımıdır. Bileşimi ham petrolün çeşidine ve harmanlandığı çeşitli saflaştırma proseslerinin çeşidine göre değişebilmektedir. Araçlara benzin dolumu sırasında kısa zamanda çok fazla miktarda benzin buharı çevreye yayılmaktadır. Benzini oluşturan önemli aromatikler benzen, toluen ve ksilendir [13-15].

Motorin

Kaynama noktaları 200-360°C aralığında olan hidrokarbonların, ham petrolden ayrılmasıyla elde edilmektedir ve dizel motorlarında yakıt olarak kullanılmaktadır. Limon sarısı renginde ve berrak bir petrol ürünüdür. Alevlenme noktası minimum 55°C'dir. Dizel içeriğinde polisiklik aromatik hidrokarbonlar (PAH'lar), kükürt, formaldehit ve asetaldehit başta olmak üzere pek çok kimyasal madde bulunmaktadır [13].

LPG

Ham petrolü meydana getiren hidrokarbonlardan propan ve bütanın normal sıcaklık ve yüksek basınç altında sıvılaştırılmasıyla elde edilmektedir. Evlerde ve endüstride geniş çapta kullanılan bu gazlar çelik tüpte doldurulmuş olarak tüketime sunulmaktadır [16].

Benzin, Motorin ve LPG Bileşiminde Bulunan Kimyasallar

Petrol, hidrokarbonların karışımından meydana gelmiş olup, her zaman sabit bir bileşimi yoktur. Doğal akaryakıt olan ham petrol, bulunduğu ülkelere göre değişen bileşimlere sahiptir. Örneğin; ABD'de özellikle Pennsylvania bölgesinde çıkarılan petroller genellikle hidrokarbon sınıfında olan bileşikleri; Rusya petrolleri, kötü kokulu naftan sınıfından bileşikleri ve Romanya petrolleri ise bu ikisinin karışımını içermektedir. Çeşitli tipteki petrollerin kendine has ağırlıkları 0.80-0.96; alevlenme noktaları 15-120°C ve ortalama ısıtma kuvvetleri 10.500 kcal/kg'dır. Ortalama elementel bileşimleri ise; karbon %84, hidrojen %12, oksijen %1 olup çok az miktarda da kükürt bulunur. Teksas ve Kaliforniya petrollerinde kükürt diğerlerine oranla fazladır. Değişik kimyasal içeriğe sahip hidrokarbonların bir

araya gelerek oluşturduğu değişik kimyevi bileşimde olan çok sayıda petrol tipi bulunmaktadır (örneğin, parafin bazlı petrol ve asfalt bazlı petrol gibi) [17].

Benzen

Benzen vücuda en fazla inhalasyon yolu ile girer. Vücuda alınan benzenin yaklaşık yarısı absorbe olurken, geri kalanı yine inhalasyon yolu ile dışarı atılır. Yiyecekler ve su içme ile oral yolla vücuda alınan benzen miktarı minimal düzeydedir. Dermal yol ile vücuda alınan benzen, organizmaya ciddi zararlar verecek kan konsantrasyonlarına ulaşamaz. Bununla birlikte deri yoluyla absorbsiyonun ciddiyeti, maruziyeti, maruziyet süresinin azaltılması ve maruz kalan deri yüzeyinin alanının azaltılması sağlanarak engellenebilir [15].

Benzen, IARC tarafından Grup 1 "insan karsinojeni" olarak sınıflandırılmıştır [18]. Benzen maruziyeti sonucu gelişen lösemi vakalarının çoğu akut myeloblastik lösemi (AML)'dir [19]. Ayrıca, benzen maruziyetinin multiple miyeloma riskini yükseltebileceği belirtilmiştir [20]. Akaryakıt çalışanlarında, çalışmayan kontrol grubuna göre multiple myeloma riskinin daha yüksek olduğu tespit edilmiştir [21].

Benzen, kromozomlarda kırıklara sebep olabilir. Bu kromozomal aberasyonlar, benzen konsantrasyonunun 100 ppm'in üzerinde olduğu ve kronik maruziyetler söz konusu olduğunda ortaya çıkabilir [22]. Yapılan bazı araştırmaların sonucunda ise benzen konsantrasyonu 10 ppm üzerinde olduğunda da kromozomlar üzerinde hasara neden olabileceği gösterilmiştir [23].

Toluen

Toluen buharları zararlıdır. Mukoz membranlarda tahrişe sebep olur. IARC tarafından Grup 3, "insanlarda karsinojen olarak sınıflandırılamaz" şeklinde sınıflandırılmaktadır [15,18]. Mutajenik ve teratojenik etkileri mevcuttur. Ayrıca embriyotoksik etkileri olduğu ve toluenin plesantadan geçerek fetusa ulaştığı gösterilmiştir. Toluenin sperm hücre anomalileri ve impotans yaptığı bilinmektedir. Benzene kıyasla daha kuvvetli akut toksisiteye sebep olmaktadır. Ortalama 200-240 ppm konsantrasyonda yaklaşık 3-7 saat maruziyetten sonra baş dönmesi, denge bozukluğu ve baş ağrısı ortaya çıkmaktadır. Daha yüksek düzeylerde maruziyet koma ile sonuçlanabilir [15].

Toluenin meydana getirdiği kronik toksisitenin belirtileri baş ağrısı, baş dönmesi, mukozada tahriş, mide bulantısı, iştah kaybı olarak sıralanabilir. Bu belirtiler genellikle maruz kalınan günün sonunda ve daha şiddetli bir şekilde maruz kalınan haftanın bitiminde belirginleşmektedir. Çalışanlar için hafta sonu veya tatillerde bu toksisite belirtileri azalır ya da kaybolur. Ayrıca çocuklarda veya tiner suistimal eden (diğer çözücülerle beraber içinde toluen içeren yapıştırıcıların buharlarını soluyan) gençlerde ani ölüm vakaları da gözlenmiştir. Toluenin tekrarlayan maruziyetleri bağımlılık meydana getirmektedir. Glue Sniffing denilen zamk koklama alışkanlığı meydana gelmektedir. Toluenin inhalasyon ile oluşan etkisi kullanılan doza göre farklılıklar göstermektedir. Toluen düşük dozda keyif verici, kendini iyi hissetme ve uyarıcı etkiler, orta seviyedeki maruziyetlerde algılama bozukluğu, konfüzyon, halüsinasyonlar, hezeyanlar, agresif ve tehlikeli davranışlar, yüksek seviyedeki maruziyetlerde ise merkezi sinir sisteminin baskılanması, konuşma bozukluğu, dalgınlık, nöbetler ve denge bozukluğuna neden olabilmektedir. Diğer semptomlar arasında çarpıntı, solunum güçlüğü, baş dönmesi, baş ve karın ağrısı, kas zayıflığı, bulantı ve burun kanaması, dışkı ve idrar tutmada güçlük olabilir [15,24].

Etilbenzen

Etilbenzen IARC tarafından Grup 2B "insanlar için olası karsinojen" olarak sınıflandırılmıştır [18]. Etilbenzen küçük konsantrasyonlar da bile mukoz membranda, burunda ve gözlerde tahriş yapar. Etil benzene 200 ppm'den 5000 ppm'e kadar artan dozlarda maruziyet gittikçe şiddetlenen şekilde gözlerde yaşarma, göz ve burunda şiddetli yaşarma ve tahrişe neden olur. Etilbenzen yüksek konsantrasyonlarda ise narkotik etki göstermektedir. Uyuşukluk, yorgunluk ve koordinasyon bozukluğu gözlenmektedir [15].

Etilbenzene kronik maruziyet sonucu yorgunluk, baş ağrısı, göz ve üst solunum sistemi tahrişi görülmektedir. Tekrarlanan maruziyet sonucunda deride kuruluk ve dermatite (egzema tarzında) yol açmaktadır. Etilbenzen sinir sisteminde ise fonksiyonel bozukluklara neden olur. Ayrıca kan sistemi ile

ilgili (lökopeni vb.), karaciğer ve safra ile ilgili problemlere, akciğer ödemi ve kanamaya neden olabileceği gözlenmiştir [7]. Letal doz 6 g/kg vücut ağırlığı olup inhalasyon yolu ile absorbsiyonda öldürücü konsantrasyon 45-55 mg/l arasındadır [15].

Ksilen

Ksilen IARC tarafından Grup 3 "insanlarda karsinojen olarak sınıflandırılamaz" şeklinde sınıflandırılmıştır [18]. Ksilen deri üzerinde çözücü etki gösterir. Ksilenin advers etkileri arasında akciğer ödemi, mide ağrısı, bulantı, karaciğer ve böbrek hasarı yer almaktadır. Ksilen, benzen gibi narkotik etkileri olan bir kimyasaldır. Ayrıca uzun süreli maruziyet sonucunda hematopoetik sistemin zarar görmesine ve sinir sisteminde bozukluklara sebep olabilen bir çözücüdür [7].

Akut zehirlenmenin klinik belirtileri benzen ile benzerlik göstermektedir. Vertigo, yorgunluk, sarhoşluk, tremor, dispne (solunum zorluğu) ve bazen mide bulantısı, kusma olur, daha ciddi vakalarda bilinç kaybı da belirebilir. Ksilen göz ve böbrek mukozasında tahrişe neden olmaktadır [25].

Ksilene kronik maruziyette ise güçsüzlük, bitkinlik, vertigo, baş ağrısı, sinirlilik hali, uykusuzluk, hafıza kaybı ve tinnittus problemleri söz konusudur. En belirgin semptomlar kalpte ritim bozukluğu, ağızda şekerimsi tat, mide bulantısı, baş dönmesi, iştah kaybı, ateş basması, gözlerde yanma ve burun akıntısıdır. Merkezi sinir sisteminde nörolojik etkiler gözlenir. Kan değerleri ile ilgili değişimler anemi, lökopeni ve trombositopeni olarak sıralanabilir [26]. Ksilenin etkileri ve ksilen duyarlılığı konusunda bireysel farklılıkların olduğuna dair birtakım bilgiler mevcuttur. Ksilene uzun süreli maruziyet organizmanın direncini azaltmaktadır. Ksilene devamlı maruziyet sonucu oluşan zehirlenme oldukça ciddi sonuçlara sebep olmaktadır. Ksilene 100 ppm 30 dakikaya kadar maruz kalma durumunda, hafif üst solunum yolu tahrişi meydana gelir. 300 ppm konsantrasyona maruz kalma ise denge kurmada bozukluk, görüş kabiliyetinde azalma ve tepki verme süresinde uzamaya neden olur. 700 ppm konsantrasyona 60 dakika süre ile maruz kalma baş ağrısı, vertigo ve mide bulantısı ile sonuçlanır [7].

Benzin İstasyonu Çalışanlarında Olası Advers Sağlık Etkileri

Tongsantia ve arkadaşları [27] tarafından yapılan bir araştırmada 41 adet akaryakıt istasyonundan toplam 151 akaryakıt istasyonu çalışanı üzerindeki benzen maruziyeti ve benzenin advers etkileri incelenmiştir. 151 akaryakıt çalışanlarından 60'ında benzen maruziyetine bağlı advers semptomlar gözlenmezken, 90 çalışanda ise belirgin advers semptomlar gözlemlenmiştir. Bu semptomlar arasında en sık görülen baş ağrısı, baş dönmesi, bitkinlik/yorgunluk, kaşıntılı cilt/kırmızı döküntü/kabarcıklar, burun tıkanıklığı ve boğaz ağrısı/boğaz kuruluğu olarak bildirilmiştir. Bu semptomların yanı sıra benzene düşük seviyede maruziyette birçok semptom daha tespit edilmiş olup bunlardan burun akıntısı, boğulma, öksürük/ses kısıklığı, kuru cilt/çatlamış cilt, iştahsızlık ve çarpıntı örnek olarak verilebilir. Orta seviyede benzene maruziyette ise göğüs ağrısı, uyuşma, kanama, yetersiz/anormal solunum, mide bulantısı ve kusma, bulanık görme, kramp oluşumu, bilinç bulanıklığı, kas zayıflığı, yanma/şişme, titreme ve depresyon görülebilmektedir. Yüksek seviyede benzene maruziyet sonucunda ise anemi görülmüştür [27]. Akaryakıt istasyonu çalışanlarında hematolojik parametreler incelenmiştir. Bu çalışmada 43 kişilik maruziyet grubu ile 77 kişilik kontrol grubu arasında karşılaştırma yapılmıştır. Her iki çalışma grubundaki hematolojik belirteçlerin normal sınırlar içinde bulunmasına rağmen, ortalama kırmızı kan hücresi sayısı, hematokrit, hemoglobin düzeyi ve ortalama trombosit sayısı, kontrol grubu ile karşılaştırıldığında maruz kalan denekler arasında önemli ölçüde daha düşük olarak tespit edilmiştir. Ayrıca ortalama hücre hemoglobini ve ortalama hücre hemoglobin konsantrasyonu, maruz kalan katılımcılarda kontrol grubu ile karşılaştırıldığında, önemli ölçüde daha yüksek olarak belirtilmiştir. Ek olarak bu çalışmada hematokrit seviyeleri, kırmızı kan hücresi sayısı, hemoglobin konsantrasyonu, trombosit sayısı, platelet hacmi, lenfosit yüzdesi ve nötrofil yüzdesi gibi hematolojik parametrelerin, maruz kalma süresi arttıkça azaldığı bildirilmiştir [28]. Diğer bir çalışmada akaryakıt istasyonu çalışanlarının benzen, ksilen, etilbenzen ve toluen gibi genotoksik kimyasallara maruziyet sonucunda meydana gelen genotoksisite profilleri incelenmiştir. Bu çalışmada genotoksik ve sitotoksik hasarı gösteren mikroçekirdek testi uygulanmıştır. 126 kişilik maruziyet grubuna karşı 21 kişilik kontrol grubu kıyaslanmıştır. 126 kişik maruziyet grubu çalışma süresi, alkol ve gargara kullanımına göre sınıflandırılmıştır. Çalışma sonucunda akaryakıtlarda bulunan genotoksik ajanların ve analiz edilen diğer değişkenlerin, akaryakıt istasyonu görevlilerinin analiz edilen hücrelerinde mikroçekirdek

sıklığının artmasına katkıda bulunduğunu göstermektedir. Alkol tükettiğini bildiren benzin istasyonu görevlilerinde de içmeyenlere göre mikroçekirdek sıklığı anlamlı olarak daha yüksek olarak tespit edilmiştir. Bu nedenle alkol tüketmenin muhtemelen mikroçekirdek sıklığını artırmaya katkıda bulunduğu bildirilmiştir [29].

Akaryakıt İstasyonlarında Alınması Gereken Önlemler

Kronik maruziyetin önemli olduğu bu tür işyerlerinde insan sağlığının korunması ve olası maruziyetleri minimize etmek için regülasyonlarla belirlendiği ve yasal denetlemelerle kontrollerin sağlandığı birtakım önlemler listelenmiştir. Bunlar arasında, kişisel koruyucu ekipmanlar kimyasal maddelerin el ile temasına neden olacak işler yapılırken yapılan iş ve kullanılan kimyasala göre uygun eldiven kullanmalıdır. Akaryakıt istasyonunda çalışanların derilerinin kimyasal yanıklardan ve yakıtlardan korumaları uygun eldivenlerle sağlanmalıdır [28,30]. Kimyasal veya çözücülerin buharlarına, toz ve dumanlara maruziyetin söz konusu olduğu işler yaparken solunum yolu koruyucuları kullanılmalıdır. Takılıp düşme olasılığı olan alanlarda kaymaz tabanlı ve parmak koruyuculu iş güvenliği ayakkabıları giyilerek olası fiziksel bir tehlikenin önlenmesi sağlanır [29,31].

SONUÇ VE TARTIŞMA

Akaryakıt istasyonlarında çalışan kişilerin maruz kaldıkları kimyasal maddeler ve çözücülerden bazıları yukarıda da detaylı açıklandığı üzere genotoksik, karsinojenik ve teratojenik etkilere neden olmaktadır ya da neden olma potansiyeline sahiptir. Bu iş kolunda çalışan kişilerin uzun süre sağlıklı çalışabilmesi için regülasyonlarla kontrol altına alınış limit maruziyet değerlerine uymaları ve gerekli olan koruyucu önlemleri alması gerekmektedir. Geçmişte ve günümüzde halen yoğun olarak kullanılan akaryakıtın olası olumsuz sağlık risklerini bilip bunları minimize etmek için iş yeri ortamında kurallara uyulmalıdır. İşyeri havasının ölçümleri yasaların önerdiği zaman aralıklarında aksatmadan yapılmalı ve yetkilendirilmiş uzmanlar tarafından denetlenmelidir.

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

ROMATOİD ARTRİT TEDAVİSİNDE HEDEFLENDİRİLMİŞ İLAÇ TAŞIYICI SİSTEMLERE GENEL BAKIŞ

AN OVERWIEW OF TARGETED DRUG DELIVERY SYSTEMS FOR RHEUMATOID ARTHRITIS TREATMENT

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ÖZ

Amaç: *Romatoid artrit eklem, kemik, kıkırdak, tendon ve bağlarda hasara sebep olabilen otoimmün bir hastalıktır. Tedavisi, semptomları hafifletmeye yönelik olarak glukokortikoidlerin, modifiye edici antiromatizmal ilaçların (DMARD) ve biyolojiklerin spesifik olmayan, sistemik uygulamalarını içerir. Geleneksel tedavi yaklaşımlarında ilaçların sık aralıklarla ve yüksek dozlarda uygulanması gerekmekte olup, bu durum hastaların yaşam kalitesini düşüren yan etkilere neden olmaktadır. Nano boyutlu ilaç taşıyıcı sistemlerin romatoid artrit tedavisi için geliştirilerek enflamasyon bölgelerine ulaştırılması, böylelikle etkin maddelerin dozunun azaltılması, sistemik yan etkilerinin en aza indirilmesi mümkün olabilmektedir.*
Sonuç ve Tartışma: *Pek çok çalışmada gösterildiği gibi çeşitli ilaç taşıyıcı sistemlerin romatoid artrit tedavisinde geleneksel tedavi yöntemlerine alternatif olarak kullanılması hastalığın semptomlarının önlenmesi ve hafifletilmesi açısından oldukça olumlu sonuçlar ortaya koymuştur. Kanser tedavisinde olduğu gibi çeşitli hedefleme yaklaşımlarından faydalanılarak gelecek yıllarda romatoid artrit tedavisinde de umut verici gelişmeler olacağı düşünülmektedir.*
Anahtar Kelimeler: *İlaç taşıyıcı sistem, hedefleme, nanopartikül, romatoid artrit*

ABSTRACT

Objective: *Rheumatoid arthritis is an autoimmune disease that can cause damage to bones, cartilage, tendons and ligaments. Its treatment includes non-specific, systemic administration of glucocorticoids, DMARDs and biologics to relieve symptoms. In traditional treatment approaches, drugs need to be administered at frequent intervals and in high doses, which causes side effects that reduce the quality of life of patients. It is possible to develop nano-sized drug delivery systems for the treatment of rheumatoid arthritis and deliver them to the areas of inflammation, thus reducing the dose of drugs and minimizing their systemic side effects.*
Result and Discussion: *As shown in many studies, the use of various drug delivery systems as an alternative to traditional treatment methods in the treatment of rheumatoid arthritis has shown very positive results in terms of prevention and alleviation of the symptoms of the disease. It is thought that there will be promising developments in the treatment of rheumatoid arthritis in the coming years utilizing of various targeting approaches as in cancer treatment.*
Keywords: *Drug delivery system, nanoparticles, rheumatoid arthritis, targeting*

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GİRİŞ

Romatoid artrit, prevalansı dünya çapında nüfusun yaklaşık %0.5-1'den fazlasını etkilediği tahmin edilen kronik ve otoimmün bir hastalıktır. Hastalık, eklem kıkırdağı, kemik, tendon ve bağlarda dejenerasyon ve sinovit ile karakterizedir [1]. 40-70 yaş aralığında görülme sıklığının arttığı, kadınlarda erkeklerden 2-3 kat daha fazla görüldüğü bildirilmiştir [2]. Yetişkinlerde yaşam boyu romatoid artrit riski %3.6 olup, kadınlar için 1/28 erkekler için ise 1/59 olarak ifade edilmiştir [3].

Romatoid artrit radikal tedavisi olmayan, hastanın yaşam kalitesini iyileştirilmesi için sürekli semptomatik tedavi gerektiren bir hastalıktır. Konvansiyonel tedavilerin; yüksek doz, sık dozlama gereksinimi, düşük terapötik etkinlik, yüksek yan etki potansiyelinin yanı sıra yüksek tedavi masrafları gibi sakıncaları bulunmaktadır. Sık ve uzun süren tedaviler, sistemik yan etkilere sebep olmakta ve bu durum hastaların yaşam kalitesini düşürebilmektedir. Söz konusu durumları düzeltmek için enflamasyonlu sinovyuma aktif veya pasif olarak hedeflenmiş ilaç taşıyıcı sistemler üzerine sayısı son yıllarda artan pek çok çalışma yapılmaktadır. Etkin madde yüklü ilaç taşıyıcılar nanometre düzeyinde partikül büyüklükleri ve çeşitli yüzey modifikasyonları sayesinde içerisine yüklenmiş etkin maddelerin istenmeyen fiziksel/kimyasal özellikleri iyileştirilebilir, suda çözünürlüğü düşük etkin maddelerin çözünürlükleri artırılabilir, biyoyararlanımı geliştirilebilir, hedeflendirme yapılabilir ve böylece daha iyi terapötik yanıtlar alınması sağlanabilmektedir [4]. Bu derlemede, romatoid artrit hastalığı ve konvansiyonel tedavi yaklaşımlarına kısaca yer verilmiş olup son yıllarda yapılmış nano boyutlu ilaç taşıyıcı sistemlere yönelik çalışmalar incelenerek hedeflendirme yaklaşımları üzerine odaklanılmıştır.

Romatoid Artrit (RA)

RA, yaygın prevalansa sahip kronik ve otoimmün bir hastalıktır. Hastalığın etiyolojisi henüz net olarak bilinmemekte olup doğuştan gelen bağışıklık sistemi, çeşitli hücreler (örneğin B hücreleri, T hücreleri, makrofajlar, sinoviyositler) ve sitokinler (TNF-a, IL1b, IL-6 ve IL-17A), kemokinler, hücre adezyon molekülleri gibi humoral faktörler ve matris metaloproteinazların yanı sıra genetik yatkınlık ve çevresel faktörler gibi etmenlerin rol aldığı düşünülmektedir [1]. Hastalığın patogenezinin ilk aşamasında, B hücreleri ve T hücreleri arasındaki etkileşim aktifleşir ve romatoid faktör, antisikliksitrünile peptit antikoru gibi otoantikorlara dönüşen anormal immün cevabı tetikler, sinovyumda da enflamasyonun aktivasyonu takip eder. Daha sonra CD4+T hücreleri, B hücreleri ve makrofajlar anjiyogenez ile sinovyumda pannus denilen hipertrofik sinoviyal zar oluşumuna yol açar. Bu aşamada prostaglandin E2 gibi tümör nekröz faktörü (TNF), interlökin-1 ve 6, çeşitli proenflamatuar sitokinler ve proteazlar sinovyal hücreler arası etkileşimler sayesinde salgılanarak, lokal eklem yapılarında pannusun saldırısı ve sinoviyal yıkıma neden olur [5]. Bu durum anormal immün cevabın tetiklenmesine neden olur. Hastalık patolojisi genetik ve çevresel faktörler gibi birbiriyle etkileşen immün cevabı artıracak çoklu mekanizmaları içerir. Bağışıklık modülatörlerinin karmaşık etkileşimi eklem dejenerasyonu ve sinovyal dokuda enflamasyona sebep olur. Eklem sıvısı artar ve eklemde pannus adı verilen şişlik meydana gelir. Eklem dokusundaki enflamasyon hücreleri eklemde kalıcı hasara neden olur. Romatoid artrit hastalarının %60'ının 20 yıl içinde yardımcı araç–gerece ihtiyaç duyan, eklem replasman ameliyatı gerektiren ya da günlük işlerinde başkasına bağımlı duruma gelen bir yaşam sürdürdükleri bilinmektedir. Romatoid artrit genellikle yaşamı tehdit eden bir hastalık olarak bilinmese de, enfeksiyonlar, kardiyovasküler hastalıklar gibi ciddi komplikasyonlarından dolayı ortalama yaşam süresini 5 ila 10 yıl arasında kısalttığı tespit edilmiştir [6].

Romatoid artrit belirtileri kişiden kişiye değişmektedir. Romatoid artritli hastalarda eklem enflamasyonu genellikle kalıcı ve tedavi edilmezse sürekli alevlenme halindedir. Romatoid artrit iştahsızlık, halsizlik, yorgunluk, kilo kaybı ve anemiye sebep olabilmektedir. Romatoid artritli hastaların deri altında romatoid nodül denilen küçük, ağrısız şişlikler ve yumrular görülmektedir. Yumrular vücudun herhangi bir yerinde bulunabileceği gibi basınca daha fazla maruz kalan yerler olan dirsekler ve dizler gibi bölgelerinde sık sık oluştuğu bilinmektedir. Romatoid artritli hastalarda akciğer ve kalp gibi hayati organlarda enflamasyon gelişmekte olup, gözyaşı ve tükürük bezlerinin enflamasyonuna bağlı olarak ağız ve gözlerde kuruluk ortaya çıkabilmektedir. Daha az sıklıkla romatoid vaskülit gelişebildiği de bildirilmiştir [7].

Romatoid Artrit Tanı ve Tedavisi

Romatoid artrit tanısında romatoid faktör (RF) ve anti-siklik sitrüline antikor (anti-CCP) belirteçleri kullanılmaktadır. RF ve anti-CCP, RA için yüksek özgünlüğe sahip olmasına rağmen miktarları diğer bazı hastalıklarda da artabilir. Romatoid artrit hastalarının kanında antinükleer antikorlar (ANA) ve anti-çift sarmallı (anti-ds) DNA antikorları da tespit edilmiştir. Vücutta enflamasyon varlığında değerleri yükselen eritrosit sedimantasyon hızı (ESR) ve C-reaktif protein (CRP) gibi akut faz reaktanlarını kullanarak hastalık aktivitesini değerlendirmek de mümkündür. CRP değerleri, IL-6 ve TNF-a ile ilişkili olduğundan ESR'ye göre daha spesifik sonuçlar sağlar [8].

RA radikal tedavisi olmayan, hastanın yaşam kalitesini iyileştirilmesi için sürekli semptomatik tedavi gerektiren bir hastalık olduğundan tedavide ilk amaç: Fonksiyon kaybını ve eklem hasarını azaltmak ve enflamasyondan kaynaklanan ağrıyı hafifletmektir. RA tedavisi için mevcut çeşitli geleneksel dozaj formları arasında tabletler, kapsüller, oral sıvılar, topikal ürünler, parenteraller, pediatrik/geriatrik ürünler ve transdermal bantlar yer alırken, topikal dozaj formları arasında merhem, krem, jeller veya patlar bulunmaktadır [4].

RA tedavisi, nonsteroidal antienflamatuar ilaçlar (NSAI) ve glukokortikoidlerden modifiye edici antiromatizmal ilaçlara (DMARD) ve modern biyolojiklere doğru ilerler. Uygulanan bu spesifik olmayan tedavilerin potansiyel olarak sıklıkla bağışıklık fonksiyon bozukluğu ile birlikte ileri vadede yaşamı tehdit edici sonuçlara yol açtığı bilinmektedir [9].

Nonsteroidal Antienflamatuar İlaçlar (NSAI'ler)

Nonsteroidal antienflamatuar ilaçlar (NSAI'ler) antienflamatuar etkileri sayesinde RA'nın başlangıç aşamalarında ağrıları azaltır. NSAI'ler etkilerini ağrı ve enflamasyonun ortaya çıkmasında görev alan siklooksijenaz 1 ve 2'yi (COX-1 ve COX-2) bloke ederek gösterir. NSAI'ler ağrıyı hafifletirler, ancak eklem hasarı ve hastalığın gelişimini önlemezler. Bu yüzden genelikle DMARD'larla birlikte kullanılmaktadırlar. Bu grup ilaçlar özellikle uzun süreli kullanımda gastrointestinal hasara ve böbrek fonksiyon bozukluklarına yol açabilmekte ve özellikle COX-2 selektif inhibitörü olanlarda gastrointestinal ve böbrek ile ilgili yan etkiler daha az görülse de kardiyovasküler hastalık riskini artırabilmektedir. Kısaca NSAI'lerin hastanın durumu dikkatli izlenerek düşük dozda kullanılması önerilmektedir [9].

Glukokortikoidler

Prednizolon, deksametazon, hidrokortizon gibi glukokortikoidler, birkaç yolak üzerinden antienflamatuar etkinlik gösterirler. Glukokortikoidler, güçlü antienflamatuar ve immünoregülatör etkilerinden dolayı tedavide kullanılan ilk seçenekler arasındadır, ancak sistemik kullanımları kısıtlıdır. Uzun süreli kullanımda insülin direnci, cilt incelmesi, osteoporoz, hipertansiyon, yara iyileşmesinde gecikme, immün sistemin baskılanması, obezite gibi yan etkilere sebep olabildikleri bildirilmiştir [10]. RA hastalarının yaklaşık %44 ila %75'i tedavilerinin ilk iki yılında glukokortikoid kullanır [11]. Yapılan çalışmalar RA tedavisinde düşük dozlu glukokortikoid kullanımının modifiye edici etkilere sahip olabileceğini göstermektedir. Erken artrit ve RA tedavisine ilişkin Avrupa Romatizma ile Savaş Topluluğu (EULAR) ve Amerikan Romatoloji Koleji (ARC)'nin son tavsiyeleri, mümkün olan en düşük dozda ve mümkün olan en kısa sürede geleneksel sentetik hastalığı modifiye edici antiromatizmal ilaçlara ek tedavi olarak glukokortikoidlerin kullanılması yönündedir [12]. Örneğin prednizolonun yalnız başına kullanımı yerine metotreksat gibi diğer antiromatoid ilaçlarla birlikte kullanımı hem kullanılacak prednizolonun dozunu düşürerek yan etki profilini düşürecek hem de romatoid artrit ilerlemesini yavaşlatacaktır [13]. Glukokortikodlerin uzun süreli kullanımına bağlı olarak hastalarda steroid bağımlılığı, yaşamı tehdit eden hastalıklar ve vaskulit ortaya çıkabilmektedir. Glukokortikoid kullanımı etkilerinin hızlı başlaması ve hastaların semptomlarında rahatlama sağlaması açısından sıklıkla tercih edilirler ancak yarar/risk oranının düşük doz glukokortikoidler kullanımı için bile sistematik değerlendirilmesi gereklidir [12].

Hastalığı Modifiye Eden Antiromatizmal İlaçlar (DMARD'lar)

DMARD'lar (Disease Modifying Anti-Rheumatoid Drugs), romatoid artrite yol açan faktörler

üzerine etkili ilaçlardır. DMARD terimi, romatologlar tarafından, bu gruptaki ilaçları NSAI'lerden (altta yatan nedeni tedavi etmeden enflamasyonu tedavi etmeyi amaçlayan) ve bağışıklık tepkisini zayıflatan ancak hastalığın ilerlemesini durduramayan steroidlerden ayırmak için uzun yıllardır kullanılmaktadır. DMARD'lar, NSAI ve glukokortikoidlerin aksine, eklem yıkımını önleyerek ya da azaltarak hastalığın ilerlemesini değiştirir. Ayrıca DMARD'lar ağrının ve enflamasyonun giderilmesinde direkt etki göstermezler. DMARD'ları sentetik ve biyolojik olarak iki grupta incelemek mümkündür. Şekil 1'de DMARD'ların sınıflandırılması ve her sınıfa ait örnekler görülmektedir. DMARD'lar, ilk kez 1980'lerin sonunda antiromatoid aktiviteye sahip ajanlar olarak kullanılmaya başlanmıştır. DMARD tedavisinin klinik çıktıları 1. aylık kullanımdan sonra ortaya çıkmaya başlatıp 6. aya kadar olan süreçte görülür. Uzun süreli kullanım sonucu klinik etkileri ortaya çıkmaya başladığından yavaş etkili antiromatoid ilaçlar olarak da bilinirler, SAARD'lar (Slow Acting Anti-Rheumatoid Drugs) olarak da isimlendirilirler [14]. Bu yüzden çok sık NSAI'ler veya glukokortikoidlerle birlikte kullanılırlar. Yakın geçmişte, RA teşhisinden sonra önerilen ilk tedavi yöntemi NSAI kullanımıydı, ancak son zamanlarda romatoid artritin çeşitli yönleri üzerine yapılan çok sayıda araştırmadan sonra, DMARD'lar RA tedavisinde modern tedavi olarak ilk tedavi yöntemi olarak kabul edilmektedir [12,15].

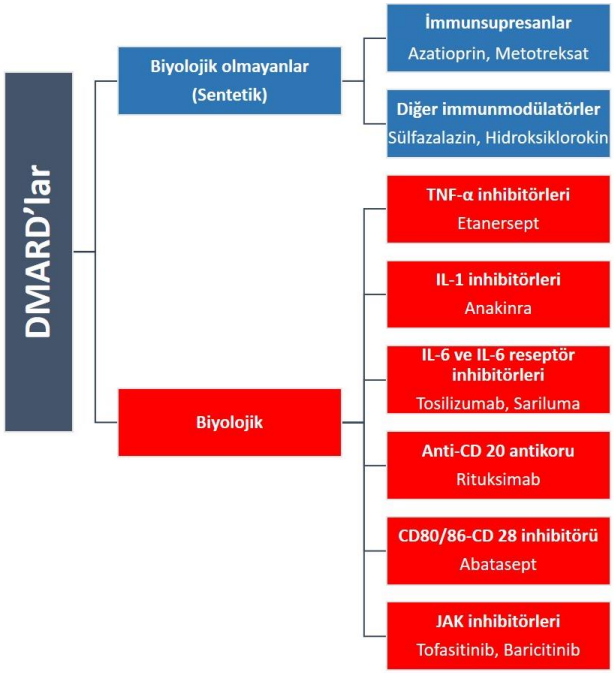
Sentetik DMARD'lar (sDMARDs)

Çeşitli DMARD ajanları mevcut olmakla birlikte nispeten hızlı etki başlangıcı, yüksek etki, düşük toksisite ve düşük maliyetinden dolayı son 20 yıldır ilk aşamada en sık kullanılan sDMARD metotreksattır. Metotreksatın antiromatoid etki mekanizması tam olarak bilinmemekte olup pürin sentezinin inhibisyonu aracılığıyla antimetabolik aktiviteye sahip olduğu düşünülmektedir. Metotreksatın major antiromatoid etkisinin ise ekstrasellüler adenozin salımı ile ilgili olduğu ifade edilmektedir [16]. Hidroksiklorokin, sülfasalazin, leflunomid ve altın tuzları, metotreksat ile kombine veya alternatif olarak kullanılan diğer DMARD'lardır [14]. Metotreksat, uzun süreli tedavide karaciğer sirozuna neden olabilmektedir [17]. Hidroksiklorokin uzun süreli kullanıma bağlı olarak dokularda birikebildiğinden, toksisite ve retina hasarı, korneal opaklık, kızarıklıklar, saçların grileşmesi, irritabl bağırsak sendromu, miyopati, nöropati gibi diğer olumsuz etkilere sebep olabilmektedir [17].

Biyolojik DMARD'lar (bDMARDs)

RA patofizyolojisi, prokaryotik ve ökaryotik hücre kültürlerinde üretilen karmaşık proteinleri temsil eden biyolojik ilaçların geliştirilmesini sağlamıştır. Biyolojik aktiviteler ve taşıyıcı anahtar sitokin veya hücre yüzey molekülü önceden belirlenmiştir. İmmünsupresif aktivitesinin düşük olması nedeniyle daha spesifik terapötik aktivite sağlanır [18]. RA'lı hastalarda klinik kullanım için onaylanan ilk biyolojik ajanlar, tümör nekroz faktörü-α (TNF-α) inhibitörleri olan etanercept, infliximab, adalimumab ve interlökin (IL)-1 reseptör antagonisti olan anakinra'dır. Mevcut biyolojik ilaçlar hedeflerine göre beş gruba ayrılabilir. Bunlar; TNF-α inhibitörleri, IL-1 inhibitörleri, IL-6 ve IL-6 reseptör antagonistleri, B hücresi baskılayıcı ajanlar ve T hücresi kostimülasyon blokörü şeklindedir. TNF-α inhibitörleri, TNF-α reseptörünü bloke ederek şişmeyi azaltırlar ve ağrıyı hafifletirler. IL-1 inhibitörleri, IL-1 reseptörünü bloke ederek eklem hasarını önlerler. IL-6 üzerine etki gösteren tosilizumab, sarilumab gibi ajanlar enflamasyon yanıtını azaltarak etki gösterirler. Anti-CD 20 antikoru, CD-20 reseptörlerine bağlanarak dolaşımdaki B hücresi sayısını azaltır, böylece T hücrelerinin aktive olmasını önler. CD80/86-CD28 inhibitörü abatasept ise CD80, CD86 ve CD28'e bağlanarak T hücrelerinin uyarılmasını azaltır. JAK (Janus-kinaz) inhibitörleri de JAK-1 ve JAK-3'ü inhibe ederek etki gösterirler. JAK çeşitli IL'lerin sinyal iletiminden sorumlu olan sitoplazmik protein tirozin kinazdır. TNF-α blokerleri, zaman zaman göğüs enfeksiyonu ile birlikte enjeksiyon bölgesinde kızarıklık, kaşıntı ve şişmeye neden olur. Anti-TNF ajanlar ciddi bakteriyel enfeksiyon riskini artırmaktadır [19]. IL-1 ise bazı hastalarda şiddetli enfeksiyonlar, WBC ve trombositlerde azalmaya ve enjeksiyon bölgesinde kaşıntı, ağrı ve kızarıklığa yol açabilmektedir. IL-6 inhibisyonu divertikülite, pürülan peritonite, gastrointestinal perforasyonu düşürmeye, fistüle, apseye, nötropeniye, trombositopeniye, hiperlipidemiye, enfeksiyonlara ve karaciğer enzim yükselmesine neden olur. Anti-CD 20 antikorları, baş ağrısı, ateş, deri döküntüleri, nefes darlığı, hipotansiyon, mide bulantısı, rinit, kaşıntı, hafif anjiyoödem ve hipogamaglobulinemiden sorumlu olabilmektedir. Bununla birlikte JAK inhibitörleri

hipotansiyon, mide bulantısı, ishal, LDL, HDL ve toplam kolesterol artışı, ven trombozu, pulmoner emboli, üst solunum yolu enfeksiyonlarına neden olabileceği bildirilmiştir.



Şekil 1. DMARD'ların sınıflandırılması [17]

Verilen bilgilerden anlaşılacağı üzere, RA yaşam boyu tedavi gerektiren bir hastalıktır. Konvansiyonel ve uzun süreli RA tedavileri yukarıda bahsedildiği gibi sistemik yan etkilere neden olmaktadır. Bu tür sorunların üstesinden gelmek amacıyla enflamasyonlu sinovyuma hedeflenmiş ilaç taşıyıcı sistemler üzerine pek çok çalışma yapılmaktadır. Etkin madde yüklü mikro/nanopartiküllerin fizikokimyasal özellikleri, terapötik etkinlikleri ve hedefleme potansiyelleri göz önüne alındığında, RA tedavisi için ilaç taşıyıcı sistemlerin tasarımının önemli fayda sağlayacağı düşünülmektedir.

Romatoid Artritte Hedefleme Yaklaşımları

Enflamasyonlu Doku

RA'daki enflamasyonlu eklemlerin vasküler geçirgenliği, tümörlerdeki artmış geçirgenlik ve tutulma (Enhanced Permeability and Retention-EPR) etkisine benzer şekilde, enflamasyonlu dokuyu pasif hedefleme için uygun hale getirir [20]. RA'daki enflamasyonlu eklemlerde tümörlerinkine benzer şekilde enflamatuar aracılar sürekli olarak aşırı eksprese edilir ve kronik enflamasyon durumunda sızıntılı damarların oluşması ile sonuçlanır. Partridge ve Malik, TNF-α'nın interendotelyal hücre boşluğu oluşumunu indüklediğini ve hücre-hücre dışı matris temaslarını azaltarak endotel geçirgenliğini artırdığını ifade etmiştir. Bu durumun enflamasyonlu dokuların geçirgenliğini artırdığı ve enflamasyonlu dokularda etkin madde yüklü taşıyıcı sistemlerin hedeflendirilmesinde etkili olabilen tümör dokularına göre daha fazla aktive olmuş makrofaj bulunduğu bildirilmiştir. Ayrıca vazoaktif intestinal peptit (VIP) reseptörlerinin, enflamasyon durumunda aktive olmuş sinoviyositlerde aşırı eksprese edildiği gösterilmiştir [20-23].

Pasif Hedefleme

Tümör tedavisinde ilaç taşıyıcı sistemler içine yüklenmiş kemoterapötiklerin tümör dokusunda birikmesi, sistemik toksisitelerinin azaltılması ve terapötik etkinliklerinin artırılması gibi amaçlarla bir pasif hedeflendirme stratejisi olarak EPR etkisinden başarıyla yararlanılmaktadır [24]. Antikorla indüklenen romatoid artrit sıçan modelinde, betametazon yüklü PEGile polimerizomların

enflamasyonlu eklemlerde biriktiği gösterilmiştir. Proenflamatuar sitokin IL-6 ekspresyonu gerçekleştiği ve romatoid artrit semptomlarının hafiflemesini işaret eden artritik skorun azaldığı tespit edilmiştir. *In vivo* görüntüleme çalışmalarında polimerizomların 96 saate kadar enflamasyonlu eklemlerde tutulduğu ve 8 gün boyunca uzatılmış betametazon salımı sağladığı gözlenmiştir [25]. Ulmansky ve arkadaşları, glukokortikoid (metil prednizolon hemisüksinat ve betametazon) yüklü PEGile nanolipozomların terapötik etkinliğini, intravenöz (i.v.) veya subkütan (s.c.) enjeksiyon yolu ile uygulanan serbest glukokortikoidlerle ve biyolojik DMARD'larla (infliximab ve etanersept) karşılaştırmıştır. Her iki etkin madde ile hazırlanan lipozomlar kulanılarak yapılan tedavinin, hastalığın alevlenme döneminde uygulandığında sıçanlarda adjuvan ile indüklenen artrit (AA) şiddetini önemli ölçüde azalttığını göstermişlerdir. Terapötik etki, formülasyonların uygulanmasından 24-48 saat sonra başlamış ve 4-13 gün sürmüştür. Serbest etkin maddeler, 2 ila 25 kat daha yüksek konsantrasyonlarda dahi etkisiz ya da çok hafif ve kısa süreli etkili olup AA skorunu anlamlı olarak azaltmadığı bildirilmiştir [26].

Aktif Hedefleme

Romatoid artritin patofizyolojisinin aydınlatılması, RA'da sağlıklı dokulara göre yüksek seviyelerde olan yeni moleküllerin keşfedilmesini sağlamıştır. Terapötik aktiviteyi artırmak ve sistemik yan ekilerini azaltmak için, enflamasyonlu sinovyumdaki moleküllere seçici olarak ilgisi olan aktif hedefleme ligandları ile konjuge edilmiş çeşitli ilaç taşıyıcı sistemler kullanılabilmektedir. Bu amaçla literatürde folat reseptörleri, anjiyogenez ve integrinler, vasküler endoltelyal büyüme faktörü (VEGF) ve reseptörleri, matriks metaloproteinaz enzimleri, selektinler, vazoaktif intestinal peptit inhibitörü (VIP), immunoglobulin ailesinden Fc-gamma reseptörleri üzerine çalışmalar yapılmıştır [27].

RA hastalarından alınan sinoviyal doku örneklerinin immünohistokimyasal yöntemlerle incelenmesiyle dokuların iç yüzeylerindeki makrofajlarda ve sinoviyal alt katmanda yüksek folat reseptör-β (FRβ) ekspresyonu izlenmiş, T hücre alanlarında veya kontrol sinoviyal dokusunda FRβ izlenmemiştir. FRβ'nın sinovyal doku ekstraktlarında ve RA monosit türevli makrofajlarda en yüksek, periferdeki T hücreleri ve monositlerde ise düşük miktarda olduğu bulunmuştur [28]. RA'da makrofajların aktive olması, hastalığın hem akut hem de kronik evrelerine büyük ölçüde katkıda bulunur. Hem hayvan modellerinde hem de RA'lı hastalarda aktive olmuş makrofajlar üzerinde folat reseptörü ekspresyonunun keşfi, patolojik hücre popülasyonuna bazı ilaçları hedeflemek için folat reseptörlerinin ligandı olan folik asitten yararlanma fikrini desteklemiştir [29]. Folat aracılı hedefleme sağlamak için folik asit; dendrimerler, lipit nanopartiküller ve kitozan nanopartikülleri gibi çeşitli nanotaşıyıcıların yüzeyine konjuge edilerek, etkin madde veya görüntüleme ajanı yüklü sistemlerin RA dokusuna hedeflenmesi sağlanmaktadır [27,30].

Endotel hücreler anjiyogenezde önemli rol oynar. αvβ3 integrinler, aktif olarak farklılaşan endotelyum içinde ve tümör dokusu çevresinde eksprese olmaktadır. Bu anlamlı artış, αvβ3 integrinleri tümöre ulaştırmak için bir hedef haline getirmiştir. Sırasıyla arjinil-glisil-aspartik asit (RGD) peptitleri αvβ3 integrinine bağlanabilir, RGD peptitleriyle veya bu motifi taşıyan peptidlerle konjuge edilen ilaç taşıyıcılar katı tümörlere hedeflendirilmiş ilaç taşıyıcı sistemlerde kullanılabilir. Özellikle siklik konformasyonda dizilen RGD peptidlerin integrine afinitelerinin arttığı bildirilmiştir [31]. Katı tümörler için iyi bilinen bu durum, RA'da enflamasyonu artan dokulara hedeflemede de kullanılabilir. Deksametazon içeren RGD ve PEG ile konjuge edilmiş lipozomların enflamasyonlu dokulardaki endotel hücreleri ile seçici olarak etkileştiği doğrulanmış ve hedeflenen lipozomlar konvansiyonel lipozomlara kıyasla önemli ölçüde yüksek etkinlik göstermiştir [32].

Diğer bir romatoid artrit ilaç taşıyıcı sistemi hedeflendiricisi, enflamasyonlu dokunun vasküler endotelyumunda yüksek oranda adhezyonunu sağlayan e-selektindir. Anti-e-selektin antikorunun romatoid artritli hastalarda karakteristik olduğu gösterilmiştir [33]. Yapılan bir çalışmada e-selektinin endotelyal hücre enflamasyonunun yüzey işaretçisi olduğundan e-selektin hedeflemeli mikropartiküller oluşturarak enflamasyonlu endotele verilmesi ile aterosklerozu önlediği tespit edilmiştir [34].

Başka bir strateji; aktif makrofajların ekspresyonunu artıran hücre yüzeyi reseptörü olan CD-44 bağlayıcı potansiyele sahip hyalüronattır. Hyalüronat kaplı metotreksat yüklü PLGA nanoparçacıkları hazırlanmış ve RAW 264.7 makrofaj hücre hattında çalışılmıştır. Serbest metotreksat hyalüronik asit kaplı nanoparçacıklara göre eklemlerde çoğalan hücrelerden hızlı bir biçimde uzaklaştığından,

hyalüronik asit kaplı nanoparçacıkların hücre proliferasyon fazında daha fazla iyileşme gösterdiği belirlenmiştir [35].

Ligand Bazlı Aktif Hedefleme

Aktif hedefleme, ilaç taşıyıcı sistemin yüzeyinde bulunan bir ligandın, hedef bölgede bulunan reseptörlere bağlanması sonucunda gerçekleşir. Romatoid artrit için aktif hedeflendirme amacıyla folat, vasküler endotelyal büyüme faktörü, vazoaktif intestinal peptit, CD-44, e-selektin, alfa ve beta integrin gibi reseptörler kullanılır. Folik asit, folat reseptörüne yüksek afinite gösterir, bu nedenle anti-romatoid etkin maddeyi enflamasyonlu bölgelere hedeflemek için yüksek afiniteli bir ligand olarak kullanılabilir. PLGA kullanılarak deksametazon fosfat nanopartikülleri hazırlanmıştır. Folik asit reseptörlerini eksprese eden hücrelere hedefleme sağlamak için polietilen glikol (PEG) ile stabilize edilmiş ve folik asit kullanılmış manyetik nanoparçacıklar geliştirilmiştir. Kurkumin yüklü nanopartiküller iki tümör hücre hattında çalışılmıştır. Sonuç olarak folik asit ile hazırlanan nanoparçacıkların kanserin yanı sıra romatoid artrit, sistemik lupus erimatozus gibi enflamasyon ile ilişkili hastalıkların tedavisinde kullanılabilecek terapötik ajanlar oldukları bildirilmiştir [36].

Hyalüronik asit, romatoid artrit hastalarında ekspresyonu artmış CD-44 reseptörüne bağlanan, biyouyumlu doğal bir polisakkarit olup kıkırdak dokudaki sinovyal sıvıda bulunmaktadır. Hyalüronik asiti hidrofobik olarak modifiye etmek için kolanik asit ile birlikte hazırlanan hyalüronik asit nanopartiküllerinin osteoartritli fare eklem kondrositlerinde CD-44 kaynaklı NF-kB aktivasyonunu inhibe ettiği belirlenmiştir [37].

Dekstran sülfat 1-6 bağlı α-D-glukopiranosilden oluşan polianyonik bir polimerdir. Kimyasal modifikasyon için potansiyel olması, immünojen olmaması, toksik olmaması, biyouyumlu ve biyodağılabilir olması gibi özelliklerinden dolayı farmasötik çalışmalarda sıkça kullanılmaktadır. Ülseratif kolit tedavisinde kullanılmak üzere antienflamatuvar ilaçların hedef bölgede salınmasını sağlayarak sistemik yan etkileri azaltmak için resveratrol yüklü PLGA nanoparçacıkları hazırlanmıştır. Sonuç olarak pH duyarlı, midedeki enflamasyon bölgesine hedefli nanopartiküllerin dekstran sodyum sülfat ile indüklenen farelerde oral kullanımı ülseratif kolitin enflamatuar belirteçlerini önemli ölçüde iyileştirmiştir [38].

Peptit bazlı aktif hedeflemede romatoid artritli hücrelerde anjiyogenezden faydalanılır. Anjiyogenez tedavisi için hedef hücreler endotel hücrelerdir. Anjiyojenik endotel hücrelerde aşırı eksprese edilen integrinler vasküler hastalıklarda hedef molekül kabul edilir. TNF-α gibi proenflamatuar sitokinler ile NFκB ve P38MAP kinazın (P38 mitojenle aktive olan protein kinaz) indüksiyonu aracılı IL-1b aktif endotel hücreler aktive olur. Aktif endotel hücreler kemokin (IL-8 ve IL-6) üretir. P38MAP kinaz (P38 mitojenle aktive olan protein kinaz) inhibitörleri kronik enflamasyon tedavisi için umut vaad etmektedir. Ancak yan etkileri nedeniyle kullanımları sınırlıdır. Titanyum dioksit parçacıklarının sıçanlarda kronik enflamasyon ve akciğer tümörü oluşumuna neden olduğu gözlenmiş ve titanyum dioksit nanopartiküllerinin doza bağlı olarak hidroksil radikali oluşumuna yol açtığı belirlenmiştir. Titanyum dioksit nanopartikülleri tarafından indüklenen AP-1 aktivasyonunun (AP-1 upregülasyonu tümörijenez sürecinde önemli role sahiptir), protein kinazları ve p38 kinazı inhibe eden ancak C-Jun N-terminal kinazları (JNKs) inhibe etmeyen spesifik inhibitörler tarafından bloke edileceği belirtilmektedir. P38MAP kinaz ve protein kinazların enflamasyon ve karsinojenez ile ilişkili olduğu bildirilmiştir [39].

Altın nanopartiküllerinin biyouyumluluk, kolay sentezlenme, yüzey modifikasyonunun basit bir biçimde yapılabilmesi gibi özellikleri sayesinde kullanımı yaygındır. Romatoid artrit gibi çeşitli hastalıklarda terapötik ajan olarak kullanılır. Altın nanopartiküllerin romatoid artrit patobiyolojisinde ana rol oynayan vasküler endotel büyüme faktörünü (VEGF) bağlayarak antianjiyojenik etki gösterdiği bildirilmiştir. Altın nanopartiküller güçlü antioksidan özellik göstermektedir. Ayrıca kemik ve kıkırdak erozyonuna neden olan osteoklast oluşumunu indükleyen reseptör aktivatör nükleer faktörü (RANKL) inhibe etmektedir. Altın nanopartiküller, bir hedef molekülü seçici olarak teşhis eden nanoprob olarak da dikkat çekicidir. DMARD'lar arasından metotreksat seçici olmaması nedeniyle potansiyel toksisite özelliklerine sahiptir. Bunun için metotreksat konjuge edilmiş altın nanopartikülleri hazırlanarak adjuvanla indüklenen artritli sıçanlarda değerlendirilmiştir. Altın nanopartiküllerine yüklü nanopartiküllerde immünomodülatör etki, sistemik enflamasyon, aterojenik profil, vasküler reaktivitede

iyileşme üzerine etkisi serbest metotreksata göre çok daha iyi olduğu ve romatoid artrit için kullanılabileceği bildirilmiştir [40].

Manyetik nanopartiküller hem teşhis ve hem de tedavide kullanılabilen avantajlı nanopartiküllerdir. Süperparamanyetik demir oksit nanopartikülleri tanısal görüntüleme ve terapötik uygulamalarda yaygın olarak kullanılmaktadır. Bu sistemlerin romatoid artrit teşhis ve tedavisinde de kullanımı üzerine çalışmalar yapılmıştır. Manyetik nanopartiküller ile enflamasyonlu bölgeye dış manyetik alan uygulanılarak uzun süreli salım ile antienflamatuar etki sağlanabileceği bildirilmiştir. Romatoid artritte meydana gelen EPR etkisi erken teşhis için önem arz eder. Manyetik demir oksit nanopartikülleri biyouyumluluk, düşük toksisite ve FDA tarafından onaylanan tek metal oksit parçacık olması gibi sebeplerden teşhis için kontrast madde olarak kullanılmaktadır [41].

Romatoid Artrit Tedavisinde İlaç Taşıyıcı Sistem Tasarımı

Romatoid artrit semptomlarını gidermek için genellikle oral ve intramüsküler yollar ile sık ve uzun süreli uygulama gerektiren geleneksel tedavi yöntemleri kullanılmaktadır. Bunun sonucunda ekstrasinoviyal birikim ve sistemik yan etkiler görülmektedir. Kortikosteroidlerin yan etkilerini azaltmak için intraartiküler enjeksiyon ile uygulama tercih edilebilmektedir. Ancak eklemlere yapılan tekrarlı enjeksiyonlar oldukça ağrılı olduğundan hasta uyuncu oldukça düşüktür. Bu nedenle kontrollü ilaç salımı sağlayan ve enflamasyonlu sinoviyal bölgede spesifik olarak salım yapan ilaç taşıyıcı sistemlerin hasta uyuncunu iyileştireceği ve sistemik yan etkileri azaltacağı düşünülmektedir [42].

RA tedavisi için ilaç taşıyıcı sistemlerin geliştirilmesinde tümörlü dokulara ilaç hedefleme yaklaşımları yol gösterici olmuştur. Tümör bölgesi ile enflamasyonlu romatoid artrit bölgesi anjiyogenez ve sızıntılı damar sistemi açısından benzerlik gösterdiğinden, tümör taşıyıcı sistemleri için kullanılan yaklaşımların romatoid artrit taşıyıcı sistemleri için de kullanılabileceği anlaşılmıştır. Romatoid artrit tedavisinde kullanılacak taşıyıcılar geliştirilirken taşıyıcı tipi dışında; partikül büyüklüğü, yüzey özellikleri, dolaşımda kalış süresi gibi faktörler önem taşımaktadır [9].

Partikül Büyüklüğü

Partikül büyüklüğünü küçültmek biyoyararlanım açısından ilaç taşıyıcı sistemler için önem arz etmektedir. İntravenöz uygulamalarda kan dolaşımında kalma, hedef dokuya taşınma, hücresel alım gibi pek çok aşamada partikül büyüklüğü kritik rol oynamaktadır. Makrofaj gibi immün hücreler yabancı hücre ve partikülleri fagosite ederek vücuttan uzaklaştırılmasını sağlarlar. Çalışmalar sinoviyal fagositik hücrelerin gerçekleştirdiği fagositozun partikül boyutuna bağlı olduğunu göstermiştir [9]. P-selektin hedefli ligandlarla kaplanmış 5-20 μm çapındaki mikroküreler hazırlanmış ve parçacıkların hedef yüzeyi tutmasında partikül büyüklüğü arttıkça akış altında adezyonun azaldığı bildirilmiştir [43]. Brownian hareketin de bir sonucu olarak çapı 500 nm altındaki partiküllerin akış odasına doğru lokalizasyonunun 500 nm çapından büyük parçacıklara göre daha iyi olduğu bildirilmiştir [44].

Enflamasyonlu ekleme pasif taşıma için de partikül büyüklüğü önemlidir. Romatoid artritin anjiyogenez ve enflamasyon süreci anormal ve sızıntılı damar açısından tümörlere benzerdir. Bu durum ekstravazasyon yoluyla sinoviyal sıvılara sızmak için serum proteini gibi makromoleküllere izin verir. Artmış permeabilite ve retansiyon (EPR etkisi) olarak bilinen bu durumdan enflamasyonlu sinovyuma pasif hedeflendirme için faydalanılır. Nanotaşıyıcılar serbest ilaçlar ile karşılaştırıldığında enflamasyonlu sinovyum içinde daha fazla birikim göstermektedir.

Sistemik uygulanan ilaçların hedef bölge olan eklemlere ulaşmadan diğer doku ve organlara da dağılarak eklem bölgelerine düşük konsantrasyonlarda ulaşmaları ilaç dozunun da artırılmasını gerektirip yan etkileri artırmaktadır. Yan etkileri azaltmak için lokal uygulamalar uzun süredir kullanılmaktadır. İlaç taşıyıcı sistemler kullanılarak sürekli/kontrollü ilaç salımının sağlanması enjeksiyon sıklığını azaltarak hasta uyuncunun artışını sağlayacaktır.

Nanopartikül Şekli

İlaç taşıyıcı sistem olarak mikro ve nanopartiküller, enerji yönünden en stabil geometriyi temsil eden küresel şekilde tasarlanırlar. Bir dizi çalışma mikro ve nanopartiküllerin fiziksel şekillerinin ilaç taşıyıcı sistemlere etkisini, makrofajlar tarafından fagositoz oranı, kan dolaşımındaki akış dinamiği, hedeflendirme yeteneği, hücresel alım ve hücre içi dağılım yönlerinden incelemiştir. Romatoid artrit

hedefli ilaç taşıyıcılarda partikül şeklinin etkisi hakkında herhangi bir rapor bulunmamasına rağmen, romatoid artrit tedavisi için mikro ve nano parçacıkların tasarımında partikül şekli önemli bir parametre olduğu düşünülmektedir. Şekil ile ilgili çalışmaların çoğu tümöre ve vasküler endotelyuma ilaç ulaştırma etkinliğini artırmayı amaçlamaktadır. Romatoid artrit sızıntılı damar sistemi, asidoz, hipoksi ve hedefleme için spesifik moleküller açısından tümörlere büyük ölçüde benzemektedir. Bu nedenle, romatoid artrit terapötiklerinin hedeflemesi ilaç taşıyıcıların şekil özellikleri dikkate alınarak önemli ölçüde kolaylaşabilir. Küresel olmayan şekil tümörlerdeki EPR etkilerine bağlı olarak daha yüksek birikime yol açabilir [45]. Örneğin, uzun-ince partiküller, küresel partiküllere kıyasla daha yüksek temas yüzey alanı ve buna bağlı olarak daha yüksek hedefleme kabiliyeti sergilerken, sfenoid, elipsoid, çubuk ve diskler gibi parçacıklar kesme akışındaki dönme hareketlerinden dolayı yanal kaymaya maruz kalır [44]. Ayrıca endositoz ve hücre içine dağılımda uzun-ince partiküllerin, küresel partiküllere göre daha yavaş hücre içine alındığı belirlenmiştir [46].

Partiküllerin şekli makrofaj ile etkileşimde belirgin bir role sahiptir. Küresel partiküllerin makrofajlar aracılığı ile fagositozunun partikül büyüklüğü ile ilgili olduğu, eğer partikül büyüklüğü makrofaj büyüklüğünden küçükse fagositoz olayının gerçekleştiği ifade edilmiştir. Eliptik disk şeklindeki parçacıkların belirli bir oryantasyonda fagositozu önlediği gösterilmiştir. Solucan şeklinde partiküller tasarlanmış ve aynı hacimdeki küresel partiküllere kıyasla önemli ölçüde daha düşük fagositoz sergilediği gösterilmiştir [47].

Dolaşımda Kalış Süresi

İntravenöz yoldan verilen yabancı partiküllerin çoğu özellikle karaciğer ve dalakta immün sistem tarafından elimine edilmektedir. Opsoninlerin partikül yüzeyine adsorbsiyonu karaciğer ve dalaktaki makrofajlar tarafından yabancı madde olarak tanınmasını sağlamakta, opsonizasyonu fagositoz ve eliminasyon takip etmektedir [48]. İmmün sistem fonksiyonlarının azalması partiküllerin kan dolaşımında bulunma süresini uzatmaktadır. Dolaşımda kalış süresi, partikül yüzeyine opsoninin adsorpsiyonunu önleyen hidrofilik yapı kazandırılarak ve yüzey yükü azaltılarak sağlanabilmektedir. Yaygın olarak kullanılan yüzey modifikasyon stratejisi partiküllerin yüzeyine polietilen glikolün bağlanmasıdır (PEGilasyon). Partikül yüzeyinde PEG'in yoğunluk, kalınlık ve konfigürasyonu gibi özellikleri opsonizasyonun etkili bir şekilde engellenmesini sağlamaktadır [49]. Romatoid artit tedavisinde PEGlenmiş ve PEGlenmemiş partiküller karşılaştırıldığında PEGlenmiş partiküllerin enflamasyonlu sinovyumda daha fazla biriktiği, karaciğer ve dalakta daha az alimine edildiği yapılan çalışmalarda gösterilmiştir [50].

Uyaran Duyarlı Özellikler

Romatoid artritte asidoz ve hipoksi iki önemli patofizyolojik değişim olarak bilinmektedir [45]. Bu da tümör hedefli ilaç taşıyıcı sistemler için kullanılan pH'a duyarlı ilaç salımı ve dePEGilasyon yöntemlerinin romatoid artrit için hedefli ilaç taşıyıcı sistemlerde de kullanılabileceğini göstermektedir. Hipoksik bölgede tümör bölgesindeki ilaç salımına benzer bir strateji geliştirilebileceği düşünülmektedir [45,50]. Romatoid artrit tedavisinde kullanılmak üzere glukokortikoid yüklenmiş PEG-lipozom yapısında bir ilacın hedef bölgelere erişilebilirliğinin iyi olduğu gösterilmiştir [51].

Romatoid Artrit Tedavisinde İlaç Taşıyıcı Sistemler

Terapötik etki için ilaçların yüksek dozlarda tüketilmesi gerekmektedir. Dozlardaki bu artış istenmeyen etkilere sebep olabilmektedir. Klinik kullanım için onaylanmış birçok nanopartikül formülasyonu bulunmaktadır. Bu nanopartiküller terapötik tedavi ve teşhis amaçlı kullanılmaktadır [52]. Nanopartiküller ilacın dolaşımda kalma sürelerini artırabilir ve kontrollü salım sağlayabilir. Uygun modifikasyonlarla ilaç yüklenen nanopartiküller enflamasyonlu dokular da dahil olmak üzere hedeflendirme yapabilmektedir [53]. Bu amaçla nanopartiküllerin yüzeyi onları istenen enflamasyonlu doku veya organa yönlendirmek için peptit, antikor, protein veya küçük moleküllü bir madde ile modifiye edilebilmektedir [54]. Terapötik amaçla ilaç hedeflendirmesi için kullanılan en yaygın nanopartikül türleri lipozomlar, polimerik nanopartiküller, nanomiseller, nanoemülsiyonlar ve dendrimerlerdir [55].

Polimerik nanopartiküller biyolojik olarak parçalanabilir, biyouyumlu, minimal düzeyde immünojenik ve hedeflemeye uygundur [56]. Romatoid artrit modeli oluşturulmuş hayvanlarda polimerik nanopartiküllerin kullanımının biyouyumluluk, kimyasal stabilite, kontrollü ilaç salımı ve enflamasyonlu dokulara seçici ilaç salımı açısından tedavide kullanılabileceği bildirilmiştir [57]. Yapılan başka bir çalışmada makrofajların intrinsik hedefleme kapasitesinden yola çıkarak romatoid artriti hedeflemek için makrofaj kaynaklı mikroveziküller elde edilerek, takrolimus yüklü PLGA nanopartikülleri kaplamak için kullanılmış, elde edilen nanopartiküller, kırmızı kan hücre membranı ile kaplanmış nanopartiküller ve kaplı olmayan nanopartiküllerle kıyaslamalı olarak incelenmiştir. Geliştirilen nanopartiküllerin enflamasyonlu HUVEC hücrelerine bağlanma yetenekleri kırmızı kan hücre membranı ile kaplanmış nanopartiküllere göre güçlü bulunmuş ve kollajenle indüklenen artrit modeli oluşturulmuş farelerde makrofaj kaynaklı mikrovezikül kaplı nanopartiküller, kaplanmamış nanopartiküllere göre daha iyi hedefleme etkisi göstermiştir. Makrofaj kaynaklı mikrovezikül kaplı nanopartiküllerin farelerde romatoid artrit semptomlarının ilerlemesini önemli ölçüde engellediği tespit edilmiştir [58].

Romatoid artritte makrofajlar, immün/enflamatuar cevaba aracılık eden M1 (proinflamatuar) ve M2 (antiinflamatuar) fenotiplere dönüşebilir. Enflamasyonlu sinovyumda ve eklemlerde bol miktarda aktif M1 fenotip makrofajları bulunur. Ayrıca folat reseptörlerinin aşırı ekspresyonu da gözlenir ki bu iki faktör hastalığın ilerlemesinde rol oynar. Folat reseptör hedefli germakron yüklü PLGA-PEG nanopartikülleri, hedeflendirilmemiş nanopartiküllere göre RAW 264.7 hücreleri tarafından daha fazla hücre içine alınarak M1 hücrelerinin M2 hücrelerine dönüşümünü desteklemiştir. Adjuvan kaynaklı artrit modeli oluşturulmuş sıçanlarda yapılan çalışmada, serbest germakron ve folat reseptör hedefli olmayan nanopartiküle yüklenmiş germakrona göre, folat reseptörüne hedeflenmiş germakron yüklü nanopartiküllerin enflamasyonlu bölgede daha çok biriktiği gözlenmiştir [59].

Lipozomlar, terapötik maddelerin verilmesi için taşıyıcı olarak yaygın şekilde kullanılan fosfolipit yapılı küresel nanoveziküllerdir. Lipozomlara hem hidrofilik hem hidrofobik ilaçlar yüklenebilmektedir. Enkapsüle edilen etkin maddeyi belirlenen hedeflerde serbest bırakabilmektedir [60]. Yapılan bir çalışmada serbest kannabidiol ve kannabidiol yüklenmiş lipozom formülasyonların osteoartritli köpek modellerinde randomize plasebo kontrollü çift kör çalışması yapılmıştır. Lipozomal formülasyonun serbest formülasyona göre ağrıyı önemli ölçüde azaltarak hareketliliği artırdığı belirlenmiştir [61]. Başka bir çalışmada tofasitinib sitratın etkinliğini artırmak ve enflamasyonlu eklemlere hedefleme sağlamak amacıyla lipozomlara pH gradyanı yöntemiyle tofasitinib sitrat yüklenmiş ve lipozomal sistemin serbest tofasitinib sitrata kıyasla enflamasyonlu hücreler tarafından daha çok alınarak artritik pençelerde daha fazla birikim gösterdiği, enflamatuar sitokin ekspresyonunu ve lipit peroksidasyonunu azalttığı gösterilmiştir [62]. Yapılan başka bir çalışmada ise triptolid yüklenmiş folat reseptörüne hedefli lipozomlar geliştirilmiştir. RAW 264.7 hücrelerinde hedeflendirilmiş lipozomlar enflamasyonlu pençelerde seçici birikim göstermiştir [63].

Nanoemülsiyonlar tipik olarak yağ ve su olmak üzere iki karışmaz sıvının dağılmasıyla hazırlanır ve uygun bir yüzey aktif madde kullanılarak stabilize edilir. Antijen ile indüklenen artrit oluşturulmuş tavşanlarda yapılan bir çalışmada metotreksat yüklü nanoemülsiyonların artritik tavşan eklemlerinde, kontrol grubu tavşan eklemlerine göre 2 kat daha fazla tutulum sergilediği gözlenmiştir [64]. Yapılan bir çalışmada rapamisin içeren nanoemülsiyon formülasyonu tasarlanmıştır. Nanoemülsiyon formülasyonu, intravenöz yol ile uygulandığında enflamasyonlu pençelerde serbest ilaca göre daha iyi tutulum göstermiş ve daha yüksek antienflamatuar etki sağlanmıştır [65]. Başka bir çalışmada romatoid artrit için yüzyıllardır kullanılan arı venomunun cilt üzerine uygulanan nanoemülsiyon formülasyonları hazırlanmıştır. Kollajen ile indüklenen artrit sıçan modelinde topikal olarak uygulanan nanoemülsiyonun kontrol grubuna göre pençe enflamasyonunu azalttığı gözlenmiştir [66]. Diğer bir çalışmada ise diflunisal ile nanoemülsiyon formulasyonları hazırlanmış ve karboksimetil selüloz sodyum, sodyum aljinat ve ksantin zamkı olmak üzere üç farklı jelleştirici ajan kullanılarak nanoemülsiyon jel formülasyonları geliştirilmiştir. Karragenin kaynaklı pençe ödemi modeli, histamin kaynaklı pençe ödemi modeli ve formalin kaynaklı pençe ödemi modeli oluşturulan hayvanlara yapılan uygulamaların, ksantin zamkı ile formüle edilmiş nanoemülsiyonun tüm modeller için en iyi antienflamatuar etkiyi yarattığı bildirilmiştir [67].

Nanomiseller amfifilik monomerler ile kendiliğinden oluşan kolloidal yapılardır. Hidrofobik çekirdekleri hidrofobik ilaçları kapsüllerken hidrofilik kabukları ilacın çözünürlüğünü artırmaya yardımcı olur. Nanomisellerin yüzeyi hücre/doku hedefleme ligandları ile modifikasyonlara uygundur [68]. Yapılan bir çalışmada hidroksiklorokin ve metotreksat yüklü nanomiseller geliştirilmiş ve romatoid artrite karşı terapötik etkileri değerlendirilmiştir. Nanomisellerin romatoid artrit sürecini modüle edebildiği, ayrıca osteoklastogenezi, ödemi ve ekleme hücre göçünü azaltabildiği, serbest formülasyonlara göre nanomisellere yüklenmiş formülasyonların 2 kat daha yüksek terapötik etki gösterdiği bildirilmiştir [69]. Başka bir çalışmada pentasiklik bir triterpen olan celastrol, dekstran sülfat ile matriks metalloproteinaz-2 (MMP-2) duyarlı peptit aracılığıyla bağlanarak nanomisel yapıda ön ilacı hazırlanmıştır. Enflamatuar eklemdeki aşırı MMP-2 üretimi nedeniyle, celastrol salımının bu bölgede tetiklenmesi amaçlanmıştır. Nanomisellerin serbest celastrole göre enflamasyon bölgesinde daha iyi celestan salımı sağladığı böylece daha iyi antiromatoid etkiye ve daha düşük sistemik toksisiteye sahip olduğu gözlenmiştir [70].

Dendrimerler bir çekirdek ve etrafında dallanmış tekrarlı polimerik gruplar ile yapıya fonksiyon kazandıran yüzey gruplarından oluşan nano boyutlu küresel yapılardır [71]. Kimyasal modifikasyonlara uygun yapıda olmaları sayesinde molekül ağırlıkları, partikül büyüklükleri, şekil-spesifik karakteristikleri, çözünürlük, stabilite ve biyouyumluk gibi özellikleri çeşitlilik gösterir [72]. Yapılan bir çalışmada apoptozu tetiklemenin yanı sıra makrofajlarda enflamatuar yanıtı inhibe etmek üzere miR-23b'yi iletmek için florlanmış poliamidoamin dendrimerleri kullanılmıştır. Romatoid artrit modelinde dendrimerlerin intravenöz enjeksiyonunu takiben nanopartiküler enflamatuar yanıtın inhibisyonu, azalmış kemik ve kıkırdak erozyonu, sinoviyosit infiltrasyonunu baskılama ve hareketiliğin geri kazanılması ile terapötik etkinlik göstermiştir. Ayrıca sinovyumda mi-R-23b ekspresyonunun restorasyonu sağlanmış ve toksisiteye neden olmamıştır [73]. Bir başka çalışmada antienflamatuar etki sağlamak için anti-TNF-α antikorları ile kaplı, kondroitin sülfat ile işlevselleştirilmiş poli(amidoamin) dendrimerler geliştirilmiş, geliştirilen dendrimerlerin ATDC5 ve THP-1 hücre hatlarının metabolik aktivitesini ve proliferasyonunu etkilemediği, sitouyumlu ve hemouyumlu olduğu ayrıca uygun TNF-α yakalama kapasitesi göstermesi sebebiyle romatoid artrit hastalarında yeni immünoterapiler için kullanılabileceği ifade edilmiştir [74]. Aynı araştırıcılar tiramin gellan zamkına geliştirdikleri dendrimerleri yükleyerek insan 3D enflamatuar kıkırdak modelinde etkinliğini değerlendirmişlerdir. 14 gün boyunca artan antienflamatuar etki ortaya çıkmıştır. Ayrıca CoII tipII'nin anti-TNF-α dendrimer nanopartikülleri ile enflamasyonlu hCH hücreleri tarafından yüksek oranda eksprese edilerek hCH hücrelerinin biyolojik fonksiyonunu korumasına katkı sağladığı gözlenmiştir [75].

SONUÇ VE TARTIŞMA

Verilen bilgiler ışığında, birçok çalışmada gösterildiği gibi çeşitli ilaç taşıyıcı sistemlerin romatoid artrit tedavisinde geleneksel tedavi yöntemlerine alternatif olarak kullanılması hastalığın semptomlarının önlenmesi ve hafifletilmesi açısından oldukça olumlu sonuçlar ortaya koymuştur. Romatoid artrit tedavisi için ilaç taşıyıcı sistem tasarlanırken temel olarak kanser tedavisinde uygulanan stratejiler pasif hedeflendirme için faydalı olmaktadır. İlaç taşıyıcı sistem tasarımında, taşıyıcı tipi ve özellikleri, partikül büyüklüğü, partikül şekli, yüzey özellikleri, dolaşımda kalış süresinin dikkate alınması gerekmektedir. Son yıllarda kanser tedavisi için oldukça ilerleme kaydedilen pH, hipoksi, redoks potansiyeli gibi internal uyaranlara duyarlı sistemlerin geliştirilmesi romatoid artrit tedavisinde yan etkilerin azaltılmasına ve hasta uyuncunun artırılmasına önemli katkılar sağlayacağı düşünülmektedir. Çeşitli hedefleme yaklaşımlarından aynı anda faydalanılması ile gelecek yıllarda kanser tedavisinde olduğu gibi romatoid artrit tedavisinde de umut verici gelişmeler olacağı düşünülmektedir.

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DERLEME MAKALE / REVIEW ARTICLE

LOKALİZE MEME KANSERİ TEDAVİLERİNDE EFEKTİF İLAÇ TAŞIYICI SİSTEMLER: ENJEKTABL HİDROJELLER

EFFECTIVE DRUG DELIVERY SYSTEMS IN LOCALIZED BREAST CANCER THERAPIES: INJECTABLE HYDROGELS

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ÖZ

Amaç: *Meme kanseri dünya genelinde kadınlarda en sık gözlenen kanser türü olup, erken teşhis ve etkili tedavi stratejilerinin geliştirilmesi için sürekli araştırmaların yapılmasını gerektiren kritik bir sağlık sorunudur. Geleneksel kemoterapi uygulamalarındaki spesifik olmayan hedefleme, sistemik toksisite, ilaç direnci, kısıtlı ilaç penetrasyonu gibi sınırlamaların aşılmasında yenilikçi tedavi yöntemlerinin geliştirilmesine ihtiyaç duyulmaktadır. İlaç taşıyıcı sistemler olarak enjektabl hidrojeller biyoparçalanır, biyouyumlu, tasarıma yönelik ayarlanabilir fizikokimyasal özelliklerinin yanı sıra etkin maddenin yüksek verimlilikte yüklenmesini ve salımını sağlayabilmesi dolayısıyla lokal kanser tedavilerinde ön plana çıkmaktadır. Enjektabl biyoparçalanır hidrojeller özellikle cerrahi sonrası tedavi sürecinde tümör nüksünü ve metastazını önlemede kritik öneme sahiptir. Bu derlemede enjektabl hidrojellerin yapıları, türleri, kanser tedavilerine ilişkin uygulamaları ve antikanser tedavi etkinliklerinin değerlendirilmesi amaçlanmıştır.*
Sonuç ve Tartışma: *Bu derlemede farmasötik ilaç taşıyıcı sistemler olarak enjektabl hidrojel yapıları, meme kanseri tedavilerine ilişkin uygulamaları ve meme kanserine yönelik antikanser tedavi etkinlikleri ele alınmıştır.*
Anahtar Kelimeler: *Enjektabl hidrojel, ilaç taşıyıcı sistem, lokal tedavi, meme kanseri*

ABSTRACT

Objective: *Breast cancer is the most common cancer in women worldwide and is a critical health problem that requires continuous research for early detection and development of effective treatment strategies. There is a need to develop innovative treatment modalities to overcome the limitations of conventional chemotherapy such as non-specific targeting, systemic toxicity, drug resistance and limited drug penetration. As drug delivery systems, injectable hydrogels have come to the forefront in local cancer treatments due to their biodegradable, biocompatible, design-adjustable physicochemical properties as well as their ability to provide highly efficient loading and release of the active substance. Injectable biodegradable hydrogels are critical in preventing cancer recurrence and metastasis, especially in the post surgical treatment process. In this review, we aimed to evaluate the structures, types, cancer treatment applications and anticancer therapeutic efficacy of injectable hydrogels.*
Result and Discussion: *In this review, the structures of injectable hydrogels as pharmaceutical drug delivery systems, their applications in breast cancer treatments and their anticancer*

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therapeutic efficacy for breast cancer were discussed.
Keywords: *Breast cancer, drug delivery system, injectable hydrogel, local therapy*

GİRİŞ

Kanser günümüzde en yüksek mortalite oranına sahip hastalıkların başında gelen dünya çapında önemli bir halk sağlığı sorunudur. Meme kanseri, kanser türleri arasında global onkoloji alanında karşılaşılan en yaygın malignitelerden biri olup kadınlar arasında kanser ilişkili mortalite oranlarını gösteren belirleyici bir faktördür. Demografik parametreler olarak kan grubu ve yaş aralığı, hamilelik ve menopoz gibi üreme ile alakalı faktörler, endokrin ve genetik belirteçler, yaşam tarzıyla ilişkili unsurlar ve advers çevresel faktörler bu patojenezde önemli risk unsurları olarak değerlendirilmektedir [1].

Meme kanseri tedavisinde kullanılan yöntemler arasında adjuvan ve neoadjuvan kemoterapi gibi konvansiyonel stratejilerin yanı sıra cerrahi müdahaleler, radyoterapi, hormon duyarlılığı olan kanser türlerinde hedefleyici uygulamalar ve moleküler tedavi yaklaşımları yer almaktadır [2]. Meme kanserine yönelik tedavilerde, erken teşhisin sağlanması ve yenilikçi tedavi yaklaşımlarının geliştirilmesi kanserin yol açtığı sağlık yükünü önemli ölçüde azaltma potansiyeline sahiptir [3]. Hastalığın özgül yapısı ve çeşitliliği nedeniyle, hastaların kanser karakteristikleri ve kanser evreleri göz önünde bulundurularak mevcut tedavi yaklaşımları kişiye özgü olarak optimize edilmelidir. Geleneksel yöntemler olarak radikal mastektomi ve modifiye radikal mastektomi gibi cerrahi yaklaşımlar, lokal tedaviler için esas teşkil ederken günümüzde meme koruyucu cerrahi prosedürler ve buna eşlik eden radyoterapi de daha yaygın bir tercih haline gelmiştir. Bu tedavi yaklaşımlarına göre lokal olarak ileri evre ve cerrahi müdahaleye uygun meme kanseri vakalarında neoadjuvan kemoterapi kritik bir rol oynamaktadır [4]. Cerrahi rezeksiyon, kemoterapi ve radyoterapi meme kanseri tedavilerinde kullanılan başlıca yöntemlerdir [5]. Özellikle cerrahi rezeksiyon uygulamaları, kanser vakalarında hastalığın tekrarlanmasını önlemek amacıyla geleneksel kemoterapi tedavileriyle desteklenebilmektedir [6]. Fakat geleneksel neoadjuvan kemoterapi uygulamalarında, antikanser ajanlardan kaynaklanan şiddetli yan etkiler tedavi etkinliğini düşüren önemli bir kısıtlayıcı faktördür [7]. Kansere yönelik uygulamalarda, karşılaşılan mevcut sorunların aşılması, daha verimli ve etkili bir tedavinin sağlanması için bu amaç doğrultusunda yeni ilaç taşıyıcı sistemlerin geliştirilmesi ve uygulanması önem arz etmektedir.

Nanopartiküler ilaç taşıyıcı sistemler, düşük toksisiteye sahip olmaları, güvenli ve etkili kontrollü salım sağlayabilmeleri, aktif veya pasif hedeflemeye olanak tanımaları, ilaç direncine karşı tedavi etkinliğini arttırabilmeleri dolayısıyla kanser tedavilerinde yaygın olarak kullanılmaktadır [8]. Birçok avantajına rağmen, nanopartikül temelli antikanser tedavilerdeki sistemik uygulamalar da sınırlı tedavi etkinliğine sahip olabilmektedir. Bu sınırlılıklar, ilaç taşıyıcı sistemlerde karşılaşılabilen ilk çıkış etkisi ve spesifik olmayan etkileşimlerin yanı sıra tümör heterojenitesi ve doku bariyerleri kaynaklı kısıtlamalardan kaynaklanabilmektedir [9]. Özelikle lokal kanser tedavileri için düşünüldüğünde, kemoterapötik ajanların küçük boyutlarından dolayı hedef bölgeden hızlı bir şekilde eliminasyona uğramaları, uzatılmış lokal etkiyi sağlamada sınırlı kapasiteye sahip olmaları dezavantaj oluşturabilmektedir [10]. Bu aşamada hidrojel temelli ilaç taşıyıcı sistemler, etkili bir lokal tedavinin gerçekleştirilmesinde ön plana çıkmaktadır. Üç boyutlu ağ yapıları, yüksek su tutma kapasiteleri, gözenekli ve geçirgen yapıları, biyouyumlu ve biyoparçalanır özellikleriyle farmasötik uygulamalarda umut vadetmektedirler [11]. Hidrojeller, etkin maddenin sistemik dağılımdan kaynaklı oluşabilecek ciddi yan etkileri önlemesi, hedef bölgede kalma süresini uzatması, tümör dokusunda etkili bir dağılımını sağlaması dolayısıyla terapötik verimliliği yükseltmesi bakımından önemli avantajlar sağlamaktadır [12]. Hidrojellerin bütün temel karakteristik avantajlarına sahip olan enjektabl hidrojeller, birçok farklı terapötik uygulamada kullanılabildığı gibi [13-16] özellikle kemoterapötik ajanların cerrahi ve implantasyon işlemleri olmaksızın, enjeksiyon yoluyla hedef bölgeye lokal olarak doğrudan uygulanmasını sağlayarak kontrollü salımı mümkün kılmaktadır [17,18]. Enjektabl hidrojeller, sağladığı avantajlar dolayısıyla lokal kanser tedavilerinde ve cerrahi sonrası tümör nükslerinin önüne geçilmesinde önemli ve işlevsel taşıyıcı sistemlerdir [19].

Bu derlemede farmasötik ilaç taşıyıcı sistemler olarak enjektabl hidrojellerin yapıları, meme kanseri tedavilerine ilişkin uygulamaları ve meme kanserine yönelik antikanser tedavi etkinliklerinin

değerlendirilmesi amaçlanmıştır.

Hidrojellerin Sınıflandırılması

Kanser tedavisine yönelik uygulamalar için yüksek terapötik etkinliğe ve verimliliğe sahip enjektabl hidrojel sistemlerinin eldesinde, biyouyumluluk, şişme oranı, geçirgenlik, mekanik dayanıklılık ve salım profili gibi karakteristik özellikler göz önünde bulundurulmalıdır. Biyouyumlu özellikteki hidrojel yapılarının, uygulama bölgesindeki parçalanma ve dağılımı sonrasında, herhangi bir akut veya kronik fizyolojik tepki ve toksisite oluşturmaması hedeflenmelidir [20]. Birçok farklı yöntem ve malzeme ile elde edilebilen hidrojeller, hammadde tipine, bileşen yapılarına, çapraz bağ türüne, elektrik yüküne, boyutlarına, şişme özelliklerine, mekanik özelliklerine, uyaran duyarlılıklarına göre sınıflandırılabilmektedir [21,22]. Bu derlemede hidrojel türleri, Şekil 1'de belirtildiği üzere bileşenlerine ve uyaran duyarlılıklarına göre incelenmiştir.

Şekil 1. Hidrojellerin bileşen yapılarına ve uyaran duyarlılığına göre sınıflandırılması

Bileşenlerine Göre Hidrojel Türleri

Doğal Polisakkarit Polimer Hidrojeller

Doğal polisakkarit polimerler, bitkilerden, hayvanlardan ve mikroorganizmalardan olmak üzere çok çeşitli kaynaklardan elde edilebilmektedir. Ekstraselüler matriks ile benzer yapıda olmaları, yüksek biyouyumluluk özellikleri ve yüksek su tutma kapasitelerinin yanı sıra hedeflenen mekanik parametrelere göre modifiye edilebilme özelliklerine sahiptirler [23]. Doğal polisakkarit polimer hidrojeller, aljinat, dekstran, gellan, hyalüronik asit, kitozan, nişasta ve selüloz yapılarıyla elde edilebilmektedir [21-30]. Lokalize kanser tedavisinin amaçlandığı bir çalışmada, immünojenik hücre ölümünü indükleyen kemoterapötik ajan oksaliplatin ve doksorubisin (DOX) etkin madde olarak kullanılmış, immün adjuvan R837 ile "kokteyl" kemoimmünoterapötik bileşikler tasarlanarak aljinat ile formüle edilmiştir. Kokteyl terapötik solüsyon, lokal tümör enjeksiyonunu takiben endojen kalsiyum iyonlarının varlığında Aljinat-Ca2+ hidrojelleri oluşturarak, terapötik bileşenlerin tümör içinde tutulmasını ve kontrollü salınmını sağlamıştır. Çalışmada lokalize kemoimmünoterapinin primer tümörleri ortadan kaldırabildiği, çevre dokularda tümör oluşumunu inhibe edebildiği ve ayrıca CT26 kolon kanser tümör modelinde tümör nüksünü önleyebildiği gösterilmiştir [28]. Oksaliplatin konjuge G5 poliamidoamin ve oksitlenmiş dekstrandan oluşan nanopartikül-hidrojel yapıdaki nanokompozit enjektabl hidrojel formülasyonun, *in vivo* modellerde üç haftadan daha uzun süre hedef bölgede kalabildiği, 4T1 meme kanseri ortotopik primer tümörlerine karşı yüksek inhibisyon etkisi sağladığı,

lenf düğümlerine ve akciğerlere metastazı önemli ölçüde azalttığı gösterilmiştir [24]. Doğal hidrojeller, birçok avantajına rağmen zayıf mekanik özelliklere sahip olması, düşük fiziksel ve kimyasal stabiliteye sahip olmaları dezavantaj oluşturabilmektedir. Bu olumsuz özelliklerini geliştirmek amacıyla doğal polimerler modifiye edilerek veya sentetik polimerlerle beraber kullanılabilmektedir [31]. Bu türden iyileştirmenin yapıldığı bir çalışmada PCL-PEG-PCL (PCEC) nanopartikülleri ile enkapsüle edilen paklitaksel (PTX), epirubisin-hyalüronik asit hidrojeli karıştırılarak formülasyon (PPNPs/EPB-HA Jel) elde edilmiştir. Bu enjektabl hidrojel formülasyonunda, 4T1 meme kanseri modelinde sistemik toksisite olmaksızın her iki terapötik ajanın da kontrollü salımı sağlanmış ve sinerjistik etki sonucunda tümör nüksünün ve akciğer metastazının etkili bir şekilde önlediği bildirilmiştir [27].

Sentetik Polimer Hidrojeller

Sentetik polimerler, yüksek mekanik stabiliteye sahip modifiye edilebilir mikro yapıları, ayarlanabilir kimyasal bileşimleri ve biyoparçalanabilir özellikleriyle hidrojel formülasyonlarında sıklıkla kullanılmaktadır [32,33]. Poli(etilen glikol) (PEG), poli(ε-kaprolakton) (PCL), poli(etilen oksit) (PEO), poli(laktik ko-glikolik asit) (PLGA), poli(vinil alkol) (PVA), poliakrilamid (PAM) ve poli(2-hidroksi metakrilat) (PHEMA) polimerleri hidrojel formülasyonlarında farmasötik uygulamalarda yaygın olarak kullanılan polimerlerdir [34-39]. Lei ve arkadaşları PTX yüklü poli(etilen glikol)-poli(ε-kaprolakton)-poli(etilen glikol) (PECE) enjektabl hidrojeli uygulanan 4T1 meme kanseri modelinde, primer tümör rezeksiyonunu takiben yara iyileşme süresinin kısaldığını, tümör proliferasyonunun inhibe olduğunu ve tümör nüksünün önlendiğini bildirmişlerdir [40]. Gliobastoma lokal tedavisinde, salinomisin yüklü poli(etilen oksit)/poli(propilen oksit)/poli(etilen oksit) (PEO-PPO-PEO) ve Pluronik F127 ile hazırlanan hidrojellerin etkinliğinin incelendiği bir çalışmada, 7 gün süreli kontrollü salım elde edilmiş, glioblastoma hücrelerinde hücre proliferasyonun inhibisyonuyla birlikte apoptoz sağlanmış ve subkütan U251 zenograft nude farelerde tümör büyümesinin 4-6 kat azaldığı gösterilmiştir [41].

Protein Temelli Hidrojeller

Protein polipeptid temelli hidrojeller, fonksiyonel üniteleri dolayısıyla yüzey modifikasyonlarına imkân sağlaması, dış uyaranlara hızlı yanıt vermesi, biyoparçalanır yapıda olması, kolay sentezlenebilir olması, düşük immünojenisite ve toksisiteye sahip olması gibi özellikleriyle öne çıkmaktadırlar [42]. Protein temelli sentezlenen hidrojeller, hücre ekstraselüler matrikse benzerlikleri dolayısıyla, sentetik polimerlere kıyasla daha yüksek biyouyumlu özellikler gösterirler [43]. Farmasötik alanda, kollajen, elastin, fibrin, ipek fibroin ve jelatin protein temelli hidrojellerin eldesinde yaygın olarak kullanılan bileşenlerdir [44-48]. Yu ve arkadaşları, metoksi poli(etilen glikol)-b-poli(γ-etil-L-glutamat) diblok kopolimeriyle (mPEG-b-PELG) C26 kolon kanserine yönelik *in situ* termo jelleştirici hidrojel sentezlemişlerdir. Kombretastatin A4 disodyum fosfat (CA4P) ve sisplatin (CDDP) etkin maddelerinin kombine tedavisini sağlayan hidrojel formülasyonu, lokal uygulamada yüksek antitümör etkinlik göstermiştir [49]. Nie ve arkadaşları, DOX'un kontrollü salımı ve filamentler arasında güçlü π-π bağ etkileşiminin sürdürülmesi için heksapeptid hidrojelatör (FEF3K) kullanarak, DOX yüklü antiparalel β-tabaka yapıda enjektabl hidrojeller elde etmişlerdir. Meme kanseri fare modelinde, cerrahi sonrası tümör nüksüne karşı yüksek terapötik etki ve hızlı yara iyileşme etkilerinin gözlendiğini bildirmişlerdir [50]. Ayrıca protein temelli hidrojellerin kemoterapi, immünoterapi, fototermal terapi uygulamalarında da etkinliğini gösteren çalışmalar da bulunmaktadır [51-53].

DNA Hidrojeller

DNA hidrojeller, üç boyutlu yapıya sahip dallanmış DNA zincirlerinin, sulu çözeltide çapraz bağlanma reaksiyonları sonucunda elde edilirler. DNA hidrojeller, kolay modifiye edilebilen, biyouyumlu, biyolojik olarak parçalanabilir ve ekonomik olarak üretilebilen yapılardır [54]. DNA hidrojel sentezinde çeşitli yaklaşımlar mevcuttur. En sık kullanılan yöntemlerden biri olarak DNA dizilerinin kullanıldığı yöntemde, DNA'nın tamamlayıcı zincirleri ile bağlayıcı gruplar kullanılır. DNA primerleri, DNA'nın hibridizasyonunda tamamlayıcı zincirle etkileşime girerek üç boyutlu DNA ağ yapısı oluşturulur. Enzimatik ligasyon tekniğinde, enzimatik polimeraz amplifikasyon reaksiyonlarından faydalanılarak DNA zincirleri birleştirilir. DNA'nın sarmal yapı oluşturması temelli yöntemde, birleşik dairesel amplifikasyon ve çoklu primer zincir amplifikasyonunun uygulanmasıyla

uzun DNA zincirlerinin sentezlenmesini sağlanır [55]. Tamamı DNA bazlı yapıtaşlarından oluşan hidrojellerde, birçok avantaja rağmen uygulamada bazı dezavantajlar da bulunmaktadır. DNA'da fonksiyonel grup sayısının az olması, ileri kimyasal modifikasyonları oldukça zorlaştırır ve hidrojelin fonksiyonel özelliklerinin ayarlanabilmesini kısıtlar. Bunun yanı sıra DNA'nın negatif yüklü olması ve stabilite sorunları da dezavantajlar arasındadır [55]. DNA ve RNA zinciri içeren hidrojeller, kanser tedavilerinde ve gen terapilerinde efektif olarak kullanılabilmektedir. Kanser metastaz faktörü kemokin reseptörü CXCR4'ün, tümör baskılayıcı ve inhibitör siRNA duplekslerinin sentetik polikatyonik reaktifler eklenmeden elde edilen RNA-üçlü sarmal hidrojel ile gen terapisinin yapıldığı bir çalışmada, *in vitro* ve *in vivo* modellerde MDA-MB-231 üçlü negatif meme kanseri modelinde miRNA ifadelenmesi baskılanarak yüksek gen susturma etkinliği sağlanmıştır [56]. Zhang ve arkadaşları lokal kanser tedavisine yönelik cerrahi sonrası uygulama için geliştirdikleri kamptotesin yüklü nükleaz ve glutatyon duyarlı enjektabl DNA hidrojeller ile HCT 116 kolon kanserinde tümör nüksüne karşı yüksek terapötik etkinlik sağladıklarını bildirmişlerdir [57].

Hibrit Hidrojeller

Hibrit hidrojeller, çeşitli polimerlerin, nanopartiküllerin, karbonhidratların, peptid ve proteinlerin farklı çapraz bağlama yöntemleri uygulanarak, tedavide hedeflenen karakteristiklere uygun olarak üretilebilen hidrojellerdir [58]. Cimen ve arkadaşları lokal meme kanseri modeline yönelik olarak geliştirdikleri formülasyonda, hidrazid fonksiyonelleştirilmiş jelatin (Gel-ADH) ve aldehit fonksiyonelleştirilmiş PEG (diBA-PEG) polimerleri arasında hidrazon bağı oluşturarak, kendini onarabilen, enjektabl, pH duyarlı özellikte elde ettikleri hidrojel ile DOX yüklü laponit (LAP) nanodisklerini entegre ederek Gel-ADH/diBA-PEG/LAP-DOX enjektabl hidrojel sistemini üretmişlerdir. Bu hibrit hidrojel formülasyonunda, LAP-DOX elektrostatik etkileşimi sonucunda DOX etkin maddesinin 10 günlük sürede pH 5.0'de % 31.76, pH 7.4'te % 25.24 salım değerleri ile pH duyarlı uzatılmış salım profili elde ettiklerini, 30 günlük süre sonrasında hidrojelin % 40 rezidüel ağırlık ile tümör pH ortamında yüksek jel stabilitesi ve uzun biyoparçalanma ömrü gösterdiğini bildirmişlerdir. [59]. Başka bir çalışmada, üçlü meme kanseri modeline yönelik lokal tedavide, katyonik/nötral DOX yüklü lipit nanopartiküller, bakteriyel selüloz matriks ile kombine edilerek (BC-NLCs-NH) hidrojel formülasyonu elde edilmiştir. Taşıyıcı sistemin tümör içi uygulamasında, kontrol DOX'a kıyasla ödem, nekroz, inflamasyon gibi yan etkiler gözlenmezken ve daha yüksek antikanser etkinlik gözlenmiştir [60].

Uyaran Duyarlılığına Göre Hidrojeller

Hidrojel sistemlerde, hidrojellerin büzülmesi veya şişmesi sonucunda etkin madde difüzyon yoluyla polimer ağı üzerinden taşınarak yer değiştirir. Buradaki jel karakteristikleri kontrollü salım dinamiklerini belirler. Hidrojellerdeki şişme davranışı, mekanik dayanıklılık veya geçirgenlik gibi nitelikler, endojen veya eksojen uyaran maruziyeti ile değişebiliyorsa bu taşıyıcı sistemler, uyarıya yanıt veren hidrojeller, akıllı hidrojeller veya uyaran duyarlı hidrojeller olarak adlandırılabilmektedir. Bu sistemlerde, uyaran duyarlı polimerler yapıya katılarak hidrojeller özelleştirilmektedir [43]. Uyaranlar türüne göre temel olarak endojen ve eksojen uyaran olarak ayrılabilmektedir. Enzimler, pH, ATP, hidrojen peroksit, redoks potansiyeli, hipoksi koşulları endojen uyaranlar arasında yer alırken, manyetik alan, sıcaklık, ultrason, ışık ve elektrik alan değişimleri eksojen uyaranlar arasında yer almaktadır [61-63]. Endojen uyaranlara kıyasla eksojen uyaranlarla elde edilen manipülasyonlar daha etkili olmaktadır [63]. Bu kısımda uyaran türüne göre hidrojeller sınıflandırılarak yapısal özelliklerine ve kanser alanında kullanımlarına yer verilmiştir.

Sıcaklık Duyarlı Hidrojeller

Sıcaklık gradyanına göre şişme ve faz değişim özellikleri gösteren hidrojeller, sıcaklık duyarlı hidrojeller olarak adlandırılmaktadır. Sıcaklık uyaranına duyarlı polimer çözeltilerde, sıcaklık değişimi sol-jel faz geçişini sağlar. Polimer çözeltisinin tek fazdan ikili faza (sulu polimer zengin faz) geçtiği sıcaklık, kritik çözelti sıcaklığıdır [64]. Çoğu ısıya duyarlı polimerin, su içinde ilgili hidrojelin hacim faz geçiş sıcaklığına benzer bir alt kritik çözelti sıcaklığı (LCST) vardır. Bu polimerler, LCST'nin altındaki sıcaklıklarda su ile karışabilir ancak sıcaklık LCST'nin üstüne çıktığında çözünürlüğün

düşmesiyle çözeltiden ayrılır ve jel yapının oluşumunu sağlar. Isıya duyarlı polimerlerin, üst kritik çözelti sıcaklığı (UCST) gösterdiği durumlarda ise ortamı UCST'den daha yüksek sıcaklığa ısıtmak, polimer zincirlerinin çözünmesini sağlar. Faz geçiş sıcaklığı vücut sıcaklığına yakın olan ısıya duyarlı polimerler, genellikle LCST tipi polimerlerdir [65]. Seçilen polimer yapısı ve konsantrasyon ile ayarlanabilen hidrofilik-hidrofobik denge, jelleşme sıcaklığını belirleyen önemli bir faktördür. Farmasötik uygulamalarda genellikle sol-jel geçiş sıcaklığı için fizyolojik sıcaklık olan 37°C hedeflenmektedir [65]. Sıcaklık duyarlı hidrojel tasarımında kitozan, selüloz türevleri gibi doğal polimerler kullanılabilirken, sentetik kaynaklı olarak da poli N-izopropilakrilamid (pNiPAm), poli (etilen oksit)-b-poli (propilen oksit)-b-poli (etilen oksit) (PEO-PPO-PEO; Poloksamer), PEG-b-PCL, PEG-b-PLGA ve PEG-b-PLA gibi polimerler kullanılabilmektedir [66-73]. Lokal kanser uygulamalarında, *in situ* jel formülasyonlarında etkin maddenin çözelti formda çözünmüş olması, formülasyonun enjeksiyon sonrasında vücut sıcaklığındaki uygulama bölgesinde jel hale geçmesi, uzun süre stabilitesini koruyabilmesi ve enjektabl özelliğini koruması hedeflenmektedir [74]. Zhao ve arkadaşları, dissolüsyon yöntemiyle hazırladıkları PLGA-PEG-PLGA ısı duyarlı hidrojele, PTX nanokristalleri ve niklosamid nanokristallerini kombine şekilde yükleyerek, üçlü negatif meme kanserine karşı tümör içi uygulamada tek dozda antiproliferatif ve anti kanser etki elde etmişlerdir [70]. PDLLA-PEG-PDLLA polimerlerinin kullanıldığı diğer bir çalışmada, stabilitenin arttırılması için üçlü blok kopolimerin fonksiyonel grupları modifiye edilerek uzatılmış salım nitelikleri geliştirilmiştir. DOX yüklü DMXAA konjuge mPEG-PLGA miseller (DOX-mPPD), PDLLA-PEG-PDLLA polimerleriyle hazırlanan sıcaklık duyarlı hidrojel ile karıştırılmış, 40 günlük sürede % 60.27 DOX, % 44.55 DMXAA oranlarda etkin maddelerin kümülatif salımı elde edilmiş, hayvan modelinde tekli doz uygulamasıyla yüksek anti vasküler ve anti kanser etki elde edildiği bildirilmiştir [75]. Fan ve arkadaşları tümör rezeksiyonu sonrasında cerrahi sonrası uygulama için tasarladıkları Poloksamer 407, Poloksamer 188, Karbomer 974P bazlı ısı duyarlı hidrojel formülasyonunu, PTX nanokristalleri karıştırılarak (PTX-NCS-Gel) cerrahi kavitedeki yara bölgesine sürerek doğrudan uygulama sağlamışlardır. 4T1-Luc üçlü negatif meme kanseri modelinde, rezidüye uygulanan PTX-NCS-Gel formülasyonunun efektif şekilde tümör nüksünü ve akciğer metastazını önlediğini bildirmişlerdir [76].

pH Duyarlı Hidrojeller

Kanser hücresi ortamı ve mikro çevresi, hücrede sitoplazmanın artan alkalileşme göstermesi ve hücre dışı ortamın asitlenmesi ile karakterize edilmektedir. Alkali sitoplazmadaki pH artışı, hücrede glikolizi indükleyebilmekte ve hücrelerin hipoksiye adaptasyonunu sağlayarak kanser hücre proliferasyonunu arttırmaktadır [77]. Kanser tedavisi uygulamalarında, etkin maddenin kanser hücreleri üzerinde etkinlik gösterirken aynı zamanda sağlıklı dokularda olumsuz yan etkilerin azaltılması amaçlanmaktadır. Özellikle lokal kanser tedavilerinde, tümör mikro çevresindeki pH değişiminden faydalanılarak, etkin maddenin kanser hücrelerinin bulunduğu bölgede salımına olanak sağlayan pH duyarlı hidrojellerin kullanımı önem arz etmektedir [78]. pH duyarlı hidrojellerin üretiminde, zayıf asidik veya bazik fonksiyonel gruplara sahip polimerler tercih edilmektedir. Bu fonksiyonel gruplar, asidik pH koşullarda proton kabul ederken, bazik pH koşullarda protonları serbest bırakma eğilimindedir. Polielektrolitlerin oluşumu, elektrostatik etkileşimlere ve pKa değerlerine dayalı yük değişimlerinden kaynaklanır. Elektrolitler, hidrofobik/hidrofilik geçişler aracılığıyla hidrojelin çözünürlük özelliklerini değiştirir. Dolayısıyla hidrojelde şişme/büzülme davranışı değişir ve farklı pH değerlerinde farklı etkin madde salım profilleri elde edilmiş olur [79].

Lokal ilaç uygulamalarında, pH duyarlı hidrojellerin tasarımı için tümör dokularının asidozu temel alınarak iki ana yönteme başvurulmaktadır. İlk yaklaşımda, katyonik yapıdaki polibazlar olarak kabul edilen, hidrofobik omurgaya bağlı, çok sayıda zayıf baz (amin vb.) grubu içeren pH duyarlı polimerler seçilir. Katyonik bir polimerin pKa değeri, görece asidik bir ortamda bulunduğunda, dolayısıyla ortamın pH'ından daha yüksek olduğunda, amin grupları protonlanarak pozitif bir yük (NH^{3+}) kazanır. Böylece bu yükler arasındaki elektrostatik itme ile polimer zincirinin genişlemesi ve hidrojelin şişmesi tetiklenir [80]. İkinci yaklaşımda, polimer matrisinde veya polimer ile etkin madde arasında, pH duyarlı bağlar oluşturmak için kararsız asit bağlarının eklenmesi gereklidir. Bu tür bağlar, enzimatik reaksiyonların etkisiyle ekstraselüler sıvıda, tümör hücresinin endozom veya lizozomlarında endositoz sonucunda parçalanır. Hidrazon gibi dinamik kovalent bağlar, fizyolojik pH seviyesinde (pH

7.4) hidrolize karşı stabil iken, hafif asidik bir pH ortamında hızla parçalanma eğilimi gösterirler [80]. Bu iki stratejide de bağların kırılması sonucunda enkapsüle veya konjuge etkin maddenin ortama salımı hedeflenir. Liu ve arkadaşları, lokal meme kanseri tedavisinde gemsitabin ve PTX antikanser ajanlarının eş zamanlı salımını sağlayan, tasarım ve sentezini yaptıkları OE (VKVKVOVK-VDPPT-KVEVKVKV-NH2) peptidi ile pH duyarlı enjektabl hidrojel elde etmişlerdir. Formülasyonda, pH değişimine bağlı olarak nötral koşullardaki β-levha ikincil yapılarının protonasyonuyla salım kinetikleri de değişiklik göstermiştir. OE hidrojelinden hidrofobik karakterdeki PTX'in pH 5.8 ve pH 7.4 ortamında salımı sırasıyla 7 gün içinde % 96.90 ve % 38.98 iken, hidrofilik karakterdeki gemsitabinin sırasıyla pH 5.8 ve pH 7.4 olan ortamlarda 3 gün içinde % 99.99 ve % 99.63 olarak salındığını bildirmişlerdir [81]. Sharma ve arkadaşları, glikoksilik hidrazon bağları aracılığıyla 8-kol PEG glikoksilik aldehit çapraz bağlanması sonucu elde ettikleri hidrojelde, kovalent olarak PEG-DOX konjugatını polimer matriksine enkapsüle etmişlerdir. Nötral pH'da yüksek hidrolitik stabilite gösteren hidrazon bağları dolayısıyla, hidrojelin şişme davranışı ve konjugatların matriksten ayrılma hızı düşük pH'ya kıyasla daha yavaş olmuştur. 40 günlük salım sonrasında DOX'un tümör koşullarında (pH 6.4) % 81.33, fizyolojik koşullarda (pH 7.4) % 42.87 kümülatif salımı sağlanmış, A549 akciğer kanseri hücrelerine karşı lokal uygulamada uzun süreli uzatılmış salım elde edilmiştir [82].

Işık Duyarlı Hidrojeller

Işık, uyaran yoğunluğu, dalga boyu, maruziyet süresi ve ışın çapı gibi parametreler ile kolaylıkla manipüle edilebilen, güvenli, hızlı etkili ve invazif olmayan bir dış uyaran olarak değerlendirilir [83]. Işığa duyarlı hidrojellerde, ışık duyarlı kimyasal grupların polimerik yapıya dahil edilmesiyle formülasyona ışıkla uyarılabilme özelliği kazandırılır. Bu taşıyıcı sistemler, optik bir uyarana yanıt olarak fiziksel veya kimyasal değişim gösterebilir. Işık uyaranı, hidrojellerdeki ışık duyarlı grupların kırılmasına, izomerizasyonuna ve dimerizasyonuna neden olabilir. Böylece hidrojel yapıdaki kısmi veya tam çapraz bağların çözülmesine, bozunmasına, şişmesine veya büzülmesine yol açar. Işık duyarlı sistemlerde, fotoizomerizasyon, fotokimyasal reaksiyon ve fototermal reaksiyon olmak üzere üç temel yanıt mekanizması bulunmaktadır [84]. Fotoizomerizasyonda, hidrojel yapısında yer alan belirli motiflerin foto-indüklenmiş izomerizasyonuyla, hidrojele ait çapraz bağ yoğunluğu, yük durumu veya hidrofilik yapı özellikleri değiştirilir. Genellikle ışıkla uyarılan hidrojellerde, trans konformasyondan cis konformasyona geçiş gerçekleşir. Bu süreçte hidrojellerin gözenek boyutları büyür ve etkin maddelerin difüzyonla matris dışına çıkışı sağlanır. Bu uygulama, hidrojel kimyasal bağlarını koparılmaksızın ve yan ürün oluşturmaksızın gerçekleştirilen, genellikle geri dönüşümlü ve tekrarlanabilir bir yöntemdir [85]. Foto kimyasal reaksiyonlarda, hidrojel yapıda meydana gelen bağ parçalanması, bağ oluşumu, bağ değişimi ile hidrojelde ağ yapıda ve konfigürasyonda değişiklikler sağlanır. Böylelikle etkin madde salımı tetiklenir. Tüm foto kimyasal reaksiyonlar arasında foto parçalanma, etkin maddenin kontrollü salımı için en yaygın olarak kullanılan yöntemdir. Bu yöntemde, foto parçalanabilir bağlayıcılar hidrojel yapısına dahil edilir ve ışıkla maruziyeti ile parçalanabilen yapılar oluşturulur [86]. Fototermal bir reaksiyonda, hedef yapıda bulunan ışığa duyarlı bileşenler, ışık enerjisini termal enerjiye çevirerek malzemenin sıcaklığını artırır. Jelin iç sıcaklığı, faz geçiş koşulunu sağladığında jel uyarana tepki gösterir ve jelin mekanik özelliklerinde modifikasyon sağlanmış olur [87]. Mi ve arkadaşları, üçlü negatif rezidüel meme kanserine yönelik lokal tedavide kullanılmak üzere fotopolimerizayon yöntemi ile sentezledikleri PEG dimetakrilat (PEG-DMA) ve serisin metakriloil (SER-MA) bazlı enjektabl hidrojele, DNA metilasyon inhibitörü desitabin (DEC) ve gambojik asitin PLGA enkapsülasyonuyla elde edilmiş nanopartiküller (GA-NP) yükleyerek, meme kanserinde tümör nüksünün önlenmesini, cerrahi operasyon sonrası yara iyileşmesinin ve doku yenilenmesinin arttırılmasını amaçlamışlardır. PEG-DMA/SER-MA hidrojel yapısında, DEC'in yaklaşık olarak tamamının salımı 2 saatlik sürede gerçekleşirken, GA-NP sayseinde gambojik asitin 48 saatlik süre ile % 60 uzatılmış salımı sağlanmıştır. Çalışma ekibi, hidrojel formülasyonlarının 4T1 hücre modelinde ve ortopik 4T1 BALB/c fare modelinde, tümör hücrelerinde piroptozla indüklenen immün cevabı arttırarak yüksek terapötik etkinlikle akciğer metastazı ve lokal tümör nüksünün inhibasyonunun sağladığını bildirmişlerdir [88].

Redoks Duyarlı Hidrojeller

Redoks duyarlı ilaç taşıyıcı sistemler, tümör mikro çevresi ile sağlıklı dokular arasındaki

redüksiyon potansiyeli farkını baz alarak tasarlanmış sistemlerdir. Hayvan hücrelerinde en bol miktarda bulunan redoks ikilisi Glutatyon (GSH)-Glutatyon disülfid (GSSG) çiftidir. GSH değerleri, sağlıklı hücrelere kıyasla tümör hücrelerinde çok daha yüksek seviyededir [89]. Redoks duyarlı hidrojeller, GSH'deki tiyol gruplarından elektron alabilen, GSH'yi indirgeyebilen reaktif grupların (disülfit vb.) yapıya eklenmesiyle elde edilirler. Temel olarak redoks duyarlı hidrojellerin eldesinde, disülfit bağlarının hidrojeli oluşturan polimer yapıya doğrudan bağlanması veya disülfid içeren çapraz bağlayıcılar aracılığıyla bağlanarak polimer yapıya dahil edilmesi yer almaktadır [90]. Disülfid bağları, GSH gibi bir indirgeyici ajan varlığında elektron verici olarak işlev görür ve GSSG'ye oksitlenir. Oksidasyon duyarlı hidrojellerde ise hidrojelin bozunması ve etkin madde salımının sağlanabilmesi reaktif oksijen türlerine (ROS) bağımlıdır. Bu oksidatif türler, endojen olarak mitokondride veya eksojen olarak bir fotosensitizör aracılığı ile üretilebilmektedir [91]. β-siklodekstrin konjuge dekstran polimerinin (βCD-Dex) ve disülfit içeren bis-adamantan-tetraetilen glikol (bis-Ada-TEG-SS) çapraz bağlayıcısının sulu çözelti ortamında kendiliğinden birleşmesiyle hidrojelin elde edildiği bir çalışmada, çapraz bağlayıcılar sayesinde hidrojele endojen indirgeyici ajan olan GSH ile etkileştiğinde parçalanabilme özelliği kazandırılmıştır. Buna ilaveten kovalent olmayan etkileşimlerle, DOX yüklü hidrojellerin, siklik peptid bazlı hedefleyici yapı olan adamantan konjuge cRGDfK (Ada-cRGD) ile tümör spesifik hedeflenmesi sağlanmıştır. Hidrojelden DOX salımının, 72 saat sonrasında ekstraselüler ortam pH 7.4'te % 20 iken, tümör mikro çevresi modellemesinin pH 5.4 ve GSH ile sağlandığı salım ortamında % 86 olduğu gösterilmiştir. Yüklü olmayan hidrojeller, kontrol hücrelerde sitotoksisite göstermezken, DOX yüklü hidrojellerin MDA-MB-231 üçlü negatif meme kanserine karşı sitotoksik etkinlik sağladığı ve en yüksek etkinliğin RGD-βCD-DOX hidrojel formülasyonlarında olduğu bildirilmiştir [92].

Manyetik Duyarlı Hidrojeller

Manyetik duyarlı hidrojeller, genellikle paramanyetik özellikli materyallerin hidrojel yapıyla birleştirilmesiyle elde edilmektedirler. Bu materyaller, manyetik alana maruz bırakıldıklarında titreşerek bulundukları bölgede lokal olarak sıcaklığı arttırır ve termal ablasyon mekanizmaları aracılığıyla terapötik etkinliğin arttırılmasını sağlarlar. Sıcaklık artışının ortamdaki etkin madde salımını tetiklediği ilaç taşıyıcı sistemlerde, kullanılan ajanın yanı sıra termal olarak da terapötik etki desteklenmiş olur [93]. Genellikle yapısında demir oksit (Fe_2O_3, Fe_3O_4) [94,95], geçiş metal ferritleri ($CoFe_2O_4$, $MnFe_2O_4$ vb.) [96, 97] gibi çeşitli nanomateryallerin, manyetik duyarlı malzemeler olarak hidrojellerin yapısına katılmasıyla manyetik duyarlı hidrojel formülasyonları elde edilmektedir. Özellikle yapısında γ-Fe_2O_3, Fe_3O_4 içeren nanopartiküller, yüksek biyouyumluluk ve manyetizasyon özellikleri dolayısıyla manyetik duyarlı hidrojellerde terapötik amaçlı olarak sıklıkla tercih edilmektedir [98]. Enjektabl ve kendi kendini tamir eden kompozit hidrojel formülasyonunun, oksitlenmiş pektin, kitozan ve nano γ-Fe_2O_3 ile elde edildiği bir çalışmada, 0.25 μm boyutlu nano γ-Fe_2O_3 yapıları, hidrojel yüzeyine yüklenerek ilaç taşıyıcı sisteme manyetik özellik kazandırılmıştır. Çalışmada 5-florourasilin (5-FU) 12 saat süreli kontrollü salım profili elde edilmiş, MCF-7 meme kanseri hücre hattına karşı yüksek sitotoksik etki sağlanmış ve γ-Fe_2O_3 yapılarının hedefleme dışında da formülasyonun antikanser özelliklerini de iyileştirdiği belirtilmiştir [99]. Gao ve arkadaşları, rezidüel meme kanserine yönelik ferro manyetik vorteks alt birim demir oksiti, kitozan bazlı dinamik hidrojele greft metoduyla birleştirerek enjektabl manyetik hidrojel elde etmişlerdir. Manyetik alanda, süpermanyetik demir oksitle fonksiyonelleştirilen hidrojellere kıyasla çok daha yüksek verimlilikte hipertermi koşullarını elde ettiklerini, manyetik hipertermi tedavisinde yardımcı ajan olarak DOX'un uzatılmış salımı sağladıklarını, hem kemotarapi hem de termal terapinin aynı anda sağlanmasıyla tümör nüksünü önlemede *in vitro* ve *in vivo* olarak yüksek terapötik etkinlik elde ettiklerini bildirmişlerdir [100].

Ultrason Duyarlı Hidrojeller

Ultrason duyarlı ilaç taşıyıcı sistemler, düşük maliyetli olması, derin dokulara penetre olabilmesi ve invazif olmaması dolayısıyla tercih edilebilmektedirler. Bu ilaç taşıyıcı sistemlerde, yapıya eklenen stres duyarlı moleküller olan mekanoforlar aracılığıyla ultrason uyaranına karşı duyarlılık kazandırılmaktadır. Mekanofor moleküllere stres uygulanması sonucunda, moleküllerin özellikle bağ yapılarında ve fiziksel özelliklerinde değişlikler meydana gelmektedir. Bu nedenle, tasarlanan ilaç

taşıyıcı sisteme uygun mekanofor moleküllerin seçilmesi gerekmektedir [101]. Klinik olarak valide ve invazif olmayan bir yöntem olan yüksek yoğunluklu odaklanmış ultrason (HIFU), polimerik materyallerde kavite etkisi olmaksızın mekanofor molekülleri aktive eder ve bu yapıların klinik kullanımlarına olanak sağlar [102]. Kim ve arkadaşları, mekanokimyasal dinamik terapi olarak isimlendirdikleri yöntemde, meme kanserine karşı HIFU ile tetiklendiğinde ortama reaktif oksijen türleri ve serbest radikal salımını sağlayan mekanoforları terapötik ajan olarak kullanmışlardır. Azo temelli mekanoforları, çapraz bağlanmış biyouyumlu PEG hidrojellerine kovalent olarak birleştirerek elde ettikleri formülasyonun etkinlik değerlendirmelerinde, 72 saat uygulama sonrası HIFU tetiklemesi yapılmayan, dolayısıyla azo mekanoforlarının aktifleşmediği kontrol hücrelerinde sitotoksisite gözlenmezken, HIFU tetiklenmesi yapılan melanom hücrelerinin neredeyse tamamında sitotoksik etki elde ettiklerini bildirmişlerdir [103].

Çoklu Duyarlı Hidrojeller

Birden fazla uyarana duyarlı olan hidrojeller, endojen kaynaklı veya eksojen kaynaklı çeşitli faktörlere yanıt oluşturabilmektedir. Çoklu yanıt veren malzemeler kullanılarak elde edilen hidrojel formülasyonları, sadece tek bir uyarana yanıt veren malzemelerle hazırlanan hidrojeller ile karşılaştırıldığında, terapötik etkinlik için hedeflenen bölgede daha gelişmiş bir kontrol sağlar ve tümör bölgesi lokal ilaç uygulamalarında potansiyel olarak daha etkili ve uyarlanabilir şartlar sunar [104]. Meme kanseri lokal tedavisi için tasarlanan pH ve NIR duyarlı PEI-AuNR/DOX/HCT/ALG formülasyonunda, poli(etilen imin) (PEI) modifiye altın nano çubuklar (PEI-AuNR), sodyum aljinat (ALG), DOX ve herseptini birleştiren hibrit *in situ* hidrojel yapısı oluşturulmuştur. Etkin madde salımı, fototermal ablasyon ve sinerjistik ilaç-antikor etkileşimi ile sağlanmıştır. Formülasyondaki pH duyarlılığı sayesinde, tümör mikro çevresinde düşen pH'la birlikte etkin madde salımının arttığı, altın nano çubuklar sayesinde de 808 nm lazer ışın (1.0 W/cm^2) maruziyeti sonucunda % 70 verimlilikte fototermal çevirim ile yakın kızılötesi ışığının absorbsiyonuyla ısı açığa çıkartılarak etkin madde salımının tetiklendiği gösterilmiştir [105]. Zhu ve arkadaşları, pH ve redoks duyarlı IC1-R (CKIKIKIK–I^{D}PPT–KIOIKIKC–NH_2) peptid ile hazırladıkları enjektabl hidrojel ile PTX'in 7 gün süreli *in vitro* salımda; % 89.63 kümülatif salım ile en yüksek salım hızının pH 5.8 GSH içeren medyada elde edilirken, % 8.02 kümülatif salım ile en düşük salım hızının pH 7.4 GSH içermeyen medyada elde edildiğini göstermişlerdir. PTX yüklü hidrojel formülasyonunun, düşük pH ortam şartlarında ve indirgeyici varlığında etkin maddenin salım hızını önemli derecede arttırdığını, *in vitro* ve *in vivo* uygulamalarda da üçlü negatif meme kanserine karşı yüksek terapötik etkinliğe sahip olduğunu bildirmişlerdir [106].

SONUÇ VE TARTIŞMA

Bu derlemede, enjektabl ilaç taşıyıcı sistemler yapılarına ve uyaran duyarlılık özelliklerine göre incelenmiş, lokal uygulamalardaki ve özellikle meme kanserindeki terapötik etkinlikleri değerlendirilmiştir. Hidrojel uygulamalarında sıcaklıkla indüklenen çözelti-jel-çözelti dönüşümlerine, pH aracılı ilaç salım mekanizmalarına, ışıkla aktive edilen kombinasyon tedavilere, manyetik ve termal olarak duyarlı sistemler dahil olmak üzere çeşitli yanıt mekanizmalarına yer verilmiştir. Enjektabl hidrojel ilaç taşıyıcı sistemlerde, yapıyı oluşturan polimer veya bileşen türüne göre formülasyon davranışları modifiye edilebilmekte, jelin biyoparçalanır ve biyouyumluluk özellikleri de bu doğrultuda değişiklik göstermektedir. Hidrojel sistem tasarımında hedeflenen karakteristiklerin sağlanmasındaki çeşitli zorluklar, nanopartikül yapılarının ve yardımcı grupların hidrojel yapılarıyla birleştirilmesi ile aşılabilmekte ve çok fonksiyonlu tedavilere olanak sağlayabilmektedir. Kemoterapötik ajanların kullanıldığı enjektabl hidrojel ilaç taşıyıcı sistemlerin, etkin maddelerin hedef dokuda yüksek düzeyde ve verimli dağılımını sağlayabilmeleri, ilk çıkış etkisinin ve hızlı sistemik eleminasyonun önüne geçerek terapötik pencere içinde uzatılmış salımı mümkün kılmaları, uyaran duyarlılığına göre cevap oluşturabilmeleri özellikle lokal meme kanseri tedavilerinde yüksek avantaj sağlamaktadır. Lokal antikanser ajan uygulamalarında eş zamanlı olarak yardımcı uyaranlarla sağlanan terapi desteği, tümör dokusunun kazınması ve yeniden tümör nüksünün önlenmesinde önemli bir rol oynamaktadır. Sonuç olarak enjektabl hidrojel bazlı ilaç taşıyıcı sistemler, kontrollü ve uzun süreli etkin madde salımını,

etkinliği artırılmış hedefleyici tedaviyi mümkün kılarak geleneksel sistemik kemoterapiye kıyasla önemli iyileştirmeler sunmaktadır.

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REVIEW ARTICLE / DERLEME MAKALE

THE PROMISING ROLE OF iNOS INHIBITORS IN ALZHEIMERS DISEASE

ALZHEIMER HASTALIĞINDA iNOS İNHİBİTÖRLERİNİN UMUT VERİCİ ROLÜ

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ABSTRACT

Objective: *This study aims to explore the role of iNOS inhibitors in Alzheimer's disease (AD), a neurodegenerative disorder affecting millions worldwide. The main symptoms of AD include memory loss, cognitive decline, and behavioral changes. While the exact cause remains uncertain, both genetic and environmental factors are believed to contribute. Recent research has emphasized the significance of nitric oxide (NO) in AD development. Specifically, the upregulation of inducible nitric oxide synthase (iNOS) in AD patients leads to excessive NO production during neuronal inflammation, exacerbating AD and dementia. Therefore, the investigation focuses on the potential of iNOS inhibitors as a novel therapeutic approach for AD treatment.*

Result and Discussion: *In this review, we present the current therapeutic strategies available for Alzheimer's disease (AD) and explore the promising potential of iNOS inhibitors in AD treatment. Specifically, we will focus on their capacity to mitigate NO production and examine their potential neuroprotective effects. Additionally, this review will offer an overview of both natural and synthetic iNOS inhibitors, emphasizing the importance of safety considerations during the development of iNOS inhibitors as therapeutic interventions for AD.*

Keywords: *Alzheimer's disease, iNOS, neuroinflammation, nitric oxide*

ÖZ

Amaç: *Bu çalışma, milyonlarca insanı etkileyen Alzheimer hastalığı (AD) ve iNOS inhibitörlerinin rolünü araştırmayı amaçlamaktadır. AD'nin temel belirtileri arasında hafıza kaybı, bilişsel gerileme ve davranış değişiklikleri bulunmaktadır. Kesin neden belirsiz olsa da, genetik ve çevresel faktörlerin katkıda bulunduğu düşünülmektedir. Son araştırmalar, nitrik oksit (NO)'nin AD gelişimindeki önemini vurgulamıştır. Özellikle, AD hastalarında indüklenebilir nitrik oksit sentaz (iNOS) aktivasyonu, nöronal iltihaplanma sırasında aşırı NO üretimine neden olarak AD ve bunamayı kötüleştirmektedir. Bu nedenle, bu araştırma, iNOS inhibitörlerinin AD tedavisinde yeni bir terapötik yaklaşım olarak potansiyelini incelemektedir.*

Sonuç ve Tartışma: *Bu derleme, Alzheimer hastalığı (AD) için mevcut terapötik stratejileri sunuyor ve AD tedavisinde iNOS inhibitörlerinin umut verici potansiyelini araştırıyoruz. Özellikle, iNOS inhibitörlerinin NO üretimini azaltma kapasitelerine odaklanacak ve potansiyel nörokoruyucu etkilerini inceleyeceğiz. Ayrıca, bu derleme doğal ve sentetik iNOS inhibitörlerinin genel bir bakışını sunacak ve AD için terapötik müdahaleler olarak iNOS inhibitörlerinin geliştirilmesi sürecinde güvenlik değerlendirmelerinin önemini vurgulayacaktır.*

Anahtar Kelimeler: *Alzheimer hastalığı, iNOS, nöroenflamasyon, nitrik oksit*

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INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disease with a complex etiology. It is also the leading cause of dementia in the elderly, which accounts for 60-80% of all cases. According to the World Health Organization (WHO), approximately 50 million people worldwide are now living with dementia today, and this number is expected to triple by 2050 [1,2]. Symptoms of AD include language impairment, progressive memory loss, and visual-spatial impairment, all of which require caregiving [3,4]. Early signs of AD may vary from patient to patient, but typically memory problems are one of the early signs of AD. However, the very early stages of AD may also be indicated by a deterioration in other cognitive abilities [5,6], including the ability to express oneself clearly, problems with vision or spatial awareness, and impaired reasoning or judgment [7,8].

Although the pathology of AD has been studied for decades, the exact cause remains unknown. Recent developments in the diagnostic strategy of AD have aided to diagnose pathological abnormalities in the early stages of the disease. The current understanding of the pathological features of AD, which are the formation of neurofibrillary tangles (NFTs) inside the neurons [9], senile plaques outside neurons, and neuronal loss, is shown in Figure 1 [10]. However, there are other critical factors that promote the onset and development of AD, such as oxidative stress, neuroinflammation, and impaired glucose metabolism in the brain. In addition, amyloid plaques and tau protein-based neurofibrillary tangles may form years before clinical dementia manifests [11,12].

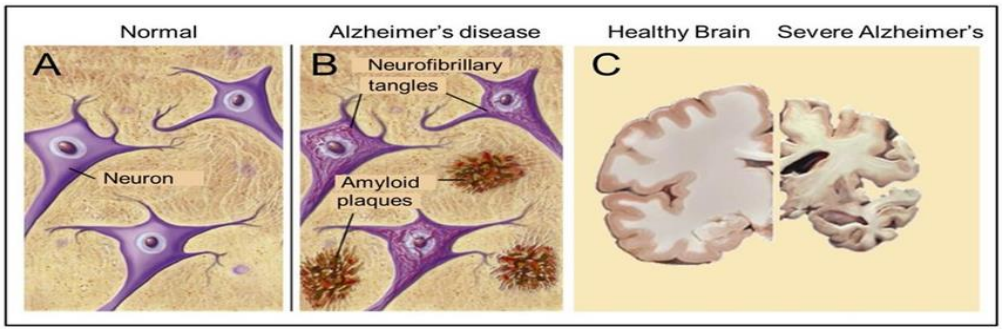


Figure 1. Difference between a healthy brain and the brain of a patient with severe AD. De Loof et al, (2019) OBM Neurobiology journal, from ref [10]

Although scientists have made great in the current decade to find a treatment that stops the progression and development of AD, we are still far from finding an effective treatment strategy [13]. This is because the currently available treatment only treats the symptoms of the disease. In recent years, scientists have focused on the role of neuroinflammation in finding an effective treatment for AD, because neuroinflammation is one of the major causes of the development and exacerbation of AD. Nitric oxide (NO) during neuroinflammation is overproduced by the inducible nitric oxide synthase (iNOS) enzyme in response to inflammatory stimuli, leading to the progression and exacerbation of AD, therefore, finding effective iNOS inhibitors will help in limiting and slowing the progression of AD [14]. This narrative review article will provide a brief overview of AD, and will highlight the promising role of iNOS inhibitors in AD.

Neuroinflammation and Alzheimer's Disease

Recent investigations have demonstrated that neuroinflammation is one of the major causes of the development and exacerbation of AD [15,16]. Interestingly, neuroinflammation could be a beneficial immune response that limits and slows disease progression [17]. For example, in its normal range, neuroinflammation modulates microglial activation and involves in the clearance of Aβ and cellular debris via microglial phagocytosis [18,19]. On the other hand, some researchers believe that neuroinflammation is merely a byproduct of the disease process and may not significantly alter its course. However, evidence from clinical studies and animal experiments has demonstrated that

enhanced neuroinflammatory cascades mediated by primed microglial cells also contribute to AD pathogenesis [20,21].

Neuroinflammation during AD is characterized by microglial activation and astrocyte release of various chemokines and cytokines, which impair blood-brain barrier (BBB) function and stimulate cognitive impairment [22,23]. Furthermore, during neuroinflammation, NO is overproduced by the enzyme iNOS in response to inflammatory stimuli, leading to an exacerbation of AD and dementia [24].

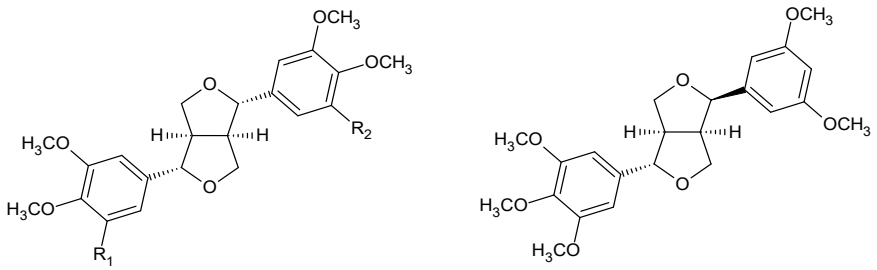
Role of iNOS Inhibitors in Neuroinflammation

NO acts as a mediator in several physiological and pathophysiological processes within the body. It is primarily produced by the inducible isoform of the enzyme, known as the iNOS, in response to inflammatory stimuli [25]. During neuroinflammation, excess NO is produced by iNOS enzymes in response to neuroinflammation. Furthermore, the overproduction of NO will lead to an exacerbation of AD and dementia [26].

To this end, many investigations and studies have been conducted by scientists to develop iNOS inhibitors, which will be an important treatment for the neuroinflammation of AD. However, many iNOS inhibitors showed neuroprotective effects, such as aminoguanidine, and two other amino acid amidines known as GW274150 and GW273629, but most iNOS inhibitors need further studies and investigations to ensure their affectivity and potency. In recent years, computational studies have been used by scientists to screen the inhibitory value of iNOS inhibitors in AD [14].

iNOS Inhibitors from Natural Sources

In recent decades, plants have been used as medicines to treat various diseases, and many plants have been used as anti-inflammatory agents that act by inhibiting the production and expression of NO. Furthermore, compounds isolated from these plants provided a medical chemist with a crucial lead to find effective and safe iNOS inhibitors for the treatment of AD [27]. The anti-neuroinflammatory effect of four ligands, (+)-eudesmin (1), (+)-magnolin (2), (+)-yangambin (3), and epimagnolin B (4) isolated from *Magnolia fargesii* flower buds were tested for their iNOS inhibitory activity against neurodegenerative disorders such as AD (Fig. 2). These four compounds showed IC_{50} values ranging from 10.9-30.0 μM. Among the group, compound 4 showed the most potent inhibition of LPS-induced NO production with an IC_{50} value of 10.9 ± 1.6 μM compared to the positive control drug NG-monomethyl-L-arginine (L-NMMA) IC_{50}=19.2 ± 1.8 μM [28].



1 : R_1, R_2 = -H
2 : R_1= -OCH_3, R_2 = -H
3 : R_1, R_2 = -OCH_3

4

Figure 2. Absolute configuration of natural iNOS inhibitors isolated from *Magnolia fargesii*

Kohno et al. investigated the potential inhibitory effects of various terpenoid coumarins such as (methyl galbanate, galbanic acid, and farnesiferol A) that have been extracted from *Ferula szowitsiana* DC. Specifically, the study aims to investigate whether these compounds can inhibit the production of NO in RAW264.7 mouse macrophage cells that have been stimulated with lipopolysaccharide (LPS) and interferon-c (IFN-c). Among the terpenoid coumarins that studied, it was observed that in the

presence of methyl galbanate 5 (Figure 3), LPS/IFN-c-induced iNOS mRNA expression was significantly reduced to 52% of the level found with LPS/IFN-c stimulation alone [29].

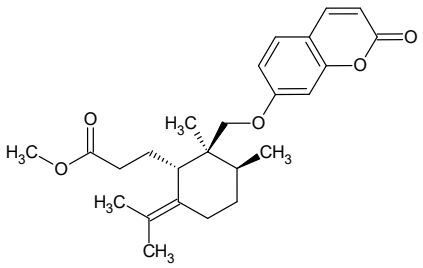


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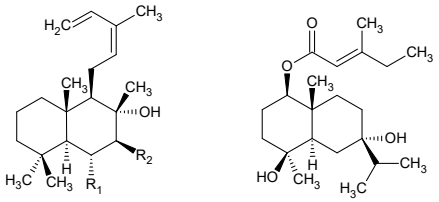
Figure 3. Chemical structure of methyl galbanate

In recent years, two other studies for the development of iNOS inhibitors from natural sources were performed by Liu et al. In both studies, the anti-neuroinflammatory effect of compounds isolated from plant sources was evaluated by inhibiting LPS-induced NO release in murine microglial BV-2 cells. In addition, 2-methyl-2- thiopseudourea, sulfate (SMT) was used as a positive control in the studies. In the first study, the anti-neuroinflammatory effects of twelve daphnane diterpenoids isolated from the twigs of *Trigonostemon thyrsoideus* were investigated. The result of this study demonstrated that all the compounds had an inhibitory activity on LPS-induced NO production with an IC_{50} value range of 3.19-24.9 μM [30]. In the second study of the group, isolated sesquiterpenes and terpenes from the flowers of *I. japonica* were evaluated as anti-neuroinflammatory agents for AD through NO inhibition. All of the compounds showed inhibitory activity against NO production, however, five of them possessed a more inhibitory effect on LPS-induced NO release with lower IC_{50} values than 10 μM [27].

Ma et al. investigated the potential inhibitory effects of one new labdane diterpenoid and three new guaiane sesquiterpenoids, alongside ten known compounds from *Blumea balsamifera*. The study focused on assessing the anti-neuroinflammatory effects by impeding the release of NO in murine microglial BV-2 cells induced by LPS. Among these derivatives, three compounds 6-8 showed IC_{50} values ranging from 15.4-22.7 μM, respectively, indicating notable inhibition against LPS-induced NO production in BV-2 cells, with their IC_{50} values falling below 30 μM (Figure 4) [31].



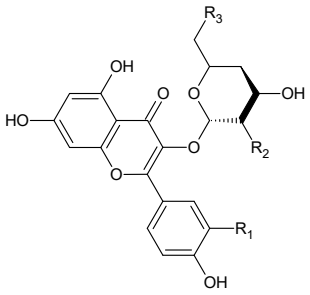
6 : R_1 = -OH, R_2 = -H
7 : R_1 = -H, R_2 = -OH

8

Figure 4. iNOS inhibitors as anti-neuroinflammatory agents for AD isolated from *Blumea balsamifera*

In 2021, two crucial studies have been done to test the anti-neuroinflammatory activity of numerous glycosides isolated from the leaves and stems of *Neoshirakia japonica*, and 7-O-1,2,3-triazole hesperetin derivatives. In the first study, the anti-neuroinflammatory effects of all the isolates were evaluated by inhibiting NO production against LPS-induced BV-2 microglial cells. Notably, three compounds 9-11 exhibited IC_{50} values ranging from 2.7 to 5.5 μM, respectively. These values indicated a higher degree of inhibitory activity compared to the positive control, minocycline, which had an IC_{50}

value of 15.6 μM. (Figure 5) [32].



9 : R_1 = -H, R_2 = -OH, R_3 = $-S_4$
10 : R_1 = -H, R_2 = -OH, R_3 = $-S_3$
11 : R_1, R_2 = -OH, R_3 = $-S_2$

Figure 5. iNOS inhibitors as anti-neuroinflammatory agents for AD isolated from *Neoshirakia japonica*

In the second study, the anti-neuroinflammatory of 7-O-1,2,3-triazole hesperetin derivatives was evaluated by Wang et al. Most of the hesperetin derivatives showed better NO inhibitory activity compared to hesperetin (IC_{50}=49.56 ± 2.39 μM). Furthermore, resveratrol (Res) was used as a positive control, and compound 12 (IC_{50}=1.04 ± 0.31 μM) had an eight fold higher NO inhibitory capacity compared to the positive control Res (IC_{50}=7.86 ± 1.49 μM) (Figure 6) [14].

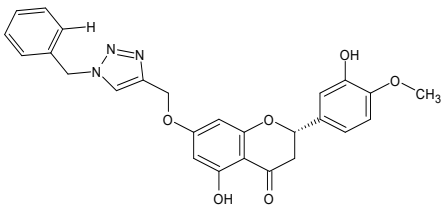


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Figure 6. Hesperetin derivative potent iNOS inhibitor

iNOS Inhibitors from Synthetic Sources

Synthetic iNOS inhibitors have been extensively studied for their potential therapeutic applications in a variety of disease, including neurodegenerative disorders. These inhibitors are designed to target the iNOS enzyme, which is responsible for the overproduction of NO in response to neuroinflammation. Overexpression of NO can lead to tissue damage and may play a role in the etiology of a number of diseases such as AD. A number of approaches, including structure-based drug design and high-throughput screening, have been used to develop synthetic iNOS inhibitors. These inhibitors have shown promising results in preclinical research, currently being evaluated in clinical trials to determine the efficacy and safety of iNOS inhibitors [26]. Synthetic iNOS inhibitors are classified based on their chemical structures into amidinic and guanidine-based compounds, quinone-based compound, oxadiazole-based iNOS inhibitors, steroidal compounds, and others.

Amidine and Guanidine-based Compounds

Amidine-based iNOS inhibitors, as the name suggests, have an amidine group (-C(=NH)-NH_2) in their structure, which can participate in hydrogen bonding and electrostatic interactions with the active site of iNOS. It has been discovered that these compounds are being investigated as potential treatments for Alzheimer's disease [26].

An example of an amidine-based iNOS inhibitor is GW274150 13 and 1400W 14, which are selective inhibitors of iNOS that have shown promising results in preclinical studies related to AD (Figure 7). GW274150 13 specifically inhibit the activity of iNOS by interfering with its enzymatic function, reducing the production of NO. Also, it has demonstrated anti-inflammatory effects and has been found to attenuate neuroinflammation and neurodegeneration in animal models of AD. Additionally, GW274150 13 has shown neuroprotective properties by reducing oxidative stress and preserving neuronal function. Similarly, 1400W 14 which is another amidine-based iNOS inhibitor is a potent and selective inhibitor of iNOS. It effectively suppresses iNOS activity and the subsequent production of NO. Preclinical studies utilizing 1400W have shown promising results in attenuating neuroinflammation, reducing amyloid-beta (Aβ) deposition, and improving cognitive function in animal models of AD. The inhibition of iNOS by 1400W has been associated with decreased oxidative stress and reduced neurodegeneration [14,26].

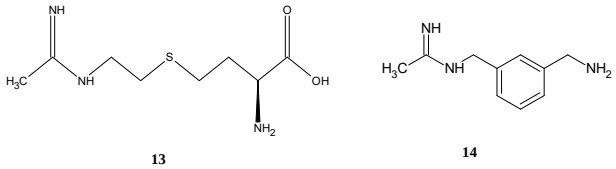

Figure 7. Amidine-based iNOS inhibitors

Guanidine-based iNOS inhibitors have a guanidine group (-C(=NH)-NH-C(=NH)-NH_2) in their structure, which can also interact with the active site of iNOS. Examples of guanidine-based iNOS inhibitors include aminoguanidine 15, and L-NG -nitro arginine methyl ester (L-NAME) 16 (Figure 8).

Two pivotal studies were conducted on guanidine-based compounds in mouse microglial for investigating their iNOS inhibitory activity in AD. The first study was conducted in 2003, during which scientists tested the inhibitory activity of two different guanidine-based iNOS inhibitors for inhibition of AGE-induced NO production in mouse microglial. Compound 15 exhibited the highest rate of iNOS inhibition, reaching 90%. On the other hand, the other guanidine-based iNOS inhibitors, compounds 16 demonstrated inhibition values of 80% for NO production in AD [33].

In addition, Esposito et al. investigated the stimulation of PC12 cells with Aβ (1-42) (1 g/ml), which resulted in a significant increase in NO formation by iNOS enzymes compared to unstimulated cells. Both selective and nonselective iNOS inhibitors significantly reduced the effect of Aβ (1-42). For example, S-methyl-isothiourea (SMT) 17 (Figure 8), a selective iNOS inhibitor, demonstrated the highest level of iNOS inhibition activity in AD, achieving an impressive 80% inhibition rate. Conversely, L-NAME 16, a non-selective iNOS inhibitor, exhibited a lower inhibition rate of 45.6% for iNOS [34].

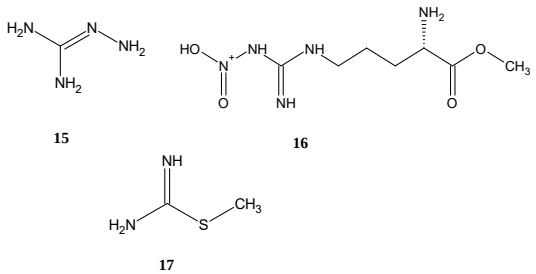

Figure 8. S-methyl-isothiourea and guanidine-based iNOS inhibitors

Quinone-based iNOS Inhibitors

Quinone-based compounds are a class of organic compounds characterized by the presence of a quinone structure in their chemical composition. Quinones are aromatic compounds that consist of a

benzene ring conjugated with two carbonyl groups (C=O) in different positions. Quinone-based derivatives have been investigated as iNOS inhibitors for AD due to their additional antioxidant properties. These compounds are designed to scavenge reactive oxygen species (ROS) and prevent oxidative damage, which is thought to contribute to the pathogenesis of AD. An example of a quinone-based iNOS inhibitor is idebenone 18 shown in (Figure 9), which has been evaluated in preclinical and clinical studies for its potential as a therapeutic agent for AD. Yan et al. conducted a study to investigate the iNOS inhibitory effects of idebenone 18 on LPS-activated BV2 cells for the treatment of neurodegenerative diseases such as AD. The researchers examined the cytotoxicity of idebenone by analyzing its effects at different concentrations (1, 2.5, and 5 μM) on LPS-activated BV2 cells. The findings revealed that idebenone exhibited a dose-dependent reduction in the LPS-stimulated NO production, and decreased mRNA expression of TNF-α, IL-1β, iNOS, and IL-6 in LPS-stimulated BV2 cells. In addition, the highest inhibition of iNOS activity was observed at the concentration of 5 μM [35].

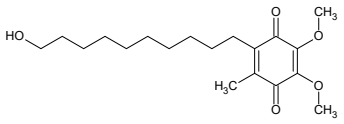

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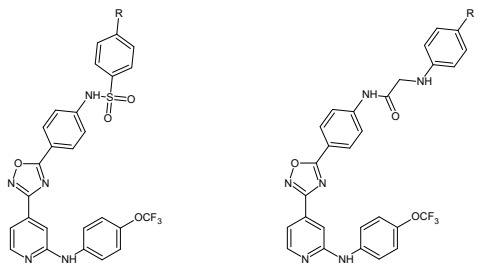
Figure 9. Quinone-based iNOS inhibitors

Oxadiazole-based iNOS Inhibitors

Oxadiazole-based iNOS inhibitors are a class of compounds being developed as potential therapeutics for various inflammatory diseases such as neuroinflammation. These compounds have the potential to inhibit iNOS activity and reduce NO production, which may have therapeutic implications for Alzheimer's disease and other inflammatory conditions [36,37].

Two important studies were conducted on 1,2,4-oxadiazole-containing compounds to investigate their NO inhibitory activity in AD. In the first study, a series of novel 3-(4-pyridyl)-5-(4- sulfamido-phenyl)-1,2,4-oxadiazole derivatives were synthesized, and the anti-neuroinflammatory activity of the compounds was assessed in LPS-induced BV2 microglial cells. All compounds showed better activity against NO compared to the positive drug, Res (IC_{50}=10.16 ± 0.12 μM). Compounds 19 and 20 showed the best inhibition rate against NO production, with an IC_{50} value range of 0.47 to 0.72 μM, which was about ten times higher than that of the positive drug Res [36]. In the second study, researchers investigated the potential anti-neuroinflammatory effect of the novel (4-(1,2,4-oxadiazol-5-yl)phenyl)-2-aminoacetamide derivatives in LPS-induced BV2 microglial cells. Among the derivatives tested, compounds 21 and 22 exhibited particularly noteworthy results, displaying more than 20-fold greater iNOS inhibitory activity compared to Res. Notably, compounds 21 and 22 demonstrated IC_{50} values ranging from 0.42 to 0.67 μM, highlighting their potent inhibitory effects on iNOS expression and NO production in AD (Figure 10) [37].



19 : R = -$COCH_3$
20 : R = -$C(CH_3)_2$
21 : R = -H
22 : R = -F

Figure 10. Oxadiazole-based iNOS inhibitors

Steroidal Compounds

Steroid compounds are a class of organic compounds that have a characteristic structure consisting of a sterane ring. These compounds have numerous biological activities, including anti-neuroinflammatory activity in AD [26].

Yang et al. synthesized novel steroidal derivatives, from readily available hyodeoxycholic acid ($C_{24}H_{40}O_4$). In the study, the anti-neuroinflammatory activity of 5α-cholestan-6-one derivatives was evaluated in LPS-stimulated BV-2 microglial cells. As a result of the study, five compounds (23-27) strongly inhibited LPS-induced NO production with a percentage inhibition range of 73.6 – 60%, without causing cell toxicity while dehydroepiandrosterone, which was used as a reference drug in the study, has 55.3% percentage inhibition (Figure 11).

Microglial activation is known to increase the expression of iNOS and COX-2, which are responsible for the production of PGE-2, and NO, and activated microglial cells also increase the production of proinflammatory cytokines such as IL-1b and TNF-a. As a further study, the effects of compound 23 on the mRNA expression of these cytokines were investigated. The result of the investigation clearly indicated that compound 23 which strongly inhibited LPS-induced expression of iNOS, IL-1b, TNF-a, and COX-2 in a dose-dependent manner [38].

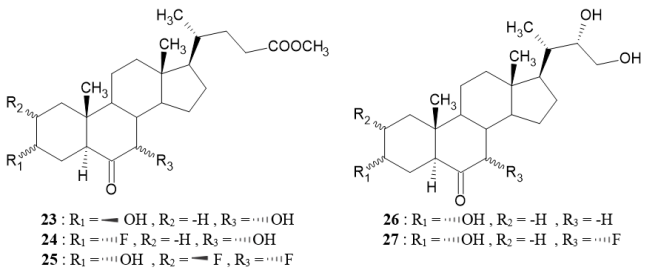


Figure 11. Steroidal compounds as inhibitors of iNOS

Others

Other studies have been conducted to test the iNOS inhibitory ability of various synthetic compounds, such as the study by Watterson et al. that investigated the role of the anti-inflammatory drug K252a 28 (Figure 12), which is a CaMKII inhibitor, that exhibits dose-dependent inhibition of lipopolysaccharide-induced increases in iNOS production and NO accumulation by the BV-2 microglial cell line. Furthermore, the study showed that the anti-inflammatory K252a inhibits the accumulation of NO and IL-1, and other cytokines during neuroinflammation in AD [39].

Zhou et al. explored the effects of rolipram 29 which is a Phosphodiesterase-4 (PDE4) inhibitor, in AD by using bilateral Aβ25– 35 injections into the hippocampus of rats (Figure 12). Different doses of rolipram were administered daily for 25 days after Aβ 25-35 injections. The results of the study demonstrated that rolipram significantly inhibited NO and iNOS pathways in the hippocampus. Furthermore, rolipram improved memory and learning abilities in the Aβ25-35-induced AD rat model [40].

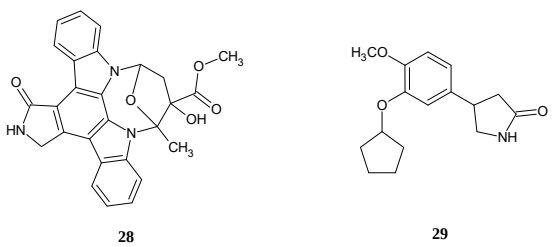


Figure 12. Other synthetic potent iNOS inhibitors

RESULT AND DISCUSSION

In conclusion, iNOS inhibitors have shown potential as therapeutic agents for AD in various studies. Studies evaluating iNOS inhibitors, including amidine, guanidine, quinone-based derivatives, and oxadiazole-based compounds, have demonstrated promising results in various *in vivo* and *in vitro* models of AD. These inhibitors have shown the ability to reduce iNOS activity, inhibit NO production, attenuate neuroinflammatory responses, and improve cognitive function. Some of these compounds have also shown effects on amyloid beta plaque accumulation and oxidative stress.

While the preclinical findings are encouraging, it's important to note that translating iNOS inhibitors into effective clinical treatments for AD has been challenging. Several factors, including the complexity of AD pathology, the multifaceted nature of iNOS signaling, and the need for targeted drug delivery to the brain, contribute to the difficulty in developing successful therapeutics.

Further research and clinical trials are needed to determine the safety, efficacy, and optimal dosing regimens of iNOS inhibitors in the treatment of AD. Combining iNOS inhibitors with other therapeutic approaches, such as anti-amyloid or anti-tau strategies, may also hold promise for improving outcomes in AD patients. Overall, iNOS inhibitors represent a promising avenue for future studies and therapeutic intervention in this major neurodegenerative disease.

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AUTHOR CONTRIBUTIONS

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

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

ALZHEİMER HASTALIĞINDA KOMPLEMAN SİSTEMİN ROLÜ

THE ROLE OF THE COMPLEMENT SYSTEM IN ALZHEIMER'S DISEASE

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ÖZ

Amaç: *Bağışıklık sistemi, vücudu enfeksiyonlara karşı savunan, yabancı maddelere tepki oluşturan ve hastalık gibi durumlarda organizmayı koruyan bir sistemdir. Merkezi sinir sistemi bağışıklık yanıtları bakımından periferik organlardan farklı benzersiz bir yapıya sahiptir. Son yıllarda gerçekleştirilen kapsamlı araştırmalar, beyin ve bağışıklık sistemi arasında karmaşık bir etkileşim olduğunu göstermiştir. Beyin bağışıklık sistemi, merkezi sinir sistemi içinde yer alan bir dizi hücresel ve moleküler mekanizmadan ve bağışıklık hücreleri ve moleküllerinin yer aldığı bir dizi yapıdan oluşan kompleks bir sistemdir. Beyindeki kronik inflamasyonun birçok nörodejeneratif hastalıkta ilerleyici nöron ölümünde önemli bir rol oynayabileceği bilinmektedir. Son yıllarda başta kadınlar olmak üzere ileri yaş popülasyonu etkileyen Alzheimer hastalığı, kısa süreli hafıza, biliş ve günlük yaşam aktivitelerinde zorluklarla ilgili sorunlarla karakterize edilen ilerleyici, nörodejeneratif bir hastalıktır. Alzheimer hastalığı genetik, immün ve çevresel etmenleri de içerdiği düşünülen kompleks bir mekanizmayla ortaya çıkar. Bu hastalığın kesin bir tedavisi yoktur ve kullanılan ilaçlar ancak semptomları geciktirir. Kompleman sistem doğuştan gelen bağışıklık sisteminin bir parçasıdır. Bu sistemin üç farklı aktive edici yolu vardır ve nihai olarak hedef hücre lizisine neden olan bir membran saldırı kompleksinin oluşumuyla sonuçlanır.*
Sonuç ve Tartışma: *Bu derlemede kompleman sistemin merkezi sinir sisteminde işleyişine ve Alzheimer hastalığı gibi nörodejeneratif bozukluklara yol açan kronik nöroinflamasyona nasıl katkıda bulunduğuna dair bilgiler paylaşılması amaçlanmıştır.*
Anahtar Kelimeler: *Alzheimer hastalığı, bağışıklık sistemi, kompleman sistem, merkezi sinir sistemi, nörodejenerasyon*

ABSTRACT

Objective: *Immune system is a system that defends the body against infections, reacts to foreign substances, and protects the organism in conditions such as illness. The central nervous system has a unique structure that differs from peripheral organs in terms of immune responses. Extensive research in recent years has shown that there is a complex interaction between the brain and immune system. Brain immune system is a complex system consisting of a number of cellular and molecular mechanisms within the central nervous system and a set of structures in which immune cells and molecules take place. It is known that chronic inflammation in the brain may play an important role in progressive neuron death in many neurodegenerative diseases. Alzheimer's disease, which has been affecting the elderly population, especially women in recent years, is a progressive, neurodegenerative disease characterized by problems related to short-term memory, cognition and difficulties in daily living activities. Alzheimer's disease occurs with a complex*

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mechanism thought to include genetic, immune and environmental factors. There is no definite cure for this disease and the drugs used only delay the symptoms. The complement system is part of the innate immune system. This system has three different activating pathways and results in the formation of a membrane attack complex that ultimately causes target cell lysis.
Result and Discussion: *In this review, we aimed to share information about the functioning of the complement system in the central nervous system and how it contributes to chronic neuroinflammation that leads to neurodegenerative disorders such as Alzheimer's disease.*
Keywords: *Alzheimer's disease, central nervous system, complement system, immune system, neurodegeneration*

GİRİŞ

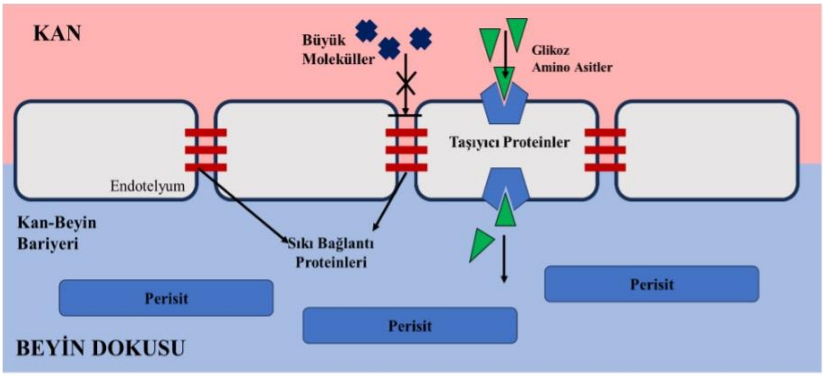
Bağışıklık sistemi, vücudu enfeksiyonlara karşı korumak ve hastalıklara yanıt vermek için tasarlanmıştır. Bununla birlikte, son araştırmalar, beyin ve bağışıklık sistemi arasında daha karmaşık bir etkileşim olduğunu göstermiştir. Beyinde bağışıklık sistemi, merkezi sinir sistemi içinde yer alan bir dizi hücresel ve moleküler mekanizmadan ve bağışıklık hücreleri ve moleküllerinin yer aldığı bir dizi yapıdan oluşan kompleks bir sistemdir [1]. Bu yapılar mikroglia, astrositler ve kan-beyin bariyeridir. Mikroglia, beyindeki en yaygın bağışıklık hücreleridir. Sinir sistemi içinde bulunan özelleşmiş makrofajlardır ve beyindeki enfeksiyonlarla mücadelede önemli bir rol oynarlar. Aynı zamanda hasarlı sinir hücrelerini temizleyerek yeniden yapılanmayı sağlarlar. Astrositler, sinir hücrelerini destekleyen ve koruyan hücrelerdir. Bağışıklık fonksiyonları da vardır ve beyin hasarında inflamasyonu düzenlemeye yardımcı olurlar. Beyin, kan-beyin bariyeri (KBB) adı verilen bir koruyucu bariyer ile çevrilidir. Bu bariyer, kan damarlarını ve sinir hücrelerini ayırarak toksik maddelerin beyne girmesini engeller. Aynı zamanda bağışıklık hücrelerinin ve moleküllerinin beyne kontrollü bir şekilde girişini sağlar. Kompleman sistem, vücudun enfeksiyonlara ve yabancı maddelere karşı savunma mekanizmasını oluşturan bir dizi serum proteini, reseptör ve düzenleyici faktörden oluşur. İmmün yanıtları başlatır, enfeksiyonları temizler ve inflamasyonu düzenler. Beyinde bağışıklık sistemi, normal beyin fonksiyonlarını düzenleme ve koruma görevi de üstlenir. Ancak aşırı aktive olduğunda veya düzensiz bir şekilde çalıştığında, nöroinflamasyon olarak bilinen bir durum ortaya çıkabilir [2,3]. Nöroinflamasyon başta Alzheimer hastalığı, Parkinson hastalığı ve multipl skleroz olmak üzere çeşitli nörolojik hastalıkların gelişiminde önemli bir rol oynayabilir. Alzheimer hastalığı hem bellekte hem de bilişsel işlevlerde yavaş ve ilerleyici bir düşüşle sonuçlanan, özellikle ileri yaşlarda görülen bir nörodejeneratif bozukluktur. Alzheimer hastalığına farklı nörokimyasal ve nöropatolojik değişikliklere yol açan çok sayıda faktör katkıda bulunur. Beyinde kronik nöroinflamasyonun Alzheimer hastalığının patofizyolojisinde önemli bir faktör olabileceği düşünülmektedir [2,3]. Bu derlemede beyin ve kan-beyin bariyeri, kompleman sistem ve Alzheimer hastalığında kompleman kaskadın rolü hakkında bilgi verilmesi amaçlanmıştır. Alzheimer hastalığında nöroinflamasyonun rolünün daha iyi anlaşılması, hastalığın patofizyolojisinin aydınlatılmasında ve yeni tedavi yaklaşımları geliştirilmesinde faydalı olacaktır.

Beyin ve Kan-Beyin Bariyeri

Kan-beyin bariyeri (KBB), dolaşım sistemi ile merkezi sinir sistemi arasında çözünen maddelerin ve kimyasalların transferini düzenleyen, böylece beyni kandaki zararlı veya istenmeyen maddelerden koruyan, yarı geçirgen bir sınırdır. Bu yapı özel beyin endoteli, vasküler perisitler, perivasküler glia ve nöronlar arasındaki iş birliğini sağlar ve bu yapılar birlikte beyin homeostazını korumak için oldukça seçici bir savunma duvarı oluşturur [4]. KBB'nin genel yapısı Şekil 1'de verilmiştir. Sağlıklı beyinde, bu duvar boyunca hücre geçişi son derece sınırlıdır. Bu nedenle, periferik bağışıklık hücreleri beyinden uzak tutulur ve kompleman proteinler dahil olmak üzere proteinlerin çoğunun plazmadan geçişi, beyin parankimi ve beyin omurilik sıvısındaki (BOS) düzeyleri tipik olarak plazmadaki düzeylerin %0,1 ila %1'i arasında olacak şekilde sınırlandırılır [5].

Kan beyin bariyeri, homeostazı sürdürmede ve beyni internal ve eksternal saldırılardan korumada ne kadar önemli olsa da hiçbir şekilde mükemmel bir engel değildir. Sağlıklı beyinde bile, özellikle hipokampus içinde ve çevresinde yer yer bariyerin yeterli olmadığı görülmektedir. Yaşlı beyinde ise,

KBB'nin bariyer özelliğinde sıklıkla aksamalar ve eksikliklerin olduğu bölgelerin varlığı bilinmektedir [6]. Beyni etkileyen hemen hemen her bozukluk ve birçok sistemik hastalık çeşitli derecelerde KBB sızıntısını tetikleyebilir. Reaktif oksijen türleri (ROS) ve doku metalloproteinazlarının aktivasyonu bu süreçte başlıca aracı olarak görülse de KBB'de yıkımına neden olan baskın aracının sistemik inflamasyon olduğu düşünülmektedir [7,8].



Şekil 1. Kan beyin bariyeri

Merkezi sinir sistemi bağışıklık yanıtları bakımından periferik organlardan farklı benzersiz bir yapıya sahiptir. Beyin uzun yıllar immünolojik olarak farklı bir organ olarak kabul edilmiştir. KBB'nin varlığı, geleneksel lenfatik drenajın olmaması ve yabancı dokuların cilt gibi periferik bölgelere aşılandığında kolayca reddedildiği, ancak beyin parankimine aşılandığında daha uzun süre hayatta kaldığı gözlemi bu inanışı güçlendirmiştir. Ayrıca, beyin periferik enflamatuvar reaksiyonlara özgü ağrı ve şişlik yanıtlarını da göstermez. Ancak, herhangi bir kronik enflamasyon süreci sağlıklı dokuya zarar verebilir ve beyin, immünolojik olarak ayrıcalıklı olmak yerine bu açıdan özellikle savunmasız hale gelebilir. Zira, nöronlar post-mitotiktir ve bir kez kaybedildiğinde yerine konamaz. Son yıllarda ortaya konan immünohistokimyasal ve moleküler biyolojik kanıtlar, beynin aktif bir endojen bağışıklık sistemine sahip olduğunu ve beyindeki kronik inflamasyonun, birçok nörodejeneratif hastalıkta ilerleyici nöron ölümünde önemli bir rol oynayabileceğini göstermektedir. Doğuştan gelen bağışıklık sisteminin ve beyinde fagositoz sürecinin önemli bir parçası olan kompleman sistem, patojenlerin ortadan kaldırılması ve hücresel bağışıklık tepkilerinin harekete geçirilmesinde kritik bir rol oynar. Merkezi sinir sisteminde birçok tamamlayıcı protein lokal olarak üretilir ve sinir sistemi gelişimini ve nöral plastisite gibi fizyolojik süreçleri düzenler. Bununla birlikte, anormal kompleman aktivasyonu, Alzheimer hastalığı da dahil olmak üzere nörodejenerasyonla ilişkilendirilmiştir [1,9-11].

Alzheimer Hastalığı

Demans, beyne doğrudan ve dolaylı olarak zarar veren birçok farklı hastalık veya hasardan kaynaklanabilir. Dünya Sağlık Örgütü'ne göre, dünya çapında demansı olan 55 milyondan fazla insan vardır ve buna dünya genelinde her yıl teşhis edilen yaklaşık 10 milyon yeni demans vakası eklenmektedir. Alzheimer hastalığı, demansın en yaygın şeklidir ve vakaların %60-70'ini oluşturmaktadır [12,13].

Alzheimer hastalığı hem bellekte hem de bilişsel işlevlerde yavaş ve ilerleyici bir düşüşle sonuçlanan, özellikle ileri yaşlarda en yaygın görülen nörodejeneratif bozukluktur [14,15]. Temel klinik özelliklerden bazıları, ilerleyici hafıza kaybı, apraksi (hareket ve jestleri gerçekleştirememe), agnozi (insanları, nesneleri ve sesleri tanıyamama ve tanımlayamama), dilde gerileme ve yönetici beyin fonksiyonlarının kaybı gibi davranış değişiklikleridir [14]. Günümüzde henüz Alzheimer hastalığının patofizyolojik süreçlerini ve hatta ilerlemesini durduracak hiçbir tedavi mevcut değildir [14,15]. Alzheimer hastalığı çok faktörlü bir hastalıktır; yaşam tarzı, çevresel ve genetik faktörler gibi çoklu risk faktörlerinin etkileşiminden kaynaklandığı düşünülmektedir. Özellikle 60-65 yaş üstü bireylerde

vakaların çoğunu oluşturan sporadik geç başlangıçlı Alzheimer hastalığının başlaması ve ilerlemesindeki değişikliklere bu faktörlerin arasındaki etkileşimler neden olur [12,16,17].

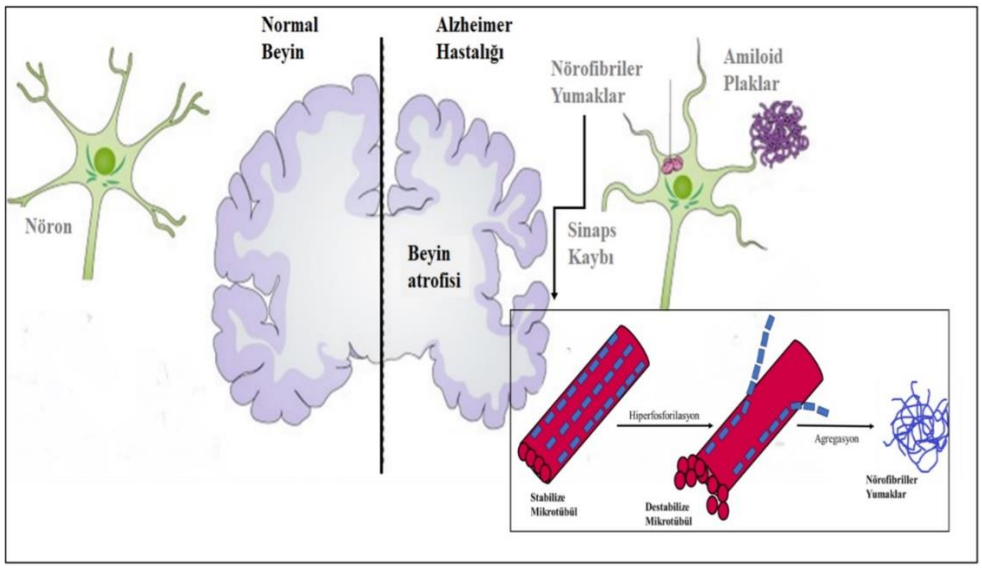
Alzheimer hastalığına yol açan bir dizi nörokimyasal (örn. kolinerjik eksiklikler) ve nöropatolojik [örn. nörofibriler yumaklar (NFT'ler) ve amiloid plaklar] değişikliğe çok sayıda farklı faktör katkıda bulunur. Nörofibriler yumaklar mikrotübüllerle ilişkili hiperfosforile hücre iskeleti proteini Tau'nun fibriler kümeleridir. NFT'ler hem hücre içi hem de hücre dışında bulunabilir. Diğer taraftan, amiloid plakların, amiloid beta (Aβ) peptidin aşırı üretiminden ve/veya azaltılmış klerensinden kaynaklandığı bilinmektedir. Aβ peptit esas olarak beyin parankiminde ve hipokampus ve korteksin leptomeningeal kan damarlarında fibriler yapılar halinde birikir. Aβ peptit, L-sekretaz(lar) ve Q-sekretaz(lar) olarak adlandırılan bazı endoproteazlar tarafından Aβ öncü proteini (LAPP)'dan proteolitik olarak türetilir. Sekretazlar sırasıyla önce LAPP'de Aβ peptidin NH_2-terminalinde, ardından Aβ peptidi salmak için COOH-terminalinde bölünür ve sonuçta 40 veya 42 amino asit uzunluğunda Aβ peptit üretirler. Aβ peptidin 42 amino asit içeren uzun formu, Alzheimer hastalarının beyinlerinin Aβ plaklarında bulunan baskın formdur [1,12].

Alzheimer hastalığında yaş, genetik ve inflamasyonun patolojik süreçte çok önemli olduğuna ilişkin kanıtlar giderek artmaktadır. Ek olarak, kardiyovasküler ve serebral vasküler hastalıklar gibi komorbiditeler veya bunların risk faktörleri, Alzheimer hastalığının klinik belirtilerinin yanı sıra klasik histopatolojik ve nörokimyasal belirteçlerin ortaya çıkışını da modüle ediyor gibi görünmektedir. Son yıllarda, Aβ peptidin merkezi sinir sisteminde fibriler yumaklar şeklinde birikmesinin Alzheimer hastalığının gelişimindeki rolü tartışılmaktadır. LAPP veya presenilin proteinleri (PS1 ve PS2) genlerinde genetik mutasyonların olduğu ailesel Alzheimer hastalığı vakaları, bu düşünceyi destekleyen en güçlü kanıtı sağlamaktadır. Bu tür mutasyonlar, Aβ peptidin üretiminin artmasına neden olur ve Alzheimer hastalığının gelişimine doğrudan yol açabilir. Ancak, Aβ peptidin toksik etkilerini tam olarak nasıl gösterdiği aydınlatılamamıştır. Klinik demans belirtileri olmayan yaşlı bireylerin beyinlerinde büyük Aβ peptit birikimlerinin meydana gelebileceği bulunmuştur. Görünüşte, bu bulgular Alzheimer gelişimi için Aβ peptide ek faktörlere ihtiyaç olduğunu düşündürmektedir. Bu iddia, merkezi sinir sisteminde insan LAPP'sini aşırı eksprese eden transgenik farelerin beyinlerinde aşırı miktarda fibriler Aβ birikintileri oluşmasına rağmen, Alzheimer patolojisinin klasik belirtilerini (yani sinir hücresi ve sinaptik kayıp) göstermiyor olmasıyla da desteklenir. Kontrol deneklerinden Aβ peptid taşıyan beyinler ile Alzheimer hastalarının beyinlerinde bulunan amiloid arasındaki ayırt edici farklılıklardan biri inflamasyon belirteçleridir. Bu nedenle, sürekli nöroinflamasyonun, Alzheimer hastalığının patogenezinde önemli bir faktör olabileceği düşünülmektedir [1].

Nöroinflamasyon enfeksiyon, travma ve nörodejeneratif hastalıklara yanıt olarak merkezi sinir sistemi içinde gelişen bir enflamatuvar yanıt olarak tanımlanabilir. Nöroinflamasyona, astrositler ve mikroglia gibi glial hücrelerin aktivasyonu, sitokinlerin [Interlökin 1 (IL-1), interlökin 6 (IL-6) ve tümör nekroz faktörü alfa (TNF-α)], kemokinlerin [C-C motifli kemokin ligand 2 (CCL2) 2, C-C motifli kemokin ligand 5 (CCL5) ve C-X-C motifli kemokin ligand 1 (CXCL1)] ve ROS'un üretimi ile kompleman sistemin aktivasyonu aracılık eder. Bu enflamatuvar mediatörler, beyin astrositlerinin ve mikroglialarının doğuştan gelen bağışıklık hücreleri tarafından üretilir. Ancak, travma veya yaşlanma KBB'de mekanik bütünlüğün kaybına neden olan bir bozulma varsa, vücudun diğer bölgelerinden de göç edebilirler (Shastri ve ark., 2013). Doğuştan gelen bağışıklık hücreleri tarafından proinflamatuvar mediatörlerin salınması, sinapsların işlev bozukluğuna, nörojenezin inhibisyonuna ve nöronal ölüme neden olabilir. Bu özellikler Alzheimer hastalığında da görülmektedir [18-20]. İnterlökin 4 (IL-4), interlökin 9 (IL-9), interlökin 10 (IL-10), interlökin 11 (IL-11) ve transforme edici büyüme faktörü 1 (TGF-1) gibi anti-inflamatuvar sitokinler de aşırı nöroinflamasyonu önlemek amacıyla homeostatik dengeyi potansiyel olarak korumak için nöroinflamatuvar olaylar sırasında üretilir [18,21,22].

Nöroinflamasyon düşük düzeyde veya kısa süreli olduğunda nöroprotektif etkileri olduğu bilinmektedir. Bağışıklık hücreleri tarafından nörotrofik faktörlerin üretilmesi, inhibitör miyelin kalıntılarının ve toksik maddelerin fagositik temizlenmesi, demir homeostazının korunması, laktat biyoyararlanımının düzenlenmesi, glial hücreler tarafından immün sürveyansı, hücreden hücreye iletimi iyileştirmeye yardımcı olan sinaptik budama ve remiyelinasyon ve doku tamiri bu nöroprotektif etkiler arasında sayılabilir [15,23,24]. Bununla birlikte, nöroprotektif mekanizmalar, mikroglia ve astrositleri yüksek derecede hiperaktif hale getiren ve Alzheimer hastalığının patolojisini şiddetlendirebilen Aß ve

NFT'ler tarafından bastırıldığında kronik nöroinflamasyon meydana gelebilir [24]. Aβ birikmesi, önemli bir nöropatolojik durumdur ve Alzheimer hastalığının patofizyolojisinde önemli bir başlatıcı olaydır. Hipokampuste Aβ plaklarının oluşumu, kısa süreli hafıza işlemeyi etkileyebildiği için kilit öneme sahiptir. Nörotoksik Aβ peptitlerinin birikmesi homeostazın bozulmasına, sinaptik işlev bozukluğuna ve astrosit ve mikroglia hiperaktivasyonuna yol açar. Aβ'nın aşırı artışı ve ortadan kaldırılmasındaki eksiklikler, α-amino-3-hidroksi-5-metil-4-izoksazolepropiyonik asit (AMPA) reseptörlerine ve kalsiyum iyon (Ca^{2+}) kanallarına bağlanmasına ve hücre içi Ca^{2+} artışına neden olur ki bu da zamanla kronik nöroinflamasyona ve mikroglia yoluyla ROS ve kompleman proteinlerin üretimine yol açar [18,25,26]. Alzheimer hastalığının patofizyolojisi Şekil 2'de özetlenmiştir.



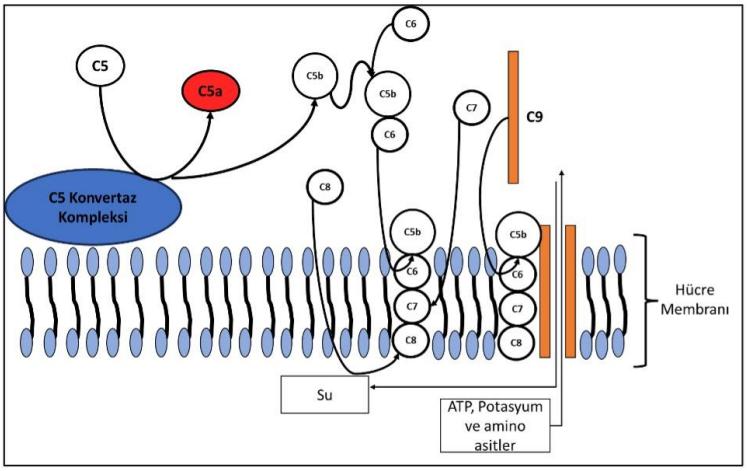
Şekil 2. Alzheimer hastalığı patofizyolojisi

Kompleman Kaskadı

Kompleman kaskadı, patojene özgü yanıtın veya basamaklı doku hasarının oluşmasından önce patojenleri, apoptotik hücreleri ve hücresel kalıntıları hızlı bir şekilde tanımak ve temizlemek için gelişmiş, 40'tan fazla proteinden oluşan doğuştan gelen bağışıklık sisteminin kritik bir efektör mekanizmasıdır. Sistem ayrıca antikor aracılı patojen öldürme ve temizlemede etkindir ve adaptif yanıtın tipini ve kapsamını yönlendirmeye katkıda bulunur [24,27].

Yetişkin veya hasar görmüş beyin dokusunda aşırı kompleman aracılı sinaptik budama (pruning) birçok nörojeneratif bozuklukta zararlı olabilmesine rağmen, bu kaskadın erken bileşenlerinden bazılarının sinir sisteminin gelişimi sırasında sinaps eliminasyonunda faydalı bir rol oynadığı da bilinmektedir [27]. Kompleman sistem, sıralı enzim aktivasyonuna, protein bölünmesine ve indüklenmiş fonksiyon sağlayan protein konformasyonel değişikliklere yol açan üç farklı yol (klasik, alternatif ve lektin) aracılığıyla aktive edilir. Klasik kompleman yolu, C1 kompleksi [kompleman bileşeni 1q (C1q), kompleman 1r2 (C1r2) ve kompleman 1s2 (C1s2)] C1q alt bileşeni aracılığıyla, immün komplekslerdeki antikorun fraksiyon kristalize (Fc) alanına ya da apoptotik hücreler veya fibriler Aß ve hiperfosforile Tau dahil diğer polianyonik maddeler gibi immünoglobulin olmayan aktivatörlere bağlandığında etkinleştirilir. Bu bağlanma, proenzim C1r'nin otoaktivasyonunu indükler, daha sonra başka bir serin proteaz olan C1s'leri ayırır ve aktive eder. Aktive edilmiş C1s, kompleman 4 (C4) bileşenini parçalayarak, kompleman 4a (C4a) ve kompleman 4b (C4b)'yi oluşturur. C4b, aktivatör yüzeyine kovalent olarak bağlanır ve kompleman 2 (C2)'yi alır. C1s daha sonra C2'yi kompleman 2a (C2a) ve kompleman 2b (C2b)'ye ayırır. C4b ve C2b, birden fazla kompleman 3 (C3) proteinini sırayla kompleman 3a (C3a) ve kompleman 3b (C3b)'ye bölebilen C3 konvertazı (C4b2b) oluşturur. C3b ayrıca yüzeye kovalent olarak bağlanır ve dijesyonu (ve ardından ölüm veya degredasyon) için fagositlere

opsonik sinyal sağlar. Parçalanan C3b'nin bir kısmı, klasik yol kompleman 5 (C5) dönüştürücü olan C4b2b3b'yi oluşturan C4b2b ile ilişkili kalır. C5 dönüştürücü daha sonra C5'i kompleman 5a (C5a) ve kompleman 5b (C5b)'ye ayırır. C5b, gözenek oluşturucu C5b, kompleman 6 (C6), kompleman 7 (C7), kompleman 8 (C8) ve kompleman 9 (C9) kompleksinin (C5b-9) oluşumunu başlatır [28-30]. Lektin yolu, tanıma bileşenleri, mannan bağlayıcı lektin (MBL), fikolinler ve kollektinler ve MBL ile ilişkili serin proteazlar, mannozla ilişkili serin proteaz 1/2 (MASP1/2)'nin spesifik karbonhidrat yapılarıyla (konakçıda normal olarak bulunmayan) etkileşime girerek aktive edilmesi dışında klasik yola oldukça benzer. MASP1/2 etkinleştirildikten sonra, C4 ve C2'yi ayırır, böylece C3 parçalayıcı enzimi (C4b2b) oluşur ve kaskatta sonraki adımlar etkinleşir. Alternatif aktivasyon yolu, bölünmüş C3b'nin genellikle bir patojen olan ancak başka yüzeyler de olabilen verici/alıcı bir yüzeye doğrudan bağlanmasının sonucudur. Sonuçta C3 parçalayıcı enzim kompleksi C3bBb ve alternatif C5 parçalayıcı enzim C3bBb3b'nin üretilmesiyle sonuçlanır [31]. Kompleman kaskadının işleyişi Şekil 3'de özetlenmiştir.



Şekil 3. Kompleman sistemin işleyişi

Kompleman sistem enfeksiyona karşı önemli bir doğal bağışıklık savunmasıdır ve dokularda etkin imha sürecine katkıda bulunur. Kompleman sistemin beyinde de aynı homeostatik roller oynaması muhtemeldir. Plazmadaki (ve dokulardaki) çoğu kompleman protein için ana kaynak karaciğerdir; bunun istisnaları, ağırlıklı olarak lökositlerde sentezlenen C1q, properdin ve kompleman 7 (C7) ile yağ dokusunda sentezlenen faktör D'dir [32,33]. Bu istisnalar dışında, çoğu durumda, plazmaya salgılanan ve dokulara sızan hepatik kaynaklı kompleman proteinleri, kompleman immün savunmasının aracılarıdır. Bununla birlikte, birçok organ ve dokuda kompleman proteinlerin çoğunun lokal olarak sentezlenebildiği bilinmektedir. Çoğu durumda bu sentezler karaciğerde gerçekleşenin yanında ihmal edilebilir düzeyde olsa da bazı durumlarda bu lokal üretim çok önemlidir. Organ nakli hastalarında yapılan çalışmalar plazma kompleman proteinlerinin yaklaşık %10'unun çeşitli ekstra hepatik kaynaklarda üretildiği göstermiştir. Bu ekstrahepatik havuza en fazla katkıda bulunan organın böbrek olduğu düşünülmektedir. Lokal olarak üretilen kompleman, dolaşım havuzuna katkıda bulunabilir. Ancak çok daha önemlisi, o organda lokal immün savunma veya sürücü patolojisi sağlayabilir. Böbrekte, lokal komplemanın bu rolü, kompleman eksikliği olan organlar ve alıcılar kullanılarak yapılan transplantasyon çalışmaları ile deneysel hastalık modellerinde gösterilmiştir [5,34,35]. Renal kompleman sentezine dair bu açık kanıtın aksine, sağlıklı beyinde lokal kompleman sentezine dair kanıtlar çok sınırlıdır. Beynin "korumalı" durumu, çoğu plazma proteininin sağlıklı beyinden uzak tutulduğunu düşündürmektedir. Sağlam bir KBB, kompleman proteinlerin periferden beyne erişimini kısıtlamakta veya engellemektedir. Bu nedenle, lokal sentez sağlıklı beyindeki doğuştan gelen bağışıklık savunması için özellikle önemli olabilir. İzole beyin hücrelerinde ve beyinden türetilen hücre hatlarında kompleman protein ekspresyonu üzerine yapılan çalışmalarda mikroglial, astroglial ve hatta nöronal kökenli hücre hatlarının *in vitro* olarak uygun şekilde uyarıldıklarında kompleman proteinlerin çoğunu veya tamamını sentezleyebildiğini ve salgılayabildiğini göstermektedir [5,32,33]. İnsan beyin

dokusunda (çoğunlukla enflamatuvar veya dejeneratif hasar görmüş) kompleman proteinler veya kritik durumlarda lokal sentezi tetikleyecek mesaj kodlayan kompleman proteinler tanımlanmıştır [36-38]. Bir çalışmada, sağlıklı ve Alzheimer hastalarının beyin dokularında C1q, C3 ve C4 için mRNA'ların ekspresyonları karşılaştırılmış ve üçünün de sağlıklı beyinde ifade edildiği, Alzheimer hastalarının beyninde mRNA düzeylerinin üç kat daha yüksek olduğu bulunmuştur [39]. Başka bir çalışmada sağlıklı beyinde C1 alt birimleri, C1 inhibitörü (C1inh), C3 ve C4'ü kodlayan genlerin ekspresyonunun Huntington hastalığına sahip beyinde 2-5 kat arttığı tespit edilmiştir [40].

Alzheimer Hastalığı ve Kompleman Sistem

Kompleman sistemin Alzheimer patofizyolojisindeki önemli rolleri, *in vitro* ve *in vivo* çalışmalardan elde edilen veriler ile desteklenmektedir. Çalışmalar, kompleman sistem proteinlerinin ekspresyonunun ve kompleman sistem aktivasyonunun, Alzheimer hastalarında görülen nöroinflamasyona, nöron ve sinaps kaybına ve ardından nörodejenerasyona yol açtığını ortaya koymuştur [12].

İn vitro çalışmalar, $A\beta_{1-42}$'nin, globüler alanı yoluyla C1q'ye bağlanarak klasik yolu doğrudan aktive edebildiğini göstermiştir. C1q ayrıca C1qA kolajen alanı yoluyla Tau'ya bağlanabilir ve klasik yolu aktive edebilir [41,42]. Bu nedenle, C1q'nun Aβ ve Tau'ya bağlanması nedeniyle kompleman aktivasyonu potansiyel olarak Alzheimer hastalığında nöroinflamasyona ve nörodejenerasyona katkıda bulunabilir [12]. McGeer ve ark. tarafından yapılan bir çalışmada Alzheimer hastalarının beyin dokularında C1q, C3 ve C4'ün immünohistokimyasal boyama ile belirlenebildiği ve bunların Aβ plakları ve NFT'lerle birlikte lokalize olduğu bulunmuştur [43]. Rogers ve ark. tarafından yapılan bir otopsi çalışmasında Alzheimer hastalarının beyin dokuları analiz edilerek kontrol numuneleri ile karşılaştırıldığında Aβ plakları ile C1q, C3 ve C4 ortak lokalizasyon düzeylerinin yükseldiği gösterilmiştir [44]. Başka bir çalışmada Alzheimer hastalarının beyinlerinin temporal korteksinde yüksek C3 ve C4 mRNA seviyeleri gözlemlenmiştir. Alzheimer hastalarının beyin dokularında C3b ve terminal membran saldırı kompleksi (MAC; C5b-C9) ürünleri gibi kompleman sistem aktivasyon ürünleri için spesifik boyama rapor edilmiştir, bu MAC'in potansiyel olarak Alzheimer'da nöronal kayıp ve nörodejenerasyona neden olabileceğini göstermektedir [39].

Kompleman disfonksiyonunun, Alzheimer hastalığı olan bir bireyde klinik semptomlar ortaya çıkmadan onlarca yıl önce nöroinflamasyona ve nörodejenerasyona katkıda bulunması muhtemeldir; bunun nedeni, tamamlayıcı sistemi aşan ve Alzheimer patolojisini yönlendiren Aβ birikimi olabilir [12].

Hayvan modellerinde yapılan çalışmalar, Alzheimer hastalığında kompleman sistemin rolü ile ilgili daha çok bilgiyi açığa çıkarmıştır. C3 genini eksprese etmeyen ($C3^{-/-}$) ve C1q genini eksprese etmeyen ($C1q^{-/-}$) fareler üzerinde yapılan bir çalışmada, öncelikle aksotomi sonrası omurilikte C3 ve C1q komplemanlarının ifadesi araştırılmış, siyatik sinir transeksiyonundan 7 gün sonra, siyatik motonöron havuzunda her iki belirteç için de immünoreaktivite artışı tespit edilmiştir. $C3^{-/-}$ farelerde siyatik sinir ezilme tipi hasarında yabanıl tip farelere kıyasla daha hızlı bir iyileşme görüldüğü gözlenmiştir. C3 eksikliğinin aksotomize motonöronlarda daha fazla sinaptik terminalin korunmasına, bu tür nöronlar tarafından daha büyük bir GAP-43 upregülasyonuna ve daha hızlı bir fonksiyonel iyileşmeye yol açtığı belirtilmiştir [45]. Başka bir çalışmada, kompleman sistemin yaşlanan farelerde sinapslar üzerindeki rolü incelenmiş, $C3^{-/-}$ erkek farelerin, yaş, soy ve cinsiyet uyumlu yabanıl tip farelere kıyasla daha iyi öğrenme, bilişsel aktivite ve hafızaya sahip olduğu gözlemlenmiştir. Yabanıl tip farelerde bölgesel ve yaşa bağlı sinaps kaybı ve bu kaybı izleyen hipokampal bölgede nöron kaybı görüldüğü ancak bu değişikliklerin $C3^{-/-}$ farelerde görülmediği de bildirilmiştir. Bu sonuçlar kompleman protein C3 veya downstream kompleman bileşenlerinin, yaşlanan beyindeki sinaptik fonksiyon ve plastisitede yaşa bağlı ve bölgeye özgü değişikliklere aracılık etmede yeni ve belirgin bir rol olduğunu düşündürmüştür [46].

Bir *in vivo* çalışmada, hipokampus ve serebral kortekste artmış Aβ birikimi düzeyleri olan ve APP'de bir mutasyon taşıyan ($APP_{K670N, M671L}$) transgenik fareler ve patolojik değişiklikleri olmayan ancak $A\beta_{1-42}$ ve $A\beta_{1-43}$ peptitlerinin hafif yüksek düzeylerini bulunduran mutant PS1 transgenik fareler arasında bir melez tür olan PS1/APP fareler kullanılmıştır [47-49]. Bu farelerde, Alzheimer hastalığının amiloid fenotipi için iyi bir model sağlayacak şekilde artmış bir Aβ birikimi ve depolanması görülmüştür [49,50]. Çalışmada C1q'nin Aβ plakları ve aktive edilmiş mikroglia ile birlikte lokalize olduğunu

bildirmiştir [49]. Bu bulgu C1q'nun Aβ plaklarına bağlandığını ve mikroglia aracılığıyla fagositoza neden olduğunu gösteren başka bir çalışma ile de desteklenmiştir [51]. Fonseca ve ark. C1q'nin rolünü, Alzheimer modeli Tg2576 farelerini (APP mutasyonu) C1q geni taşımayan $C1q^{-/-}$ fareler ile çaprazlayarak, Alzheimer hastalığının patolojisi sergileyen ancak C1q'den yoksun $APPQ^{-/-}$ fareler üretmişler ve bunları APP fareleri ile karşılaştırmışlardır. İlerleyen yaşlarda her iki fare türünde de Aβ oluşmuştur. Bununla birlikte, aktive edilmiş mikroglia formlarının, Tg2576 fareleri ile karşılaştırıldığında $APPQ^{-/-}$ farelerinde önemli ölçüde daha düşük olduğu tespit edilmiştir. Bu durum C1q'nun hem mikrogliayı hem de klasik yolu aktive ederek nöronlar üzerinde potansiyel zararlı bir etkiye sahip olabileceğini göstermiştir [52]. Hong ve ark. ailesel Alzheimer hastalığı ile bağlantılı mutasyonları olan (İsveç ve Indiana mutasyonları) ve insan APP (hAPP) genini aşırı eksprese eden transgenik farelerde (J20) kompleman sistem ve mikroglianın rolünü incelemişlerdir. Bu fare modelinde erken yaşlarda çok miktarda amiloid plak oluşmaktadır. Bu transgenik farelerde, yabanıl tip farelere kıyasla yaklaşık 1 aylıktan itibaren klasik kompleman kaskadının başlatıcı proteinlerinden biri olan C1q'nun artış gösterdiği ve bunun amiloid plaklarının oluşumundan önce olduğu tespit edilmiştir. J20 farelerine, C1q artışının çözünür Aβ seviyelerine bağlı olup olmadığının test edilmesi için α-sekretaz inhibitörü olan bir bileşik uygulandığında, Aβ birikiminin azaldığı ve buna paralel olarak C1q düzeylerinde de belirgin bir azalma olduğu görülmüştür. Yabanıl tip farelere C1q artışının çözünür Aβ'ye bağlı olup olmadığını ve eğer öyleyse hangi formda olduğunu belirlemek için çözünür Aβ oligomerleri ve monomerleri enjekte edilmiştir. Doğası gereği prefibriler olan ve Alzheimer hastalığında sinaps kaybı ve işlev bozukluğunun bir aracısı olarak işlev gören oligomerik Aβ C1q birikimini indüklerken, nispeten zararsız monomerik Aβ'de bu durum görülmemiştir. Aβ oligomerleri enjekte edildiğinde yabanıl tip farelerde 72 saat içinde sinaptik yoğunlukta bir kayıp olduğu ancak bu durumun $C1q^{-/-}$ farelerde görülmediği tespit edilmiştir. Bu da oligomerik Aβ'nin neden olduğu sinaptik kaybın C1q'ya bağımlı olduğunu göstermektedir. Sonuç olarak, C1q aracılı klasik kompleman yolağı aktivasyonunun, nörodejenerasyona yol açan downstream yolağı tetikleyen Aβ peptitleri tarafından arttırıldığı bildirilmiştir [53]. Zhou ve ark. fareler üzerinde yaptıkları çalışmada da artan C1q seviyelerinin artan hiperfosforile Tau proteini seviyeleriylepozitif körele olduğunu göstermiştir [54]. C1q proteinin ayrıca sinapslar üzerinde çözünür Aβ oligomerlerinin toksisitesinin yoğunlaştırılmasında rol oynadığı ve bu mekanizma üzerinden de Alzheimer Hastalığı patofizyolojisine katkı sağlayabileceği bulunmuştur [55]. C1q ile bağlantılı olarak sinaps disfonksiyonu ve sinaps kaybı arasındaki ilişkiyi inceleyen bir çalışmada APP/PS1 fareleri ve kontrol olarak yabanıl tip fareler kullanılmıştır ve her iki fare tipinde C1q immünofloresans yöntemiyle işaretlenmiştir. APP/PS1 farelerde mitokondriyal fonksiyonda bir düşüş ve C1q'nun işaretlendiği alanda sinaptik iletime yardımcı olan septin protein yapısında değişiklikler gözlemlenmiştir ki bu da sinaps kaybına kompleman sisteminin aracılık ettiğini düşündürmüştür [56]. Litvinchuk ve ark. yaptıkları çalışmada Alzheimer hastalığına sahip farelerde C3a reseptör inaktivasyonunun tau patolojisini azalttığını göstermiştir [57].

SONUÇ VE TARTIŞMA

Son yıllarda Alzheimer ve diğer nörodejeneratif hastalıklarda inflamasyonun önemini anlamaya ilişkin çalışmalarda önemli bir artış olmuştur. Güçlü bir proinflamatuar ve sitotoksik sistem olan komplemanın merkezi sinir sistemi homeostazında karmaşık rolleri olduğu ve bu hastalıklarda hem koruyucu hem de şiddetlendirici olarak yer aldığı düşünülmektedir. Kompleman sistemin bir yandan amiloid plak oluşumunu kısıtladığını ve plak bileşenlerinin temizlenmesine yardımcı olduğu diğer yandan mikroglia ve astrositlerin patolojiyi yönlendiren aktive nörotoksik hücrelere dönüşmesine de katkıda bulunduğunu gösteren çalışmalar bulunmaktadır. Alzheimer hastalığının patofizyolojisine ilişkin mevcut kanıtlar, kompleman sistemin hem nöroprotektif hem de nöroinflamatuar bir rol oynadığını göstermektedir. Alzheimer hastalığında kompleman sistem amiloid plaklarının oluşumunda ve nöronların hasar görmesinde rol oynar. Aβ, beyinde doğal olarak bulunan bir proteindir, ancak Alzheimer hastalığında anormal miktarlarda üretilir. Amiloid plakları, Aβ proteininin birikmesi sonucu oluşur. Amiloid plakları beynin farklı bölgelerinde birikir ve nöronların işlevini bozar. Kompleman sistem, Aβ proteinine bağlanır ve onu parçalamaya çalışır. Ancak, bu süreç, Aβ'nın daha küçük parçalara ayrılmasına neden olur ve bu parçalar, nöronlar için daha toksik hale gelir. Kompleman

sistemin Alzheimer ve diğer nörodejeneratif hastalıkların patofizyolojisindeki rolünü anlamak tedavi hedefi olarak kullanılması açısından önem taşımaktadır. Çok erken evrelerde uygulanmadıkça kompleman sistemi hedef alan tedavilerin Alzheimer etkilerini iyileştirmesi mümkün görünmese de semptomlar ortaya çıkmadan önce beynin kronik nöroinflamasyondan korunmasının hastalığın ilerlemesini potansiyel olarak değiştirebileceği veya yavaşlatabileceği düşünülmektedir. Kompleman sistemin Alzheimer hastalığındaki rolünün tam olarak anlaşılması hastalığın tedavisi için yeni yaklaşımların geliştirilmesi için de önem taşımaktadır. Bu nedenle bu konuda yapılacak kapsamlı ve mekanistik *in vitro* ve *in vivo* çalışmalara ihtiyaç vardır.

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

TOPLUM ECZACILARININ BİRİNCİ BASAMAK SAĞLIK HİZMETLERİNE ENTEGRE EDİLMESİ: TÜRKİYE İÇİN BİR POLİTİKA ÖNERİSİ

INTEGRATION OF COMMUNITY PHARMACISTS INTO PRIMARY HEALTH SERVICES: A POLICY RECOMMENDATION FOR TURKEY

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ÖZ

Amaç: *Toplum eczacılığı hizmeti, son yıllarda ilaç satış ve dağıtımının ötesine geçerek farmasötik bakım odaklı hale gelmiştir. Toplum eczaneleri, en kolay erişilebilir durumdaki sağlık profesyoneli grubu olması sebebiyle pekçok kişi için sağlık hizmetlerinde ilk ve bazen de tek temas noktası haline gelmiştir. Sağlık insangücü eksikliği, nüfusun yaşlanması, kronik hastalıkların artması gibi pekçok faktör sağlık sistemlerinde birinci basamak sağlık hizmetlerinin sunumu konusunda baskı oluşturmaktadır. Bazı ülkeler birinci basamak sağlık hizmetlerinin sunumunu rahatlatabilmek için toplum eczacılarından daha fazla yararlanmaya başlamıştır. Bu kapsamda, birinci basamak sağlık hizmetlerinde dünya genelinde eczacıların rolünün genişlediği görülmektedir. Bu çalışmanın amacı, toplum eczacılarının birinci basamak sağlık hizmetlerinde aktif olarak kullanılması konusunu Türkiye düzleminde incelemektir.*

Sonuç ve Tartışma: *Sonuç olarak, Türkiye'de toplum eczacılarının birinci basamak sağlık hizmetleri sisteminde aktif olarak yer almadığı görülmüştür. İlerleyen dönemlerde, mevcut ülke örneklerinin de incelenerek gerekli politik zeminin oluşturulmasıyla toplum eczacılarının birinci basamak sağlık hizmetleri sunumuna aktif katılımlarının sağlanabileceği düşünülmektedir.*

Anahtar Kelimeler: *Birinci basamak sağlık hizmetleri, sağlık politikası, toplum eczacılığı*

ABSTRACT

Objective: *In recent years, the community pharmacy profession has moved beyond the sale and distribution of drugs to become pharmaceutical care oriented. Community pharmacies have become the first and sometimes the only point of contact in healthcare for many people, as they are the most easily accessible group of healthcare professionals. Many factors such as lack of health manpower, aging of the population, increase in chronic diseases put pressure on the provision of primary healthcare services in health systems. Some countries have started to benefit more from community pharmacists in order to ease the delivery of primary healthcare services. In this context, it is seen that the role of pharmacists in primary healthcare services is expanding worldwide. The aim of this study is to examine the active use of community pharmacists in primary healthcare services in Turkey.*

Result and Discussion: *As a result, it has been observed that community pharmacists are not actively involved in the primary healthcare system in Turkey. In the following periods, it is thought*

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that the active participation of community pharmacists in the provision of primary healthcare services can be ensured by examining the existing country examples and establishing the necessary political ground.
Keywords: *Community pharmacy, health policy, primary care*

GİRİŞ

Dünya nüfusu hızla yaşlanmaktadır. Yirminci yüzyılda sağlık hizmetlerinde yaşanan gelişmeler, insanların daha uzun ve sağlıklı yaşamasına katkıda bulunmuştur. Ancak yaşam beklentisinin uzaması, demans gibi yaşa bağlı hastalıkları olan kişilerin sayısında da artışa neden olmuştur. Bunun yanı sıra, beslenme biçiminin değişmesi hareketsizlik gibi etkenlerle birlikte kronik rahatsızlıklarda artış yaşanmıştır. Bahsi geçen faktörler birinci basamak sağlık hizmetlerine yönelik artan talep ve baskıya neden olmuştur [1]. Bu baskı, sağlık hizmetleri sürecinde mevcut tüm paydaşların avantajlarından yararlanmak için yeni stratejilerin uygulanmasını gerektirmektedir. Bu kapsamda, toplum eczacıları grubunun, toplumdaki kronik sağlık problemlerinin bakımıyla ilgili pekçok faaliyeti tamamlayarak birinci basamak sağlık hizmetleri üzerindeki baskıyı hafifletebileceği düşünülmektedir [2].

Sağlık harcamalarının sürdürülebilirliğine ilişkin endişeler, toplum eczacılarının hasta merkezli sorumluluklarının genişletilmesi yönündeki ilgiyi artırmıştır. Toplum eczacıları, sağlık insangücünün önemli bir bölümünü oluşturmaktadır. Özellikle birinci basamak hekim ve hemşirelerinin yoğunluğu göz önüne alındığında, bazı ülkelerde politika yapıcılar, ilaçların doğru ve maliyet etkili kullanımına katkı sağlayabilecek diğer sağlık profesyonellerinin potansiyelinden de yararlanmaya başlamıştır [3]. Eczacılık uygulaması, ilaç sunumunun geleneksel rolünden, bugün farmasötik bakım olarak bilinen yöntemle hastaya daha iyi hizmetler sunmayı içeren daha hasta merkezli ve sonuç odaklı bir modele doğru evrilmiştir.

Bu çalışmanın amacı, birinci basamak sağlık hizmetlerinde toplum eczacılarının kullanımı konusuna dikkat çekerek Türkiye için uygulanabilirliğini incelemektir. Bu kapsamda öncelikle toplum eczacılığı kavramı açıklanmış ve toplum eczacılığının gelişimine yer verilmiştir. Daha sonrasında toplum eczacılarının birinci basamak sağlık hizmetlerinde kullanımının faydaları ve karşılaşılan engellere değinilmiştir. Çalışmanın sonraki bölümünde dünya genelinde toplum eczacılarının birinci basamak sağlık hizmetlerinde kullanımıyla ilgili bilgiler yer almaktadır. Son olarak Türkiye'deki mevcut durumdan bahsedilerek bazı önerilerde bulunulmuştur. Çalışmanın, toplum eczacılarının birinci basamak sağlık hizmetleri için önemi konusunda farkındalık oluşturarak gerekli politik müdahalelerin hayata geçirilmesi için politika yapıcılara fikir sağlayacağı düşünülmektedir.

Toplum Eczacılığı Kavramı ve Toplum Eczacılığının Gelişimi

Eczacıların profesyonel rolleri ve sorumlulukları, tarihsel olarak, ilaç hazırlama ve dağıtma odaklı olmaktan genişletilmiş farmasötik bakım hizmetlerine doğru evrilmiştir [4]. Farmasötik bakım, eczacıların *"hastanın ilaç tedavisinden olumlu sonuçlar elde etmesini sağlamak için mümkün olan her şeyi yapmaya söz verdikleri"* bir eczane uygulaması felsefesidir [5].

Uluslararası Eczacılık Federasyonu (International Pharmaceutical Federation-FIP)'na göre toplum eczacılığı küresel anlamda evrim sürecindedir. Bu sürece katkıda bulunan belli başlı faktörler şu şekilde sıralanabilir [6]:

- Sağlık sistemleri ve hastaların değişen ihtiyaçları,
- Covid-19 pandemisinin yarattığı etki,
- Hızlı yanıt üretme ve adaptasyon ihtiyacı,
- Öz bakımda yeni akımlar,
- Yeni teknolojilerin ve dijital unsurların ortaya çıkması,
- Hukuki modellerde yeni akımlar,
- Mesleki hizmet ve ödeme modellerindeki yenilikler,
- Toplum eczanelerinin birinci basamak sağlık hizmetlerinde artan rolü.

Küresel anlamda eczacıların hasta bakımındaki rolü son otuz kırk yıl içinde değişmiştir [7]. Son yıllarda eczacıların profesyonel figürü, rolleri, görevleri ve sorumluluklarının kademeli olarak genişlediği bilinmektedir. İlk dönemlerinde, ürün odaklı, hastaya dönük ve reçeteli ilaç dağıtımından

oluşan eczacı rolleri, daha sonrasında hizmetlere dayalı ve hasta merkezli bir yöne doğru evrilmiştir. Günümüzde ise, hasta danışmanlığı ve ilaç dağıtımının ötesinde hizmetlere doğru genişleyen bir hale gelmiştir [8]. Tablo 1'de görüldüğü üzere günümüzde toplum eczacılarının görev alanı oldukça genişlemiştir.

Tablo 1. Hizmet Kategorileri ile Toplum Eczaneleri [9]

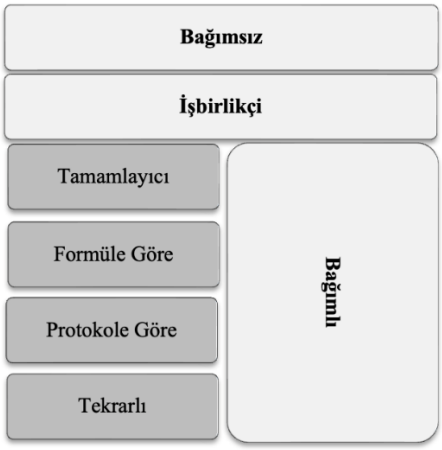
Dağıtım Hizmetleri	Gece hizmeti
	Reçeteyle satılan ilaçların olağanüstü durumlarda reçetesiz tedariki
	Reçeteyle satılan ilaçların acil durumlarda reçetesiz tedariki
	Kronik hastalıklarda reçete yenileme ve dağıtma
	Bağımsız reçetelendirme
	Jenerik / eşdeğer önerme
	Güvenlik sebebiyle ilaç vermeyi reddetme
	Eve teslimat hizmeti
Sağlıklı Yaşamı Teşvik Eden Hizmetler	Miadı dolmuş veya istenmeyen ilaçların imha ve idaresi
	İğne/şırınga değişimi
	Seyahat gibi durumlarda hastalara sunulan ilaç-eczacılık hizmeti
	Eczacı tarafından aşı uygulaması
	Kilo kontrolü
	Sigara bıraktırma
	Sağlık eğitimi
Tarama ve Sevk Hizmetleri	İlaç kullanmayan risk altındaki bireylerin taranması (Ör: diyabet, hipertansiyon, lipid bozuklukları, HIV, Hep B, Hep C, kolon kanseri, COVID 19)
	Ek izleme altındaki ilaçlar için farmakovijilans (Ör: siyah üçgen ilaçlar için tarama soruları)
	Diğer sağlık hizmeti sağlayıcılarına sevk
	Son kullanma tarihi geçmiş veya istenmeyen ilaçların ele alınması ve imhası
Hastalık Yönetimi Hizmetleri	Enjektabl ilaçların uygulanması (Örn: antibiyotikler)
	Denetimli ilaç kullanımı
	İlaçların, ilaç kullanımında kullanılan cihazların, kendi kendine izlem cihazlarının kullanım eğitimleri
	İlaç tedavisine uyum desteği
	Eczacılar tarafından telekonsültasyon
	Kronik hastalıkların yönetimi (Ör: hipertansiyon, hiperlipidemi, diyabet, astım, KOAH, yeni oral antikoagülanlar)
	Diğer: hastalara koşullar/tedaviler hakkında bilgi verilmesi
Kişiye Özgü Tedavi Yönetimi Hizmetleri	Reçetelendirilmiş tedavinin ayarlanması (doz, formülasyon vs değişimi)
	Doz uygulama yardımı
	İlaç incelemesi
	Evde ya da bakımevinde ilaç incelemesi
	İlaç uzlaşısı
	Terapötik ikame
	İlaç kesme/ ilaç vermeme (Örn: benzodiazepinler, antidepresanlar)
	Birinci basamak sağlık hizmeti ile entegre bakım yolları/protokoller/kalite çemberleri
	Yüksek maliyetli ilaç dağıtımı ve yönetimi (Ör: onkoloji, HIV, romatoid artrit, multiple skleroz)
	İlaç doz titrasyonu (Ör: insülin)
	Majistral formülasyon
Sağlık Teknolojisi Değerlendirmesine Dayalı Hizmetler	Yeni ilaçların etkinliğine dair veri toplama

Kronik hastalık yönetiminde pek çok hasta, tavsiye almak ve semptomlarını tedavi etmek veya hafifletmek ya da kronik sağlık problemlerinin ilerlemesini önlemek için ilaç almak üzere düzenli olarak

eczaneleri ziyaret etmektedir. Son derece erişilebilir olarak görülen toplum eczanelerinin ve eczacıların [10,11] toplum sağlığının iyileştirilmesine açık bir rolü bulunmaktadır [12]. Eczacılık mesleği dünya genelinde pek çok ülkede astım, artrit, mental rahatsızlıklar, diyabet, kardiyovasküler rahatsızlıklar ve osteoporoz gibi kronik durumların yönetimi konusunda sağlık sistemleri için destekleyici işlev görerek daha hasta merkezli bir hizmet sunumuna doğru ilerlemektedir [13]. Eczacılık hizmetlerinin madde kullanımı, enfeksiyon kontrolü, eğitim ve tedavi, bağışıklama, sigarayı bırakma, koroner kalp hastalığı, cilt kanseri önleme, cinsel sağlık, aile planlaması, ruh sağlığı, kaza yaralanmalarını önleme, diyabet, kolestrol yönetimi, beslenme ve fiziksel aktivite gibi alanlarda sağlığın geliştirilmesine katkı sağladığına ilişkin kanıtlar bulunmaktadır. Sağlığın geliştirilmesi ve teşviki, toplum eczacısının rolünün bir parçası olarak açıkça tanımlanmamıştır. Bununla birlikte, sağlığın teşviki ve geliştirilmesi unsurları, genel sağlık danışmanlığı, hastalara ilaçlar ve kendi kendine ilaç tedavisi konusunda danışmanlık ve sağlık eğitimi gibi görevlere dahil edilir. Sağlık hizmet sistemlerinde devam eden değişim nedeniyle, profesyonel eczacı dernekleri, mesleği toplumun halk sağlığı ihtiyaçlarına daha iyi hizmet edecek şekilde yirmi birinci yüzyılda etkili bir şekilde konumlandırmanın bir yolu olarak sağlığın teşviki ve geliştirilmesi ve hastalıkların önlenmesi ile giderek daha fazla ilgilenmeye başlamıştır [14].

FIP [15], kronik hastalık yönetiminde farmasötik bakımı da içeren eczacıların rolüne ilişkin bir bildiri yayınlamıştır. Farmasötik bakım, eczacılara kronik hastalıkları olan kişilerin bakımında daha büyük bir role sahip olma fırsatı sunmaktadır [16]. Farmasötik bakımda eczacılar, hasta için ekonomik, klinik ve insani sonuçları iyileştirecek en uygun bakımı sağlamak için hasta ve diğer sağlık profesyonelleriyle iş birliği yapmaktadır [5]. Bu hizmet anlayışı, ilaç sağlamanın ötesine geçerek hastaya değer katan diğer hizmetleri sunmaya geçtiği anlamına gelmektedir. Bu katma değerli eczacılık hizmetleri, hastaların sağlıkları için faydalı olan ve farmasötik bakım sonuçlarını iyileştiren geleneksel ilaç verme ve profesyonel danışmanlık hizmetlerine ek olarak hastaların aldığı kaliteli hizmetlerdir. Diğerlerinin yanı sıra ilaç tedavisi yönetimi, bağışıklama hizmetleri, yaşam tarzı yönetimi, hastalık tarama ve yönetim programları gibi bu hizmetler, hastanın geleneksel durumunu değerlendirmek için bir fırsat sunar ve daha uygun sağlık maliyetleri ile klinik ve insancıl sonuçlar da dahil olmak üzere artan farmasötik bakım ile ilişkilendirilmiştir [17-19].

Eczacılık hizmetlerinin gelişimiyle birlikte, bazı ülkelerde eczacılar reçete yazma yetkisi kazanmıştır. Dünyadaki toplum eczacı uygulamaları bölümünde bu uygulamaya geçen ülkelere yer verilmiştir. Literatürde eczacılar için farklı reçete yazma modelleri tanımlanmıştır. Şekil 1'de gösterildiği üzere eczacı reçete yazma türleri bağımlı, bağımsız, işbirlikçi ve ortak olarak sınıflandırılmaktadır. Eczacıların ilaç reçeteleme konusunda en fazla özerk olduğu bağımsız reçetelemedir ve bu reçeteleme türünde eczacı ve hekim arasında işbirlikçi bir uygulama ilişkisi bulunmaktadır. Buna göre, hekim hastaya teşhisi koyar ve ilk tedavi kararlarını verirken, eczacı da uygun şekilde tedaviyi seçer, izler, değiştirir, devam ettirir veya sonlandırır. Bağımlı reçete yazma, ilaç reçetesini protokollere veya formüllere göre sınırlandırarak tıbbi olmayan reçete yazan kişiye daha fazla kısıtlama getirmektedir. Bağımlı reçete yazmanın protokole göre reçete yazma, formüle göre reçete yazma, tekrarlı reçete yazma ve tamamlayıcı reçete yazma gibi farklı türleri bulunmaktadır. Protokolle reçete yazmada, yazılı bir kılavuz, tıbbi olamayan profesyoneller tarafından gerçekleştirilebilecek faaliyetleri açık ve ayrıntılı bir şekilde açıklar. Protokol, uygulayıcının reçete edebileceği hastalıkların ve ilaç sınıflarının sınırlı bir listesini içerir. Protokol ayrıca ilaçları önerilen dozlarla birlikte tercihli sıraya göre listeleyebilir ve doz değişikliğinin ne zaman düşünülmesi gerektiğine ilişkin öneriler sunabilir. Ayrıntılı protokoller ayrıca hasta için yapılması gereken laboratuvar testleri veya tanısal testler gibi ek klinik bilgileri de içerir. Formüler reçetelemede, tıbbi olmayan reçete yazanlar, belirli tıbbi durumlar için önceden tanımlanmış bir ilaç listesinden reçete yazabilmektedir. Bu durumda listede yer almayan ilaçlar reçete edilemez. Tekrar reçete yazma, kliniklerdeki eczacıların doktorlarıyla bir sonraki randevularından önce reçeteye devam etmesi gereken hastalara reçete yazdığı bir reçeteleme hizmetidir. Ek reçete yazmada, hekimin ilk değerlendirmeyi üstlendiği ve eczacının, hekimin belgelenmiş bakım planına uygun olarak reçete yazdığı, hekim ve eczacı arasında gönüllü bir ortaklık mevcuttur. Bakım planı, hekim ve hasta tarafından üzerinde anlaşılan tedavi seçeneklerini açıkça özetlemektedir [20-23].



Şekil 1. Eczacı Reçete Yazma Modellerinin Özerkliği [20]

Eczacılar, sağlık sisteminde yeni roller üstlenmek için gerekli yeterliliklere sahiptir. Geleneksel rolleri olan ilaçların dağıtımının yanı sıra, hasta merkezli sorumluluklar üstlenmek, mesleğin kapsamlı eğitimi ve profesyonelliği ile uyumludur. Eczacıların eğitim gereklilikleri ülkelere göre farklılık gösterse de resmi müfredat eğitiminin toplam süresi genellikle beş veya altı yıl olup, uzmanlaşma için ek iki veya üç yıllık bir süre istenmektedir. Bunun yanı sıra, eczacılara çalışma hakkı verilmeden önce genellikle ulusal veya bölgesel bir sınav yoluyla lisans alınması gerekmektedir. Klinik sağlık hizmetlerine entegre çalışan ve hastalara sundukları sağlık hizmeti karşılığında ödüllendirilen hastane eczacılarına karşılık, toplum eczacıları geleneksel olarak ilaç satış ve dağıtımında birinci basamak sağlık insangücünün geri kalanından ayrı olarak çalışmaktadırlar. Bu kapsamda, toplum eczacılarının sundukları sağlık hizmeti karşılığında ödüllendirilmeyen tek sağlık profesyoneli grubu olduğunu söylemek mümkündür [3]. Toplum eczacılarının birinci basamak sağlık hizmetlerinde kullanılmasının sağlık sistemleri için pek çok faydası bulunmaktadır. Bu faydalara bir sonraki başlıkta yer verilmiştir.

Toplum Eczacıların Birinci Basamak Sağlık Hizmetlerinde Üstlenebilecekleri Yeni Roller ve Bu Rollerin Sağlık Sistemi ve Hasta Sağlığı Üzerine Faydaları

Toplum eczacıları, sağlığı geliştirerek, hastalıkları önleyerek ve olumsuz olaylardan kaçınarak hastaların sonuçlarını iyileştirme ve bakım maliyetlerini azaltma potansiyeline sahiptir [24]. Eczacılar, hekim ve hemşirelerden sonra nicelik açısından dünyanın üçüncü sağlık profesyoneli grubudur [25]. Toplum eczacıları bazen hastaların sağlık sistemiyle ilk ve tek teması olabilmektedir [26] ve genellikle tüm sağlık profesyonelleri arasında en erişilebiliri olarak tanımlanmaktadır [27,28]. Mekânsal yakınlığın sağlık hizmetlerine erişimi ve genel sağlık sonuçlarını iyileştirdiği gösterildiğinden, toplum eczacılarının birinci basamak sağlık hizmetine katkılarını en üst düzeye çıkarmanın evrensel sağlık kapsamına ulaşılmasını önemli ölçüde artıracağı çıkarımı yapılabilir [29-31].

Eczacılar, aşılar, sağlığın teşviki ve geliştirilmesi, sağlık eğitimi, hasta ve ilaç danışmanlığı, ilaç tedavisi uzlaşması, hastalık öz yönetimi eğitimi, taramalar ve acil durumlara hazırlık ve afet yönetiminde müdahale dahil olmak üzere çeşitli yollarla halk sağlığının iyileştirilmesine katkıda bulunmuştur [32,33] ve bulunmaya devam etmektedir. Eczacılar, yetersiz ilaç kullanımı ve yan etkiler nedeniyle ortaya çıkan hastaneye yatışlar ve acil servis ziyaretleri gibi pahalı tedavi biçimlerinin oluşumunu azaltarak sağlık hizmetleriyle ilişkili maliyetlerin düşürülmesine yardımcı olurlar [34].

Bulunabilirlik, erişim, rahatlık ve maliyet temelinde bazı araştırmacılar, küçük rahatsızlıkların tedavisi için toplum eczanelerinin kullanımının, pratisyen hekimlerden randevular gibi daha maliyetli sağlık hizmeti seçeneklerine olan talebi azaltarak sağlık kaynaklarının optimize edilmesine yardımcı olabileceğini ileri sürmüştür [35-37].

Eczacıların hastaneye yatışlar, acil servis başvuruları ve aile hekimine başvuruları azalarak sağlık hizmet kullanımını azalttığını gösteren çalışmalar mevcuttur [38-41]. Birleşik Krallık'ta yapılan bir araştırma, aile hekimliğinin %13, acil servis saatlerinin ise %5'inin toplum eczanesi ortamında

yönetilebilecek küçük rahatsızlıklara harcandığını göstermiştir [42]. Yine sağlık profesyonelleri arasındaki iş birliğinin çalışan memnuniyetini ve daha uygun ilaç kullanımı yoluyla maliyet tasarrufunu artırdığı gösterilmiştir. Özellikle birinci basamak sağlık hizmetlerinde eczacı hekim iş birliklerinin; kolestrol düşürme ve kardiyak risk azaltma, kan basıncı kontrolü, diyabet yönetimi, kalp yetmezliği yönetimi, depresyon, ağrı, astım kontrolü ve palyatif bakım açısından başarılı sonuçlar sağladığı bildirilmiştir [43].

Bazı çalışmalar da toplum eczanelerinin hastalığa özgü hizmetler sunması ve bu hizmetlerin erişilebilirliği ve rahatlığı nedeniyle kullanıcılar tarafından da genel olarak olumlu karşılandığını göstermiştir [44-46]. Toplum eczaneleri, hizmetlerini kullanan nüfusun yüksek hacmi nedeniyle sağlığın teşvik ve geliştirilmesi için oldukça uygun ortamlar olarak kabul edilmektedir [47]. Avustralya'da, toplum eczaneleri, nüfusun %90'ından fazlasının her yıl en az bir kez ziyaret etmesiyle en erişilebilir merkezlerdir [48]. Gelişmiş ülkelerde, toplum eczacıları sağlık danışmanları ve genel halk için son derece güvenilir sağlık bilgileri kaynakları olarak tanımlanmıştır [14].

Ebola ve Zika gibi küresel sağlık güvenliği riskleri oluşturan pek çok pandemide ve sağlık krizlerinde olağanüstü roller üstlenen eczacılar sağlık hizmetlerinin ayrılmaz bir [49]. Aynı şekilde COVID-19 pandemisinin önlenmesi, hazır olma durumuna ve müdahalesine katkıda bulunarak toplum eczacıları bu krizle başa çıkmada halk sağlığına yönelik rollerini yerine getirmişlerdir [50]. Pek çok ülkede eczaneler, halka erişimi artırmak ve ilaçların eve teslimini sağlamak için uluslararası insani yardım kuruluşları ve yerel toplum çalışanlarıyla yakın iş birliği içinde çalışmıştır [51-52]. Pandemi ve kriz durumlarında eczacıların sağlık sistemlerine katkılarına dünya genelindeki toplum eczacılığı uygulamaları başlığında ayrıntılı olarak yer verilmiştir. Bu bölümde, toplum eczacılarının birinci basamak sağlık hizmetleri için faydaları açıklanmıştır. Bahsedilen faydalar, toplum eczacılarının sağlık sistemlerinde daha aktif rol alması için önemli argümanlar oluştursa da bu konuda bazı engellerin bulunduğu görülmektedir. Söz konusu engellere bir sonraki başlıkta yer verilmiştir.

Toplum Eczacılarının Birinci Basamak Hizmetlerde Aktif Rol Almasının Önündeki Engeller

Eczacıların kronik hastalık yönetimindeki gelişen rolüne rağmen, sağlık hizmeti profesyoneli olarak yeterince kullanılmadıkları görülmektedir [53]. Bu yetersiz kullanımın birçok sebebi vardır ancak en temel sorun, hastaların eczacıların sahip olduğu becerilerin genişliği konusunda farkındalığının olmamasıdır [54,55].

Eczacılar bazı sağlık hizmetleri kullanıcıları için ilk temas noktası olabilseler de nispeten az kullanılan bir kaynaktırlar ve son sağlık hizmetleri politikalarında neredeyse görünmez konumdadırlar [56-58]. Toplum eczacılarının rolünün genişletilmesi pek çok ülkede önemli bir politika hedefi olsa da bu tür politikaların kanıt temeli kapsamlı bir şekilde değerlendirilmemiştir. Konu ile ilgili pek çok sistematik derleme mevcuttur ancak bunların politika oluşturma açısından yeterli olup olmadığı açık değildir [27].

Diğer sağlık profesyonelleri ile zayıf iş birliğine ve eczacılar tarafından sağlanan çeşitli hizmetlere ilişkin ortak vizyon eksikliğine ek olarak, ücretlendirme eksikliğinin, eczacıların hizmetlerinin yetersiz kullanılmasına önemli bir katkıda bulunduğu tespit edilmiştir [59, 3]. Düşük ve orta gelirli ülkelerde toplum eczacılarının kronik hastalık yönetimi sürecine katkıları konusunda bazı temel engeller bulunumaktadır. Okoro ve Nduaguba [16] bu engelleri şu şekilde sıralamıştır:

- Yasal düzenlemelerin eksikliği,
- Toplum eczacısı sayısının yetersizliği,
- Kronik hastalık yönetimine ilişkin özellikli eğitimin eksikliği veya yetersizliği,
- Hekimlerle iş birliğine dayalı uygulamaların eksikliği,
- Klinik hizmetler için geri ödeme mekanizmalarının eksikliği.

Hastaların toplum eczacılarını birinci basamak sağlık hizmeti sunucularına göre 1,5 ile 10 kat daha sık kullandıkları tahmin edilmektedir [60]. Erişilebilir olmalarına rağmen toplum eczacılarından klinik hizmet sağlama konusunda yeterince yararlanılmadığı görülmektedir. Tıbbi kayıt verilerinin eksikliği ve toplum eczanesi uygulamaları ile reçete yazan aile hekimleri arasındaki koordinasyonsuzluk, toplum ortamında çalışan bir eczacının sağlık sistemi üzerinde anlamlı değişim yaratmasını zorlaştırmaktadır [61].

Dünya Genelinde Toplum Eczacılığı Uygulamaları

Tıbbi olarak yönetilebilen durumlar için, tanıyı genellikle durumu tedavi etmek veya durumla ilişkili semptomları hafifletmek için ilaçların reçete edilmesi takip eder. Geleneksel olarak reçete yazma eylemi hekimlerle ilişkilendirilmiştir. Hekim dışı reçete yazma; reçete yazma haklarının hemşireler, eczacılar, göz doktorları ve podologlar da dahil olmak üzere diğer belirli mesleklere genişletilmesidir. Bu reçete yazma sistemi, başlangıçta hastaların ilaçlara erişimini iyileştirmeye yardımcı olmak ve hekimlerin üzerindeki iş yükünü hafifletmek amacıyla ilaçların reçetelenmesi, temini ve uygulanması konusunda daha esnek bir sistem sağlamak amacıyla uygulanmaya konulmuştur [20,21,62].

Uluslararası pek çok kuruluş ve ulusal düzeyde bazı eczacı birlikleri, eczacılığın geleceği ve eczacıların rolü hakkında vizyon ifadeleri ve stratejik planlar geliştirme sürecindedir. Genel görüş, toplum eczanesi ve eczacılığının geleceği konusunda, her tür ilacın dağıtımını ve tedarikini sürdürürken, ücretli profesyonel hizmetlerin de uygulanması yönündedir. Toplum eczaneleri, hastalar ve tüketiciler için "sağlık merkezi" ve "birincil sağlık hizmeti varış noktası" olarak konumlandırılmıştır [63]. Dünya üzerinde toplum eczanesi profesyonel hizmetleri, farklı hizmet türlerini kapsayacak şekilde tanımlanmaktadır [18].

"Birinci basamak eczacıları" olarak tanımlanan toplum eczacılarının, kronik hastalıkların yönetimi dahil olmak üzere birinci basamak sağlık hizmetlerinin sunulmasına katkıları artık dünya genelinde kabul görmeye başlamıştır [56, 64-67]. Bu hizmetleri, İskoçya'da küçük rahatsızlıkların çözümü, Avustralya ve bazı Avrupa ülkelerinde aşılama ve bağışıklama, Birleşik Krallık'ta hastalık taraması, Kanada, İngiltere, Amerika Birleşik Devletleri ve Avustralya'da birçok türde ilaç incelemesi ve ilaç yönetimi ve pek çok ülkede görüldüğü gibi afet ve salgın müdahaleleri şeklinde özetlemek mümkündür [68-70]. Toplum eczanelerindeki farmasötik hizmetler, gelişmiş ülkelerde sağlık sistemlerinin temel ve bütünleşmiş parçaları haline gelmiştir. Örneğin, Avrupa'da bu tür eczacılık hizmetleri gelişmeye ve pek çok ülkede yayılım göstermeye devam etmektedir [71]. Bununla birlikte, gelişmekte olan ülkelerde ise eczacılık hizmetleri geleneksel anlamdaki ürün odaklı rolünü muhafaza etmektedir [72].

Son yirmi yılda dünyanın çeşitli ülkelerinde tıbbi olmayan reçete yazmaya izin veren mevzuat değişiklikleri meydana gelmiştir [73,74,22]. Eczacıların reçete yazması şu anda Kanada, Yeni Zellanda, Birleşik Krallık ve ABD'de yasaldır [62, 23]. Birleşik Krallık'ta 2003 yılında sınırlı reçete yazma hakkı getirilmiş, bunu 2006 yılında bağımsız reçete yazma takip etmiştir [20,75].

Büyük Britanya'da (İngiltere, İskoçya, Galler) eczacılar birkaç yıldır aile hekimlerinin yanında ileri düzeyde çalışmaktadırlar [76,77]. Yakın zamana kadar toplum eczacılarının ileri düzeyde uygulama yapma yeteneği, ilaç tedarikini teşvik eden toplum eczanesi sözleşmeleri nedeniyle büyük ölçüde sınırlıydı [78]. Bununla birlikte, toplum eczacılarının uygulama kapsamı son on yılda ücretsiz reçete almaya uygun hastaların reçetesiz muayene sonrasında ücretsiz ilaç aldığı, kamu tarafından finanse edilen küçük rahatsızlıklar programlarını (İskoçya'da "Önce Eczane"- "Pharmacy First" in Scotland), yeni ilaç hizmetleri ve ilaç kullanımı incelemelerini kapsayacak şekilde genişlemiştir [79,80]. Toplum eczacıları ayrıca kronik hastalıkları olan hastaları desteklemiş ve acil ve öncelikli sağlık hizmetlerinin yerini başarıyla almıştır [81-85]. İngiltere'de, toplum eczacılarına, halka ve diğer sağlık profesyonellerine kanıta dayalı ve maliyet etkili destek sağlamak için güvenilmektedir [86,87]. İngiltere'de 2020 yılında Aile Hekimliği Toplum Eczacısı Danışma Hizmeti (GP CPCS) başlatılmış ve bu hizmet, bazı kronik koşullara sahip hastaların doğrudan eczaneye yönlendirilmesine olanak tanımıştır [88]. 2020 yılında, eczacıların bağımsız reçete yazma uzmanlığını kullanarak, Pharmacy First kapsamı dışında kalan akut yaygın rahatsızlıkları olan hastalar için Pharmacy First Plus, İskoçya'da hayata geçmiştir [89,90].

Ürdün'de Sağlık Bakanlığı, eczacıların lisanslı olması, Ürdün Eczacı Birliği'ne kayıtlı olması, iyi derecede İngilizce bilmesi ve iyi bir hasta takip ve doğruluk becerilerine sahip olması gereklilikleri ile devletin birinci basamak sağlık merkezlerinde çalışması için eczacılar istihdam etmektedir. Bahsi geçen gereklilikler dışında eczacılardan her türlü tıbbi malzemeyi kullanabilmesi, eczacılık mesleğini icra ederken uluslararası standartları uygulayabilmesi, mesleğin tabi olduğu yönetmelik ve kanunları iyi bilmesi, yerel sivil savunma ve kendi alanlarıyla ilgili teknik kurslara ek olarak kardiyopulmoner

resüsitasyon (CPR) gibi kurslara da katılması, iletişim ve kişilerarası becerilere sahip olması istenmektedir [91,92].

Gelişmiş ülkelerdeki durumun aksine, gelişmekte olan ülkelerdeki eczacılardan hala yeterince yararlanılmadığı görülmektedir. Bu ülkelerde eczacılar sağlık sistemine katkıda bulunmadaki rollerinin tanınması için mücadele etmektedirler [93]. FIP, gelişmekte olan ülkelerde iyi eczacılık uygulamalarının başarılması için Dünya Sağlık Örgütü (WHO) tarafından tanınan kılavuzları benimsemiştir [14].

Düşük ve orta gelirli ülkeler (LMIC), hastaların konsültasyon için doktor ücretlerini karşılayamadığı durumlarda eczacıların desteğine daha fazla ihtiyaç duymaktadır. Standart bir tedavinin yokluğunda, COVID-19 vakalarını yöneten eczacılar tarafından farmasötik bakım sağlanmasının önemi kat kat artmıştır [51,94]. Bulaşıcı hastalık ve salgın durumlarında eczacılar, hastaları test için uygun sağlık kuruluşuna yönlendirebilir, onları daha hızlı tedavi aramaya teşvik edebilir ve hastalığın yayılmasını sınırlamak için bilgi sağlayabilir [95]. Ancak pandemi gibi halk sağlığı krizleri sırasında eczacıların rolü iyi tanımlanmamıştır [96,97]. Eczacılık uygulamalarının kapsamının son 20 yılda afetler sırasında genişlediği ve lojistiğin ötesine geçtiğini gösteren çalışmalara rağmen, eczacıların önceki sağlık krizlerindeki klinik rolleri yeterince tanınmamıştır [98]. Hastane ve toplum sağlığı hizmetleri arasındaki genel koordinasyon eksikliği, toplum eczacılarının sağlık acil durum müdahalesine entegrasyonu ve bu krizler sırasında klinik hizmetlerin sağlanması konusunda bir zorluk teşkil etmektedir. Bununla birlikte, 2018'de afet sağlığı ve eczacılık alanlarındaki kilit paydaşlardan oluşan uluslararası bir uzman paneliyle yürütülen bir Delphi araştırması, acil durum yönetiminin dört aşamasının (önleme/azaltma, hazırlık, iyileşme, yanıt verilebilirlik) tamamındaki roller de dahil olmak üzere eczacıların afetlerde üstlenebilecekleri 43 rol tanımlamıştır [99,100]. Yine de bu tür kriz durumlarında eczacıların aktif ve planlı olarak kullanılmadığı görülmektedir.

Türkiye'de Toplum Eczacılarının Durumu

Türkiye'de toplum eczacılarının durumuna değinmeden önce ülkenin sağlık sistemiyle ilgili bazı güncel bilgileri aktarmanın faydalı olacağı düşünülmüştür. Türkiye 85.279.553 nüfusa sahip bir ülkedir. Toplam nüfusun %9.9'unu 65 yaş ve üzeri nüfus oluşturmaktadır [101]. Türkiye mevcut nüfusuyla, nüfus büyüklüğüne göre sıralamada 194 ülke arasında 18. sırada yerini almıştır [102]. Doğuşta beklenen yaşam süresi erkeklerde 75 yıl, kadınlarda ise 80.5 yıldır [102]. Toplam sağlık harcaması gayrisafi yurtiçi hasılanın (GSYH) %4.9'unu oluşturmaktadır [103]. Pek çok gelişmiş ve gelişmekte olan ülkelerdeki gibi Türkiye sağlık sistemi de kronik hastalıklardaki artış, yaşlanan nüfus, sağlık insangücünün feminize olması, artan uzmanlaşma, artan sağlık hizmetleri maliyetleri, artan hasta beklentileri, sağlık kaygısı ile ilgili bir dizi zorlukla karşı karşıyadır. Bahsi geçen faktörlerle sağlık sisteminin üzerindeki baskı da artmaya devam etmektedir.

Türkiye Cumhuriyeti Anayasası'na göre sağlık hizmetleri kamu malı niteliğinde olup, bu hizmetlerin sunumu devletin görevlerinden biridir ve sağlık hizmetlerinden Sağlık Bakanlığı sorumludur. Türkiye'de sağlık hizmetleri kamu, yarı kamu, özel ve kâr amacı gütmeyen vakıf kuruluşlarıyla sağlanmaktadır. Sağlık hizmetlerinin finansmanında ise karma sistem benimsenmiştir. Finansman vergiler, sosyal güvenlik primleri, özel sigorta primleri ve cepten ödemelerle karşılanmaktadır [104,105].

Türkiye'de birinci basamak sağlık hizmeti sunan kuruluşlar başta aile hekimlikleri olmak üzere, verem savaş dispanserleri, ÇEKÜS (çocuk, ergen, kadın ve üreme sağlığı) birimleri, toplum sağlığı merkezleri, özel poliklinikler ve E2-E3 entegre ilçe hastaneleridir. Toplum eczaneleri de 2019 yılından itibaren Türkiye'de birinci basamak sağlık hizmeti sunan kuruluşlar arasında kabul edilmektedir [106]. Türkiye'de aile hekimliği birimi başına düşen ortalama nüfus, 2021 verilerine göre 3.145 olarak bildirilmiştir [107]. Yine aynı yıl verilerine göre birinci basamak sağlık kuruluşlarındaki hekimlere toplam müracaat sayısı 245.525.320'dir. Kişi başına düşen birinci basamak hekimlerine düşen müracaat sayısı ise 2.9'dur.

Türkiye'de toplam eczacı sayısı 37.211'dir. Yıllara göre 100000 kişiye düşen eczacı sayısı 2017 yılından sonra artış göstermeye başlayarak 2021 yılında 43.9'a kadar yükselmiştir [107]. Yine de Türkiye'nin bu oranlarla AB (89) ve OECD (85) ortalamalarının çok altında olduğu görülmektedir. Türkiye'de toplum eczanelerinde çalışan kayıtlı eczacı sayısı 31.935'tir. Toplum eczanesi sayısı ise 28.692'dir [9].

Türkiye'deki toplum eczaneleri özel kuruluşlardır ve yasa gereğince eczacıların yönetimi ve mülkiyetinde olmaları gerekmektedir. Yönetmelik bir eczacının yalnızca bir eczaneye sahip olmasına ve/veya işletmesine izin vermektedir. Tüm eczane sahipleri ve yöneticiler bölgesel eczacılar kuruluna kayıtlı olmalıdır. Bölge kurullarının tamamı Türk Eczacılar Birliği'nin (TEB) çatısı altındadır [108].

Türkiye'de toplum eczacılarının birinci basamak sağlık hizmetlerinde bazı ülkelerdeki gibi resmi sorumluluk ve aktif rol sahibi oldukları bir politik müdahale henüz mevcut değildir. Yalnızca TEB tarafından 2015 yılında pilot uygulama ile, 2016 yılında ise ülke genelinde yaygınlaştırılan kronik hastalıklarda eczacılık hizmetlerine yönelik mesleki gelişim programı olan Rehber Eczanem programı toplum eczacılarının gönüllü katılımlarıyla sürdürülmektedir. Bunun yanı sıra 2019 yılında TEB tarafından yaşlı hastalara yönelik tedavi uyumunu destekleyen bir mesleki gelişim programı yürütülmüştür. Bu çalışma sonucunda yaşlı hastaların tedavi uyum problemlerine yönelik sunulan farmasötik bakım hizmeti sonucunda yaşlı hastaların tedaviye uyum oranlarının arttığı görülmüştür [9, 109]. Bahsi geçen bu programlar ve diğer ülkelerde yer alan uygulamaların sonuçları değerlendirilerek ilerleyen dönemlerde Türkiye'de de birinci basamak sağlık hizmetlerinin sunumunda düzenlemelerin yapılması mümkün olabilir. Konuyla ilgili neler yapılabileceğine ilişkin sonuç ve öneriler bölümünde yer verilmiştir.

SONUÇ VE TARTIŞMA

Eczacıların ilaçla tedavi hakkındaki eşsiz bilgileri ve sağlık hizmetlerinde sunabilecekleri katkılar ve yeni hizmetler konusunda artan farkındalık sonucu, bazı ülkeler son yıllarda koordineli sağlık hizmeti sunumunu kolaylaştırmak için toplum eczacılarının rollerini genişleten politikalar uygulamaya başlamışlardır. Bu reformlar, odak noktaları ve kapsamları açısından farklılık gösterse de birinci basamak sağlık hizmeti uzmanlarının eczacılardan yararlanma amaçları açısından benzerlik göstermektedir. Sağlık profesyonellerinin rollerini, hedeflerini ve teşviklerini uyumlu hale getirme ve toplum eczacıları için genişletilmiş bir rol tasarlamak için sistem genelinde bir politika gündemine ihtiyaç bulunmaktadır. Bu tür yaklaşımlar, yavaş yavaş da olsa ortaya çıkmaya başlamıştır.

Toplum eczacılarının birinci basamak sağlık hizmetleri sürecine entegre edilmesine yönelik Türkiye'de uygun politik reform ortamının hazırlanması ve yasal zeminin oluşturulması gerekmektedir. Bu çalışmada daha önce de belirtildiği üzere, toplum eczacılarının birinci basamak sağlık hizmetlerine ilişkin pek çok konudaki desteğine ihtiyaç bulunmaktadır. Birinci basamak sağlık hizmetlerinin ülke genelinde etkili bir şekilde yürütülebilmesi için, toplum eczacılarının bireylere etkili, kullanışlı ve kolay erişilebilir hizmetler sunma potansiyeline sahip olduğu unutulmamalıdır. Yine bu sayede, birinci basamak sağlık hizmetlerine ilişkin reçete yazımı, sağlık kuruluşuna yeniden başvuru veya yeniden yatış gibi iş tekrarlarının azaltılmasına, daha geniş çerçevede sağlık ve sosyal bakım sistemleri üzerindeki baskının hafifletilmesine ve halk sağlığının temel motivasyonu olan herkes için sağlık konusuna yeniden odaklanılmasına yardımcı olacağı göz önünde bulundurulmalıdır.

Toplum eczacıları ve hekimler geleneksel olarak birbirlerinden izole bir şekilde çalışsalar da birçok çalışma her iki sağlık profesyoneli arasındaki iş birliğinin artmasının ilaç yönetimini iyileştirebileceğini ve bunun da olumlu hasta sağlığı sonuçlarına yol açabileceğini ortaya koymuştur. Pratisyen hekimler ve eczacılar arasında daha fazla iş birliğinin hasta bakımını iyileştirebileceğine dair kanıtlar artmaktadır. Türkiye'de eczacıların reçete yazma hakkını üstlenmesi durumunda hekimler ve eczacılar arasında yakın iş birliği ve iletişim ortamının olduğu bir koordinasyon sağlanmalıdır.

Toplum eczacılarının koruyucu ve önleyici hizmetlerin sunulması sürecinde aktif hale gelmeleri için Türkiye'nin mevcut konjonktürde henüz hazır olmadığı görülmektedir. Bu kapsamda, öncelikle politik aktörlerin toplum eczacılarının kullanılmayan potansiyeline ilişkin farkındalık düzeylerinin artması sağlanmalıdır. Politik olarak gerekli motivasyon oluştuktan sonra, toplum eczacılığı uygulamasının hukuki kapsamınının genişletilmesi yönünde adımlar atılmalıdır. Bunun yanı sıra toplum eczacıları birinci basamak sağlık hizmetlerine entegre etmek için sunulan hizmetler kapsamında geri ödeme mekanizmasının oluşturulması gerekmektedir. Son olarak, nüfusun sağlık ihtiyaçları doğrultusunda birinci basamak sağlık hizmetlerine, koruyucu ve önleyici sağlık hizmetlerine yapılan yatırımların artırılmasına odaklanılmalıdır.

YAZAR KATKILARI

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ÇIKAR ÇATIŞMASI BEYANI

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

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DERLEME MAKALE / REVIEW ARTICLE

İLAÇ TASARIMINDA YAPAY ZEKÂ UYGULAMALARI

ARTIFICIAL INTELLIGENCE APPLICATIONS IN DRUG DESIGN

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ÖZ

Amaç: *Yapay zekâ üzerindeki çalışmaların giderek artması, her alanda olduğu gibi ilaç endüstrisinin de bu çalışmalardan faydalanmasına sebep olmaktadır. Bu çalışmada, yapay zeka uygulamalarının ilaç tasarımı ve geliştirilmesi üzerinde nasıl bir rol aldığının incelenmesi amaçlanmıştır.*
Sonuç ve Tartışma: *Yeni biyolojik olarak aktif bileşiklere ihtiyacın giderek arttığı günümüzde, yapay zekada sürekli yeni algoritmaların ortaya çıkması, güçlü hesaplama yeteneği, elde edilen kimyasal ve biyolojik verilerin birikmesi, ilaç tasarımında yapay zekâ kullanımına olanak sunmaktadır. İlaç tasarım aşamalarının neredeyse tüm basamaklarında uygulanabilen yapay zekâ yöntemleriyle, yeni ilaç geliştirilmesindeki uzun zaman gereksinimi ve yüksek maliyet gibi zorluklar azaltılmaya çalışılmaktadır. Bu çalışma sonucunda, yapay zekâ teknolojisinin ilaç tasarım sürecindeki uygulamaları ve geleneksel yöntemlere göre avantajları kapsamlı bir şekilde analiz edilerek karşılaştırılmıştır.*
Anahtar Kelimeler: *Derin öğrenme, ilaç tasarımı, makine öğrenimi, yapay zekâ*

ABSTRACT

Objective: *The increasing number of studies on artificial intelligence causes the pharmaceutical industry to benefit from these studies, as in every other field. This study is aimed at examining how artificial intelligence applications play a role in drug design and development.*
Result and Discussion: *In today's world, where the need for new biologically active compounds is increasing, the continuous emergence of new algorithms in artificial intelligence, strong computational ability, and accumulation of obtained chemical and biological data allow the use of artificial intelligence in drug design. With artificial intelligence methods that can be applied at almost all stages of drug design, difficulties such as long time requirements and high costs in developing new drugs are tried to be reduced. As a result of this study, the applications of artificial intelligence technology in the drug design process and its advantages over traditional methods have been extensively analyzed and compared.*
Keywords: *Artificial intelligence, deep learning, drug design, machine learning*

GİRİŞ

İlaçlar; canlılarda mevcut hastalıkların tedavi edilmesini veya semptomların azaltılmasını aynı zamanda hastalıklardan korunmayı amaçlayan maddelerdir. Her hastalık için spesifik etkili olan ilaçlar üzerinde durulması önemli bir kavramdır. Her geçen gün yeni hastalıklarla karşılaşılmakla birlikte bilinen hastalıklar ve tedavileri için mevcut ilaçların her zaman tam olarak istenen özelliklerde ve

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yeterlilikte olmamasından dolayı yeni ilaçlara olan ihtiyaç da artmaktadır. Bu nedenle ilaç tasarımı, yeni ilaç adayı olabilecek yeni biyoaktif maddelerin keşfi ve tasarımı, aynı zamanda mevcut ilaçların geliştirilmesi için kullanılan bir süreç olmaktadır. Bu kapsamda ilaç tasarımı, ilaç-reseptör ve ilaç-enzim etkileşimlerini anlamak ve simüle eden hesaplama yöntemlerini kullanan bir disiplin olarak ilerlemiştir.

İlaç tasarımında tercih edilen metotlar; araştırma ve geliştirme çalışmalarında, pre klinik çalışmalarda ve klinik çalışmalar (faz çalışmaları) aşamalarında büyük önem taşımaktadır. Bu aşamalarda, genellikle bir öncü bileşikle başlanarak ilaçların etki mekanizmasını iyileştirmek için bir dizi *in vitro* ve hücre bazlı test yapılmaktadır. Daha sonra, uygun *in vivo* testler ve fare modelleri kullanılarak ilacın farmakokinetik özellikleri değerlendirilmektedir. İlaçların hastalık hedeflerine yeterince bağlanma afinitesi göstermesi yeterli değildir, aynı zamanda hedefe ulaşmak için fizyolojik bariyerleri aşması gerekmektedir. İlaç tasarımında, istenmeyen proteinlerle hedef dışı etkileşimler ve genetik varyasyonun ilaç yanıtlarını etkileyen farmakogenetik etkileşimler gibi diğer sorunlar da göz önünde bulundurulmalıdır. Bu nedenle, ilaç tasarımı, araştırmacılar için oldukça zor bir çalışma sürecini içermektedir [1]. Ciddi zaman alan bu zorlu sürecin aynı zamanda yüksek maliyet gerektirmesi nedeniyle, çok daha etkin ilaç tasarımı ve geliştirme yöntemlerine ihtiyaç duyulmakta, bu amaçla çeşitli multidisipliner çalışmalar sürdürülmektedir [2].

Günümüze kadar ilaç tasarımında, ligand bazlı ve yapı bazlı moleküler tasarım metotları olan iki temel yaklaşım ile birlikte, kimyasal moleküllerin üç boyutlu özelliklerini inceleyerek hesaplamalar yapan bilgisayar destekli ilaç tasarım yöntemleri (CADD) karşımıza çıkmaktadır. Gelişen bilgi işleme gücü, hesaplamalı kimya ve biyolojinin hızlı gelişimi sayesinde de CADD, araştırma sürecini hızlandırmakla birlikte, klinik öncesi ve klinik çalışmalarla ilgili maliyet ve riski azaltmak için ilaç tasarım ve geliştirme sürecinin hemen hemen her aşamasında başarıyla uygulanmaktadır [3]. Son yıllarda, ilaç tasarımının çok karmaşık olması ve hibrit tekniklerin kullanılmasını gerektirmesinden dolayı, ilaç tasarım araçları olarak yeni teknolojik gelişmelerden özellikle yapay zekâ ve makine öğrenimi tekniklerinin (MLT) uygulanmasına olan ilgi artmaktadır [4].

Yapay zekâ (AI), makinelerin gösterdiği zekâ olarak kabul edilmektedir. Bu terim, bir makine öğrenimi veya problem çözümü gibi insanlarla ilişkili bilişsel davranışlar gösterdiğinde kullanılmaktadır. AI, yeni özelliklerin öğrenilmesi ve tahmin edilmesi için iyi kurulmuş makine öğrenimi gibi teknolojileri içermektedir. Özellikle, derin sinir ağları (DNN) veya tekrarlayan sinir ağları (RNN) gibi yapay sinir ağlarının gelişmesiyle yapay zekanın evrimsel nitelikteki ilerleyişi gerçekleşmiştir. Bu gelişmeler ilaç tasarımında yapay zekanın kullanılmasını kaçınılmaz kılmaktadır. MLT'den elde edilen, örnek olarak destek Vektör Makineleri (SVM), Rastgele Ormanlar (RF) ve Bayes Öğrenimi modelleri, özellikle sanal tarama çalışmalarında, molekül filtrelerinin geliştirilmesi/keşfedilmesi için kullanılabilmektedir. Bu sayede ilaç tasarımı alanındaki önemli zorluklardan olan farmakokinetik ve toksisite özelliklerinin tahmin edilmesiyle klinik fazlardaki başarısızlıklar önlenebilmektedir [4].

Son zamanlarda derin sinir ağları gibi yapay sinir ağlarının gelişmesiyle fizikokimyasal ve ADME/T özellikleri gibi özellik veya aktivite tahminlerinde çok sayıda uygulama ortaya çıkmış ve bu teknolojinin nicel yapı-özellik ilişkilerinde (QSPR) veya nicel yapı-aktivite ilişkilerinde (QSAR) hesaplama gücünü ve netliğini arttırmıştır. Aynı zamanda de novo tasarımda yapay zekâ kullanımıyla, yeni ve biyolojik olarak aktif moleküllerin üretimi, istenen özellikler doğrultusunda tasarlanabilmektedir [5].

Sonuç olarak, Al teknolojisi ve makine öğrenimi teorisinin gelişmesiyle birlikte farmakolojik verilerin birikmesi sayesinde, sanal tarama, aktivite puanlama, kantitatif yapı-aktivite ilişkisi (QSAR) analizi, de novo ilaç tasarımı ile absorpsiyon, dağılım, metabolizma, atılım ve toksisite (ADME/T) özelliklerinin in silico değerlendirilmesi gibi birçok ilaç tasarımı alanlarında Al tabanlı modeller kullanılmaktadır. AI tabanlı modeller ilaç tasarımını çok yönlü çerçeveler aracılığıyla yönlendirmeye yardımcı olmak için büyük bir güç olarak kullanılmaktadır. Son zamanlarda, güçlü genelleme yeteneği ve güçlü özellik çıkarma yeteneği nedeniyle, moleküler özelliklerin tahmin edilmesinde ve istenen moleküllerin üretilmesinde derin öğrenme yöntemleri kullanılmıştır ve bu da AI teknolojilerinin ilaç tasarımı alanında uygulanmasını daha da teşvik etmektedir [3].

Yaşanan gelişmeler ilaç tasarımı ve yapay zekanın ortak bir noktada birleşmesi zorunluluğunu doğurmaktadır. Bu sebeple bu alanlarda yapılan çalışmaları anlamak ve güncel çalışmaları takip etmek

büyük bir önem taşımaktadır. Yapılan bu çalışmada, yapay zekada uygulanan yöntemler ile son teknolojik gelişmelerle birlikte ilaç tasarımındaki yapay zekâ uygulamalarına ve önemine değinilmektedir.

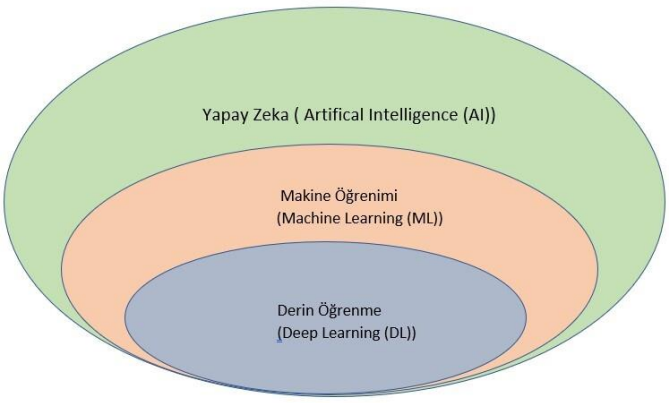
Yapay Zekâ

Yapay zekâ 'normalde insan zekâsı gerektiren görevleri yerine getirebilen bilgisayar sistemlerinin teorisi ve gelişimi' olarak tanımlanmaktadır. Farklı yeteneklerin (yani, öğrenme, akıl yürütme, problem çözme, algılama ve dili kullanma) bir kombinasyonu olan insan zekasını taklit etmek üzere yapay (veya makine) zekâ, aynı yetenekleri makinelerde kullanmak için farklı bilim ve mühendislik disiplinlerinden oluşan yöntem ve teknikleri kullanmaktadır. Yapay zekâ genellikle makine öğrenimi ile karıştırılmaktadır. Aslında, yapay zekada öğrenme (makine/derin öğrenme), makinelerdeki öğrenme yeteneklerini özümsemek için yöntem ve tekniklerle ilgilenen bir alt alandır. Yapay zekâ, makine öğrenimi, matematik, istatistik ve bilgisayar bilimlerinden bazı görevlerle (yani problemin doğası); ilgili deneyimlerden (tarihsel veriler) öğrenmek ve performansı ölçmek (performans matrisi) ve geliştirmek (yeniden uygulama) için yöntem ve teknikleri içeren multidisipliner bir alt alan olarak karşımıza çıkmaktadır [6].

Yapay zekâ, basit bir "koşula bağlı kurallar" olarak başlayıp, yıllar içinde insan beynine benzer şekilde çalışan daha karmaşık algoritmaları içerecek şekilde ilerlemiştir [7].

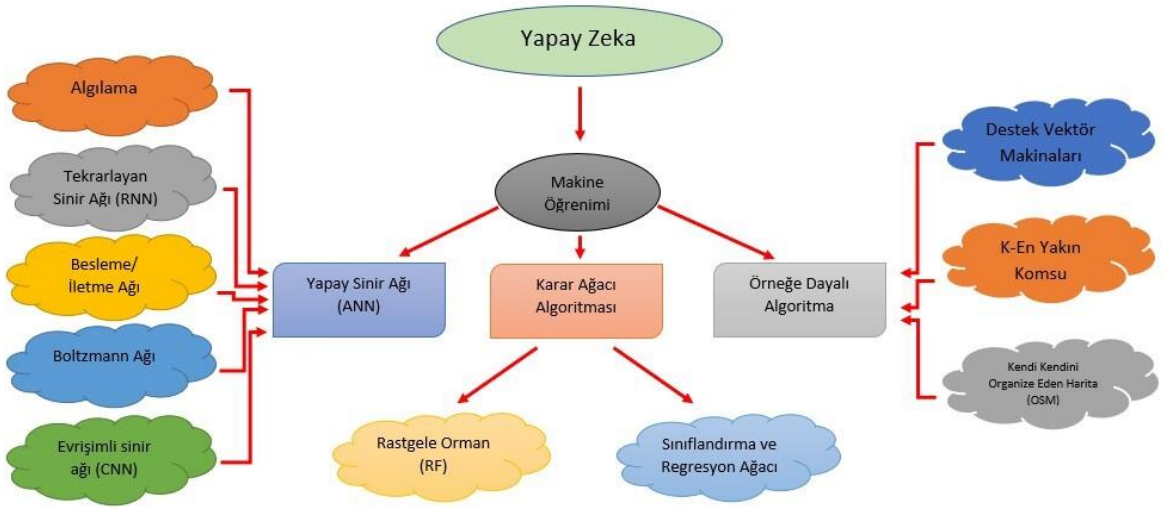
Yapay zekâ teknolojisi, verileri değerlendirmek için bilgisayarlı görüş, derin öğrenme, makine öğrenimi, doğal dil işleme, robotik, konuşma, denetimli öğrenme ve denetimsiz öğrenme gibi uygulamaları kullanmaktadır. Makine öğrenimi (Machine Learning (ML)), belirli bir durumu analiz etmek için kullanılabilecek kalıpları tanımlamak için belirli özelliklerin kullanılmasıdır. Makine, tanımlanan bilgiyi "öğrenebilir" ve bu bilgileri gelecekteki benzer senaryolara uygulayabilmektedir. ML yapılandırılmış ve yapılandırılmamış verilerden öğrenen, gizli kalıpları belirleyen, sınıflandırmalar yapan ve ileri adımları öngören bilgisayar algoritmalarından oluşmaktadır. Derin öğrenme (Deep Learning (DL)), konuşma, görüntü ve video gibi kalıpları tanımlamak ve tüm bu verileri ayırt etmek için sinir ağlarından oluşan makine öğrenimi tabanlı bir yaklaşımdır. İnsan beynine benzer şekilde, öğrenebilen ve kendi kendine karar verebilen bir yapay sinir ağı (YSA) oluşturmak için algoritmalardan oluşan DL, ML'nin daha geliştirilmiş şekli olarak karşımıza çıkmaktadır. Doğal dil işleme (Natural Language Processing (NLP)), hesaplama tekniklerinin doğal dil ve konuşmanın analiz ve sentezini yapmaktadır [8-11]. Geleneksel ML, ham verilerden elde edilen özelliklerin manuel olarak oluşturulmasını ve sıralanmasını gerektirirken DL, yüksek boyutlu, çok sayıda ve heterojen olan ham verilerden en önemli soyut özllikleri, sürekli olarak oluşturabilen çok sayıda gizli katman ve nörona sahiptir. Standart veya aşırı zor testlerde üstün sonuçların elde edilebilmesi için pratik olarak hiçbir manuel müdahale gerektirmeyen bu süreç, sadece genellemelerden kaynaklı küçük bir hata payına sahiptir [12].

Yapay zekâ, makine öğrenimi ve derin öğrenme arasındaki kavramsal ilişki Şekil 1'de verilmektedir [13].



Şekil 1. Yapay zekâ, makine öğrenimi ve derin öğrenme arasındaki ilişki [13]

Makine öğreniminin dört temel alt türü bulunmaktadır. Bunlar; denetimli öğrenme, denetimsiz öğrenme, yarı denetimli öğrenme ve güçlendirilmiş öğrenme olarak karşımıza çıkmaktadır. Makine öğreniminin alt dallarında yaygın kullanılan sinir ağları ve algoritmalar Şekil 2'de verilmektedir.



Şekil 2. Yapay zekanın alt dallarında yaygın kullanılan sinir ağları ve algoritmalar [13]

Denetimli öğrenme (Supervised learning), görev odaklıdır. Örnek veri noktalarını etiketleri bilinmektedir. Model, test örneklerinin etiketlerini tahmin etmek için bu bilinen örnekler üzerinde eğitilmektedir [14]. Denetimli öğrenmede yapay sinir ağı için hem girdi hem de çıktı değerleri belirlenmektedir. Ağın ürettiği çıktı ile istenen çıktı arasındaki fark sıfır ya da sıfıra yakın bir değere ulaşıncaya kadar yapay sinir ağının ağırlıkları değiştirilmektedir. Algoritma kabul edilebilir doğruluğa geldiğinde ya da eğitim veri kümesi tükendiğinde eğitim sonlandırılmaktadır. Yaygın olarak kullanılan denetimli makine öğrenimi algoritmalarına, Karar Ağaçları, Rastgele Ormanlar (RF), Destek Vektör Makineleri (SVM), Doğrusal ve Lojistik Regresyon, Naive Bayes, Doğrusal Diskriminant Analizi, k-En Yakın Komşu (KNN) algoritmaları ve Sinir Ağları örnekleri verilebilmektedir [15].

Denetimli makine öğreniminde sınıflandırma ve regresyon olmak üzere iki alt alan bulunmaktadır. Öngörülen çıktıların sonuçlarını değerlendirirken, problem kategorik olduğunda sınıflandırma modelleri kullanılmaktadır. Problem bir aralık içindeki sayısal bir değeri öngörmeyi içerdiğinde regresyon modelleri kullanılmaktadır. Rastgele Orman, Otomatik Kodlayıcılar (AutoEncoder (AE)) ve Evrişimli Sinir Ağları (CNN) gibi çeşitli farklı türde makine öğrenimi uygulamaları bulunmaktadır [16].

Denetimsiz öğrenme (unsupervised learning), verilerle ilişki bulmak için etiketli verilerle eğitime bağlı değildir. Veri odaklı olup, gizli yapıları bulmak için verilerdeki kalıpları öğrenmeyi amaçlamaktadır [14]. Denetimsiz öğrenmede, ağa sadece girdi verileri öğretilir. Girdi değerlerine uygun bir çıktı üretilinceye kadar bağlantı ağırlıkları değiştirilir. Bu metot görüntü işleme, işaret işleme, kontrol problemlerinde etkin olarak kullanılmaktadır. Denetimsiz öğrenmede Hiyerarşik Kümeleme (Hierarchical Clustering), K-Ortalama Kümeleme (k-Means Clustering), Gaussian Karışım Modelleri (Gaussian Mixture Modeling (GMM)), Kendi Kendini Düzenleyen Haritalar (The Self Organizing Map (SOM)) ve Gizli Markov Modelleri (Hidden Markov Model (HMM)) en yaygın kullanılan modellemelerdir [15].

Makine öğreniminde, etiketli veri, bir veri kümesindeki her örneğin doğru cevabının veya sınıflandırmasının önceden belirlenmiş olduğu verilerdir. Bu tür veriler, makine öğrenimi algoritmalarının eğitiminde kullanılmakta ve bu sayede algoritmaların doğru sonuçlar üretmesi için gerekli olan doğru cevaplar sağlanmaktadır. Örneğin, bir resim sınıflandırma modeli eğitiliyorsa, etiketli veriler resimlerin hangi nesneleri içerdiğini belirten etiketlerdir. Yarı denetimli öğrenme (Semi-

supervised learning), daha küçük bir etiketlenmiş veri kümesi ile bir modelin birkaç veriden tahmin edilmesi gereken ve daha az maliyetli olan daha büyük bir etiketlenmemiş veri kümesinin içindedir. Eğitim için etiketli veri noktalarını kullanmakta ve etiketli verilerden gelen bilgileri kullanarak bilinmeyen örneklerin etiketlerini tahmin etmeyi amaçlamaktadır.

Güçlendirilmiş öğrenme (Reinforced learning), çevre ile etkileşim kurarak sürekli öğrenebilen ve belirli bir veri setini kullanmayı amaçlayan bir sistemdir. Yarı denetimli öğrenmede, denetimli öğrenmeye benzer algoritmalar kullanılmıştır. Güçlendirilmiş öğrenme için kullanılan algoritmalara Q-Öğrenme (Q-Learning) ve Derin Öğrenme örnek olarak verilebilmektedir [17]. Derin Öğrenme, veri modellemelerini öğrenmek için birçok doğrusal olmayan işlem birimi katmanıyla yapay sinir ağlarını (Artificial Neural Networks (ANN)) kullanan makine öğreniminin alt dallarındandır. İnsan merkezi sinir sistemini taklit etmek amacıyla, bağlı yapay nöronlardan oluşan 'yapay sinir ağı' adı verilen geleneksel makine öğrenimi algoritmalarının yeniden tasarlanıp kullanılmasıdır [18,19]. Derin öğrenme yöntemleri, temel olarak derin sinir ağlarını kullanır ve bu arın farklı türleri vardır. Örneğin, Evrişimli Sinir Ağları (Convolutional Neural Networks, CNN), görsel verileri işlemek için kullanılırken, tekrarlayan sinir ağları (Recurrent Neural Networks, RNN) sıralı verileri işlemek için kullanılmaktadır. Otomatik kodlayıcılar (Autoencoders) veri boyutunu azaltmak ve özellik çıkarmak için kullanılabilmektedir. Kısıtlı Boltzmann Makineleri (Restricted Boltzmann Machines) ise yapay sinir ağı modellerinin eğitimi için kullanılmaktadır.

Derin Sinir Ağları (Deep Neural Networks (DNN)), birden çok gizli katmana sahip birbirine tamamen bağlı sinir ağlarıdır. Her gizli katman, birden fazla doğrusal olmayan işlem birimini içermektedir. Böylece DNN'ler, istenen özellikteki verileri otomatik olarak çıkarabilmek için birçok farklı katmanda birden çok nöron kullanabilmektedirler. Bu özellikleri sayesinde DNN'ler, görüntü işleme, doğal dil işleme, ses tanıma ve diğer pek çok uygulama alanında oldukça etkili bir şekilde kullanılabilmektedir [14].

CNN, derin öğrenmedeki en temel yöntemlerden biridir. Evrişimli Sinir Ağı, görüntü işleme, nesne sınıflandırması ve doğal dil işleme gibi alanlarda kullanılan ileri beslemeli bir sinir ağıdır. CNN'ler, özellikle görüntü işlemede oldukça etkilidirler ve çok boyutlu diziler biçiminde olan verileri işlemek için tasarlanmışlardır. Bu nedenle, gen ekspresyon veri analizi gibi diğer uygulama alanlarında da kullanılmaktadırlar. CNN'lerin temel özelliği, evrişim ve havuzlama katmanlarından oluşmasıdır. Evrişim katmanları, özellikle görüntülerdeki özellikleri çıkarmak için kullanılırken, havuzlama katmanları ise boyut azaltma işlemi yaparak hesaplama yükünü azaltmaktadırlar [20].

CNN, 1980'lerde Fukushima tarafından, Hubel ve Wiesel'in bir kedinin görsel korteksindeki alıcı alan (receptive field) araştırmalarından esinlenen, çok katmanlı yapay sinir ağı olan Neocognitron'un geliştirilmesiyle oluşturulmuştur [21,22].

CNN, görsel sinyalleri işlerken, yerel nöron kalıpları duyusal boşluktaki belirli bölgeleri algılamaktadır. Aynı zamanda evrişimli katmanlarda seyrek bağlanabilirlik ve paylaşılan ağırlıklar olmak üzere iki ana karakter geliştirerek bunların özelliklerini taklit edebilmektedir [19].

RNN, derin öğrenmedeki diğer bir modelleme türüdür. Özellikle sekans verilerini işlemeyi amaçlayan RNN, NLP'de yaygın olarak kullanılmış ve büyük başarılar elde edilmiştir. RNN'ler tahmin modellerinde ve dil modellemelerinde yaygın olarak kullanılmaktadır. RNN, bir katmanın çıktısını kaydetme ve katmanın çıktısını tahmin etmek için girişi geri besleme prensibiyle çalışmaktadır [19].

1950'lerden 1970'lere Yapay Zekanın Hayatımıza Girişi

Akıllı davranışı ve eleştirel düşünceyi simüle etmek için bilgisayarları kullanma fikri ilk olarak 1950'de Alan Turing tarafından ortaya konulmuştur [23]. Bilgisayarlar ve Zekâ kitabında Turing tarafından, bilgisayarların yetenekli olup olmadığını belirlemek için "Turing testi" olarak bilinen basit bir testi tanımlanmıştır [9]. Bundan altı yıl sonra ise John McCarthy yapay zekâ (AI) terimini "akıllı makineler yapma bilimi ve mühendisliği" olarak nitelendirmiştir [24,25].

Yapay zekâ, erken dönemdeki AI çalışmalarında yalnızca insanların yapabildiği çıkarımlar yapma veya karar verme yeteneğine sahip makinelerin geliştirilmesine odaklanmıştır. İlk endüstriyel robot kolu (Unimate; Unimation, Danbury, Conn, ABD) 1961'de General Motors'taki montaj hattına katılmış ve otomatik döküm gerçekleştirilmiştir [26]. Birkaç yıl sonra (1964) Joseph Weizenbaum tarafından, bir makine olan Eliza tanıtılmıştır. Doğal dil işlemeyi kullanan Eliza, kalıp eşleştirme ve

değiştirme kullanarak, insan konuşmasını taklit etme metodolojisiyle sohbet robotları olma görevini görmüştür [27]. 1966 yılında ise "ilk elektronik insan" olan Shakey geliştirilmiştir. Shakey, Stanford Araştırma Enstitüsü'nde talimatları yorumlayabilen ilk mobil robottur. Sadece 1 adımlık komutları takip etmek yerine, daha karmaşık talimatları işleyerek uygun eylemleri saptamıştır. Bu gelişme yapay zekada bir dönüm noktası olmuştur [28].

1970'lerden 2000'lere Al Kışı

Yapay zekâ adına daha az önemli gelişmelerin yaşanması nedeniyle, fonlamanın azaltılarak faizlerin artırıldığı bu yıllar 'Al kışı' dönemi olarak tanımlanmıştır [9]. 1970'lerin sonlarında başlayan ve 1990'ların başlarına kadar uzanan, uzman dijital bilgi veri tabanlarının geliştirilmesi ve sürdürülmesindeki aşırı maliyet nedeniyle bu dönemlerde Al ile ilgili gelişmeler yavaşlamıştır. Buna rağmen, 1971'de Saul Amarel tarafından Rutgers Üniversitesi'nde 'Biyotıpta Bilgisayarlarda Araştırma Kaynağı' üzerine çalışılmış ve 1973'te Stanford Üniversitesi Tıp Bölümünde, çeşitli kurumlardan biyomedikal araştırmacılar tarafından, gelişmiş ağ oluşturma yeteneği olan paylaşımlı bir bilgisayar sistemi 'Tıpta Deneysel-Yapay Zekâ' oluşturulmuştur [29].

Bunların dışında 2000'li yıllara kadar kayda değer başka gelişme yaşanmamıştır.

2000'den Günümüze; Çığır Açan Gelişmeler

2007 yılında IBM (International Business Machines) tarafından geliştirilen ve doğal dilde sorulan sorulara cevap vermek için tasarlanan, bir yapay zekâ programı olan 'Watson' geliştirilmiştir. Watson telesekreter sistemi, 2011'de insanlarla yarışan 'Jepordy!' adlı oyun programında birinciliği kazanmıştır. DeepQA adı verilen bu teknoloji, ileriye dönük akıl yürütme (verilerden sonuçlara kadar takip etme) ve geriye dönük akıl yürütme (takip etme) kullanılan geleneksel sistemlerin aksine, olası cevaplar oluşturmak için yapılandırılmamış içerik üzerinde verileri analiz etmek için doğal dil işlemeyi ve çeşitli veri tabanlarını kullanmıştır [30]. Ardından yüzeysel iletişimi kullanan sohbet robotlarından (Eliza) sonra anlamlı konuşma tabanlı arayüzleri kullanan Apple'ın sanal asistanı Siri (2011) ve Amazon'un sanal asistanı Alexa'ya (2014) doğal dil işleme teknolojisi uygulanmıştır [31,32].

Mart 2016'da ise AlphaGo, dünyanın en iyi Go oyuncularından biri olan Lee Sedol'u yenerek yapay zekayı (AI) yeniden kamuoyunun dikkatine sunmuş ve büyük ilgi uyandırmıştır [33]. IBM tarafından geliştirilen ve satranç oynayan bir bilgisayar olan Deep Blue ile karşılaştırıldığında, AlphaGo, en başarılı bilgisayarlardan biri olan evrişimli sinir ağı (CNN) adı verilen sinir ağlarında (NN'ler) derin öğrenme (DL) algoritmalarının uygulamalarını kullanan gelişmiş ve yenilikçi bir mimariyi entegre etmiştir [34].

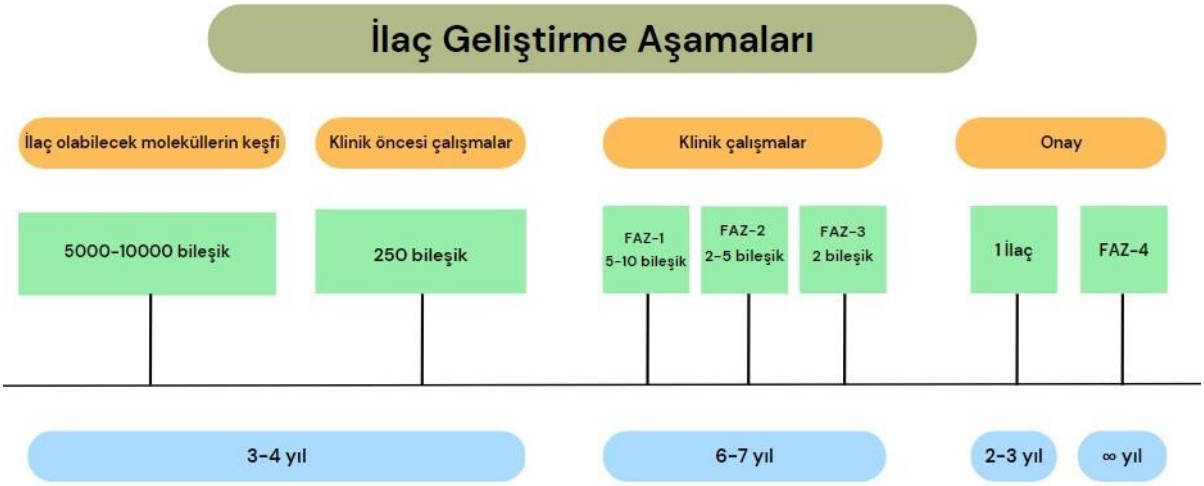
Ardından, 2017 yılında Fabien Beckers tarafından, bulut/derin öğrenme girişimi olan, görüntü tabanlı hassas tıp cihazları geliştiren Arterys'in kurulumunun ardından ilk Gıda ve İlaç Dairesi (FDA) onaylı bulut tabanlı yapay zekâ destekli kardiyak görüntüleme ilk kez ABD'de sağlık sektöründe kullanılmaya başlanmıştır.

Yine 2017 yılında yapay zekayla geliştirilen, otomatik olarak hasta alımı yapan 'Chatbot Mandy' teknolojisinden sonra, 2018-2020 yılları arasında karar destek sistemi, görüntüleme ve tanı koyma gibi konularda birçok AI destekli cihaz ve teknoloji pediatri, gastroenteroloji, kardiyoloji, oftalmoloji gibi alanlarda kullanılması yaygınlanmıştır [7,35,36]

OpenAI tarafından 2022 yılında, metin tabanlı sorgulara yanıt vermek ve doğal dilde yanıtlar oluşturmak için tasarlanmış bir dil modeli 'ChatGPT' geliştirilmiştir. ChatGPT, dildeki kalıpları öğrenmeyi, tutarlı ve insan benzeri metinler oluşturmayı sağlayan Transformer adlı derin bir öğrenme mimarisi kullanılarak oluşturulmuştur. Çok büyük bir metin verisi dizileri üzerinde eğitilmiştir ve bu nedenle genel bilgi sorularından daha karmaşık konuşma konularına kadar çok çeşitli taleplere yanıtlar üretebilmektedir. Genel olarak, ChatGPT, NLP alanında önemli bir ilerlemeyi temsil etmekle birlikte bilgisayarlar ve dijital sistemlerle etkileşim biçimimizde devrim yaratma potansiyeline sahiptir [37]. Mart 2023'e gelindiğinde ise OpenAI'nin derin öğrenmeyi ölçeklendirme çabalarındaki en son kilometre taşı olan GPT-4 geliştirilmiştir. GPT-4, insanlardan daha az yetenekli olmasına rağmen, çeşitli profesyonel ve akademik kriterlerde insan düzeyinde performans sergileyen, görüntü ve metin girişlerini kabul edip metin çıktıları yayan, büyük birçok modlu model olarak karşımıza çıkmaktadır [38].

İlaç Tasarımı

İlaç tasarımı, daha önceden tanımlanmış yapı-aktivite ilişkilerinden yararlanarak, farmakolojik aktivitesi öngörülebilen potansiyel ilaç moleküllerinin tasarlanmasıdır. Yeni ilaç molekülleri geliştirmenin amacı, var olandan daha güçlü, daha az toksik ve yan etkileri en aza indirilmiş, yararlı terapötik bileşikler geliştirmektir. Var olan ilaçların hemen hemen hiçbiri her yönüyle istenilen özelliklere sahip olmadığından, araştırma kurumlarında ve ilaç endüstrisinin araştırma-geliştirme laboratuvarlarında, sürekli olarak ilaç olabileceği düşünülen yeni bileşikler geliştirilerek elde edilmekte ve birçok farmakolojik aktivite testlerine tâbi tutulmaktadır. Günümüzde tedaviye girmiş 14.000'in üzerinde ilaç bulunmakta ve yeni ilaç molekülleri geliştirmek üzere yapılan çalışmaların sayısı giderek artmaktadır [39]. İlaç geliştirme yöntemlerindeki ilerlemelerle, terapötik etkileri iyileştirilmiş yeni ilaçlar tedaviye sunulabilmektedir. Geliştirilen bu ilaçlar, 20-30 yıl önce yaygın olarak kullanılan birçok ilacın yerini alabilmektedir. Ayrıca, tıp ve biyolojik bilimlerdeki gelişmelerle, nedeni bilinmeyen bazı hastalıkların mekanizmaları açıklanmakta ve henüz tedavisi olmayan hastalıklarda kullanılmak üzere geliştirilen yeni ilaçlar da tedaviye girmektedir. Ancak yeni bir ilacın geliştirilme aşamasından klinik kullanıma sunulmasına kadar uzun ve zorlu bir süreç gerekmektedir. Bu zorlu sürecin Şekil 3'te görüldüğü gibi farklı aşamaları bulunmaktadır [40].



Şekil 3. İlaç geliştirme aşamaları [40]: Klinik öncesi çalışmalarda yeni bileşiğin etkinliği ve güvenliğiyle ilgili çalışmalar, klinik çalışmalarda faz 1, faz 2 ve faz 3 aşamaları (Faz 1 de az sayıda sağlıklı kişide yan etkiler ve güvenliğin doğrulanması ile ilgili çalışmalar yapılırken faz 2 de az sayıda hastada etkililik, güvenli dozaj ve uygulama yönteminin doğrulanması yapılır. Faz 3 aşamasında çok sayıda hastada mevcut ilaçlara kıyasla etkinlik ve güvenlik doğrulaması yapılır. Faz 4 aşamasında ise onaylanmış ilacın toplumda uygulanmasıyla uzun dönemdeki etki ve güvenlik sonuçları toplanır)

İlaç geliştirmede farmasötik tarama testlerinden önce etkin olabilecek aday bileşiklerin geliştirilmesinde iki önemli aşama söz konusudur. İlk olarak, belli bir farmakolojik aktivite için model (prototip) olan öncü bileşiğin bulunması gerekmektedir. Genellikle bu bileşik istenen farmakolojik aktiviteyi göstermekle birlikte etkisi yetersiz olabilmekte ve/veya istenmeyen farmasötik, farmakokinetik ve toksik özelliklere sahip olabilmektedir. Bu nedenle ikinci aşamada, bu öncü bileşiklerden yola çıkılarak optimizasyon çalışmaları yapılmaktadır. Öncü bileşiği bulabilmek için pek çok yol izlenebilmektedir. Bunlar geçmişten beri bilinen, doğal kaynaklardan molekül elde etme, tesadüfen bulma, klinik gözlemler, farmakolojik tarama testleri, ilaç metabolizma çalışmaları ve etkin endojen moleküllerin yapılarını taklit etme ve akılcı ilaç tasarımı gibi yöntemlerdir. Günümüzde bu yöntemler içinden akılcı (rasyonel) ilaç tasarımı daha çok ön plana çıkmıştır. Akılcı ilaç tasarımında, yeni ilaç molekülleri geliştirmek üzere hem öncü bileşiğin bulunması hem de optimizasyon aşamalarında çeşitli yöntemler kullanılmaktadır. Yöntemlerden biri olan bilgisayar destekli ilaç

tasarımı, ilaç aktivitesinde önemli fizikokimyasal parametreleri, kantitatif yapı-aktivite ilişkilerini ve kuantum kimyasal modelleri kullanmaktadır. Diğer yöntem olan moleküler modellemede ise öncü molekül veya analog tasarımı için çeşitli bilgisayar programları, X-ışınları kristalografisi gibi yöntemlerle ilacın moleküler şekli ve konformasyonu araştırılmaktadır. Bunlarla birlikte, ilacın hedefle olan etkileşimin gücü ve uyumu değerlendirilerek etkin maddelerin tasarlanması için reseptör tanımlama yöntemleri kullanılmaktadır. İlacın geliştirilmesinde tüm aşamalarda etkin olarak kullanılan bilgisayar yardımlı ilaç tasarımı, günümüzde en yaygın kullanılan teknik olmuştur [40].

Bir ilaç molekülünün tasarımı ve geliştirilmesi uzun zaman alan, yüksek maliyetli ve kompleks aşamalar içeren zorlu bir süreçtir. Son zamanlarda, bilgisayar destekli teknolojilerde ve yapay zekanın uygulama alanlarındaki gelişmelerin ilaç tasarlama ve geliştirme alanına da yansımasıyla daha başarılı ilaç adayı moleküllerin, daha kısa zamanda ve daha az maliyetle elde edilmesine olanak sağlamaktadır.

İlaç Tasarımında Yapay Zekâ Uygulamaları

Yapay zekâ (AI), çeşitli sektörlerde en yeni uygulamaları kullanıma sunmaktadır. Bilgisayar destekli ilaç tasarım teknolojisi, yüksek verimlilik, düşük maliyet ve hızlı sonuç verme gibi avantajlarıyla ilaç keşfinde ve tasarımında önemli bir rol oynamaktadır. Bilgi işlemedeki önemli ilerlemeler, üst düzey algoritmaların geliştirilmesi, biyoloji ve kimya alanlarındaki verilerin çoğalması da bunu desteklemektedir. Son yıllarda, makine öğrenimi (ML) algoritmalarındaki hızlı ilerleme nedeniyle, AI, ilaç tasarımında çeşitli aşamalarda yaygın olarak kullanılmaktadır. Makine öğrenimi algoritmalarının genelleme yeteneği güçlenerek, daha etkili ve daha büyük veri işleme yeteneğine sahip olmasıyla derin öğrenme tekniğine dönüşmüştür; bu durum, yapay zekâ teknolojisi ile bilgisayar destekli ilaç tasarım teknolojisinin entegrasyonunu daha da teşvik ederek en yeni ilaçların tasarımını ve keşfini hızlandırmaktadır [41].

İlaç tasarımı ve keşfinin tüm işlemleri, hedef tanıma, hedef bulma, potansiyel hasta grupların optimizasyonu, klinik öncesi ilaç adaylarının tanınması, klinik öncesi çalışmalar ve klinik araştırmayı içermektedir. Raporlara göre, daha yeni bir ruhsatlı ilacın standart araştırma ve geliştirme döngüsü yaklaşık 10-17 yıl süreye ve ortalama 2.558 milyar dolar bütçeye ihtiyaç duymaktadır [42,43]. Bununla birlikte, uzun zaman ve yüksek maliyet harcanmasına rağmen, küçük moleküllü ilaçlar için onaylanarak ruhsat alma oranları %10'dan azdır. Bilgisayar destekli ilaç tasarımı yaklaşımları ile ilaç tasarım çalışmalarında önemli akılcı ipuçları elde edilerek daha etkin bileşiklere daha uygun maliyetlerle ulaşılabilmektedir [41].

Günümüzde teknolojinin ve yapay zekâ çalışmalarının ilerlemesi ile geliştirilen çeşitli algoritmalar ve sinir ağları bilgisayar destekli ilaç tasarım çalışmalarında da kullanılmaya başlanmıştır. Makine öğrenimi ve derin öğrenmedeki ilerlemelerle, teknolojinin her alanında çarpıcı gelişmeler ortaya konulmasıyla, yapay zekâ disiplinler arası ortak bir alan olmuştur. AI tabanlı hesaplamalı modelleme, bileşikler için biyolojik hedef aranması ve toksisiteleri açısından değerlendirilmesi için umut verici bir yöntem olarak karşımıza çıkmaktadır. Kantitatif yapı-aktivite ilişkisi (QSAR) tabanlı yaklaşımlar da dahil olmak üzere mevcut hesaplama modelleri, çeşitli biyolojik hedefler için çok sayıda yeni molekülün hızlı bir şekilde ön görülmesinde kullanılabilmektedir [41].

Yapay zekanın alt gruplarından olan makine öğrenimiyle birlikte, yıllardır, Naive Bayes Sınıflandırıcısı (NB), Lojistik Regresyon (LR), En Yakın K Komşusu (KNN) algoritması, Destek Vektör Makineleri (SVM), Çoklu Doğrusal Regresyon (MLR), Gauss Süreci (GP), Rastgele Ormanlar (RF), Boosting ve Karar Ağacı, ilaç tasarımında yaygın olarak kullanılan diğer uygulamalardır [44-49].

Umut verici gelişmelerin ardından ilaç tasarımında günümüze kadar aktif kullanılan programlar ile kullanım amaçlarına ve ilaç şirketleriyle yapay zekâ şirketlerinin ortak çalışmalarını içeren tablolar (Tablo 1 ve Tablo 2) aşağıda verilmektedir.

Tablo 1. İlaç tasarımında aktif kullanılan programlar ve kullanım amaçları [41]

Programlar	**Kullanım Amaçları**
PPICurator (https://ppicurator.hupo.org.cn)	Bu araç, geniş kapsamlı PPI'leri araştırmak için kullanılmaktadır.
BioRAT (http://bioinfadmin.cs.ucl.ac.uk/biorat/docs/index)	Tüm metin madenciliği çalışmaları için kullanılmaktadır.
DeepChem (https://github.com/deepchem/deepchem)	Phyton tabanlı, Al sistemi kullanan MLP modelleme yöntemiyle yeni ilaç adayı bulunmaktadır.
DeepNeuralNetQSAR (https://github.com/Merck/DeepNeuralNet-QSAR)	Phyton tabanlı bu sistem, hesaplama araçlarıyla birlikte, bileşiklerin moleküler aktivitesinin saptanmasına yardımcı olmaktadır.
DeepTox (www.bioinf.jku.at/research/DeepTox)	12.000 ilacın toksisitesini tahmin eden bir yazılımdır.
GeneWays (http://geneways.genomeleft.columbia.edu)	Bu araç, biyolojik yolakları saptamaktadır.
PotentialNet (https://pubs.acs.org/doi/full/10.1021/acscentsci.8b00507)	NN'leri kullanarak ligandların bağlanma afinitelerini tahmin etmektedir.
ORGANIC (https://github.com/aspuru-guzik-group/ORGANIC)	İstenen özelliklere sahip moleküllerin üretilmesine yardımcı olan bir molekül oluşturma aracıdır.
AlphaFold (https://deepmind.com/blog/alphafold)	Proteinlerin 3 boyutlu yapılarını tahmin etmektedir.
CancerDR (http://crdd.osdd.net/raghava/cancerdr/)	148 antikanser ilacı ve bunların yaklaşık 1000 kanser hücre hattına karşı etkinliğini kapsayan kanser ilaç direnci veri tabanıdır.
BRENDA (http://www.brenda-enzymes.org)	Enzim ve enzim-ligand veri tabanıdır.
UniProt (http://www.uniprot.Org)	Proteinlerle ilgili bilgi merkezidir.
InterPro (http://www.ebi.ac.uk/interpro)	Protein bölgesiyle ilgi verileri bulunduran bilgi merkezidir.
ChEMBL (https://www.ebi.ac.uk/chembl)	Küçük ilaç benzeri moleküllerin birincil yayınlanmış literatürüne ve bunların biyoaktif özelliklerine dayanan veri tabanıdır.
TDR targets (http://tdrtargets.org/)	İhmal edilen tropikal hastalıklar için geliştirilmiş kemogenomik bir veri tabanıdır.
MATADOR (http://matador.embl.de/)	Protein-kimyasal etkileşimlerle ilgili veri tabanıdır.

Tablo 2. İlaç şirketleriyle yapay zekâ şirketlerinin ortak olarak yürüttüğü çalışmalar [41]

İlaç Şirketleri	**Yapay Zekâ Şirketleri**	**Ortak Çalışmaları**
Pfizer	IBM Watson	İmmüno onkolojide yeni ilaç hedeflerini, kombinasyon terapilerini ve hasta seçim stratejilerinin belirlenmesi
Pfizer	XtalPi	Moleküllerin mekanik ve kimyasal özelliklerinin yanı sıra 3B yapıyı tahmin etmek için bulut bilgi işlem çerçevesiyle kuantum mekaniği (QM) ve makine öğrenimi algoritmalarının kullanılması, Moleküllerin proteinlerle bağlanmasının incelenmesi

Tablo 2 (*devamı*). İlaç şirketleriyle yapay zekâ şirketlerinin ortak olarak yürüttüğü çalışmalar [41]

İlaç Şirketleri	Yapay Zekâ Şirketleri	Ortak Çalışmaları
BAYER	Exscientia	Exscientia'nın, onkolojik ve kardiyovasküler hastalıkları tedavisi ve olası ilaç adayı için en yeni öncü yapılarını optimize etmek amacıyla Centaur Chemist™ AI ilaç bulma platformunu kullanması
BAYER	Sensyne Health	Kariyovasküler hastalıklar için Sensyne Health'in tescilli, klinik yapay zeka teknoloji platformunu kullanarak yeni tedavilerin geliştirilmesi
NOVARTIS	Microsoft	Yeşil kimyanın, hücre ve gen tabanlı tedavilerin, görüntü bölümlendirmenin, akıllı ve özelleştirilmiş tedavi sunumunun araştırılması
NOVARTIS	IBM Watson	Meme kanseri hastaları için tedavi çalışmalarının yürütülmesi
SANOFI	Exscientia	Diyabet ve komorbiditeleri için bispesifik küçük molekülün bulunması
AstraZeneca	BenevolentAI	İdiyopatik pulmoner fibrozis ve kronik böbrek hastalıklarının tedavisi için sinir ağları çerçeveleri temelinde yeni tedavilerin geliştirilmesi
Janssen	BenevolentAI	BenevolentAI, Janssen'in daha önce Parkinson hastalarında Bavisant'ın faz IIb denemelerinde klinik veri sağlamak için kullanılan yenilikçi klinik faz ilaç adaylarını geliştirmesi
Takeda	Numerate	Gastroenteroloji, onkoloji ve merkezi sinir sistemi temelli bozukluklarda tedavi için ilaç moleküllerinin hedef yerinin belirlenmesi
Eli Lilly	Atomwise	Henüz bilinmeyen protein hedeflerine yönelik ilaçların geliştirilmesi
Roche	OWKIN	ML ağlarına dayalı klinik deneyler ve ilaç tasarımının yapılması

Tarihsel Gelişim

1950 yıllarında Al kavramının doğuşunun ardından 1960'lı yıllarda QSAR üzerine ilk çalışmalara başlanmış ve ilaç tasarımında kullanılmıştır [50]. 1990'lardan önce, ilaç tasarımında modeller tasarlamak için olağan hesaplama yöntemleri, yani doğrusal regresyonlar kullanılmıştır. Bu erken çalışmalarda, modelleme için kullanılan kimyasal tanımlayıcılar, atomik tip ve parçalı tanımlayıcılar gibi kimyasal ve yapısal özellikleri tanımlamakla sınırlı olmaktaydı [51].

Yapay zekanın ilaç tasarımı ve geliştirmesindeki ilerlemesi, ilk olarak, son teknoloji kimyasal tanımlayıcıların yani moleküler parmak izlerinin oluşturulmasıyla ve eğitim setlerinde tanımlayıcı kategorileri önemli ölçüde arttıran topolojik tanımlayıcılarla hızlanmıştır [12,52-54]

İlaç tasarımında ilk olarak 1989'da sinir ağlarının kullanılması üzerine çalışılmıştır [55]. Kabul edilen ilk yaklaşım, yani yapay sinir ağı (YSA), değişkenlerin seçim sürecini temel almaktadır [56,57]. YSA'lar, değişkenler arasında doğrusal olmayan bağlantılar kurmak ve biyolojik etkinliklere odaklanmak için çok iyi bir makine öğrenimi yaklaşımını temsil etmektedir [58]. YSA'lar da dahil olmak üzere çeşitli makine öğrenimi yaklaşımlarını kullanan modern hesaplama modelleri, sağlam bilgisayarlar edinmiş ve 1990'larda bilgisayar donanımlarındaki gelişmelerden yararlanmıştır [59]. Bu gelişmelerle birlikte 1980 yıllarında YSA ile birlikte temel olarak DL kavramı da tanıtılmıştır [60].

1990'lardan 2000'lere kadar modelleme değerlendirmelerinde, doğrusal regresyon kullanmak yerine, doğrusal olmayan modelleme algoritmaları, yani KNN, RF ve SVM temelinde oluşturulan gelişmiş ML algoritmaları geleneksel olarak kullanılmıştır. Yine aynı dönemde, model doğrulamanın önemi vurgulanmış ve modellemenin olmazsa olmaz bir parçası olarak kabul edilmiştir. Yapay zekanın ilerlemesiyle birlikte, 2000'li yılların başında QSAR modellemelerinde köklü değişimler yaşanmıştır. Bu gelişmenin ardından hesaplama kapasitesi ve modellemelerde verilerin donanım ve erişilebilirliğinde önemli derecede iyileşmeler yaşanmıştır [59]. Merck tarafından desteklenen QSAR çalışmaları sırasında, ilaç tasarımı için kullanılan DL algoritmaları, kullanılan ML algoritmalarınından daha güçlü ve üstün bulunmuştur [61].

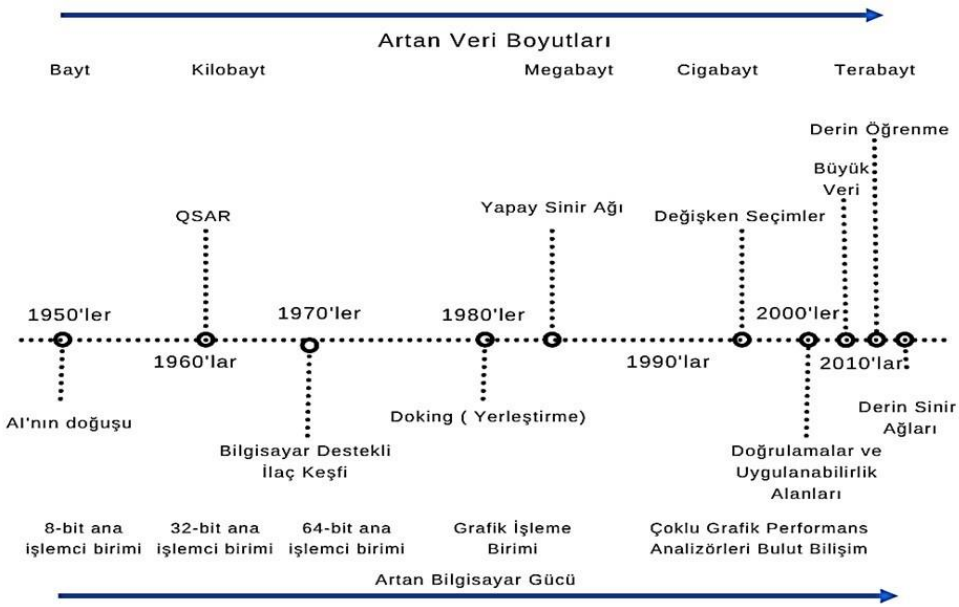
Donanımdaki hızlı gelişmeler, yani 2010'larda grafik işlem birimi olan GPU'ların ve bulut bilişimin kullanımı, NN modelleme araştırmalarını doğrudan teşvik etmiştir [60]. Çok sayıda gizli katmana sahip DNN'ler kurulmuş ve 2015 yılında gelişmeleriyle çığır açan DL ile ilgili makale yayınlanmıştır [54]. Yine DeepTox (DNN'lere dayalı bir hesaplamalı toksiklik modeli), diğer ML yaklaşımına dayalı modelleri geride bırakmıştır [62]. DNN modelleri yüksek başarı oranı ile büyük veri kümelerini modellemek ve anlamlı özellikleri seçmek için DL yaklaşımları avantajlarından dolayı ilaç tasarımında uygulanmaya ve geliştirilmeye devam etmiştir.

Daha sonra, Pande ve ark., 2018 yılında moleküler makine öğrenimi için kapsamlı bir ölçüt olan MoleculeNet'i tanıtmışlardır [63]. MoleculeNet, bir Al yazılım aracı olan DeepChem'e bir verileri bölme mekanizmaları kitaplığı sunmakta ve tüm algoritmaları, çeşitli veri bölme seçenekleriyle değerlendirmektedir. Aynı zamanda MoleculeNet, veri seti koleksiyonlarındaki çeşitli özelliklerden yararlanarak makine öğrenimi algoritmalarının kıyaslanmasını yapmaktadır [41].

Son zamanlarda, Allen ve ark., ATOM Modelleme Boru Hattını (AMPL) geliştirerek ilerlemelere büyük katkıda bulunmuşlardır [64]. AMPL, silico ilaç tasarımında ilerlemede, modeller oluşturma ve paylaşmada açık kaynaklı, esnek ve genişletilebilir bir yazılım boru hattıdır. Model eğitimi ve değerlendirmesinde, veri setlerini bölmede çeşitli seçenekleri desteklemektedir. Ancak bölme, iç içe küme çapraz doğrulamaya benzer bir süreci takip etmektedir [65].

Veri oluşturma; eğitim, model seçimi (yani doğrulama) ve performans değerlendirmesi (yani test) olmak üzere veri setleri birbiriyle örtüşmeyen üç bölmeye bölünmüştür. AMPL ise, eğitim verilerinden alışılmışın dışında kimyasal uzaya kadar genelleşen modeller oluşturmanın önündeki engellere çeşitli yaklaşımlar sunan çok sayıda veri seti bölme algoritması sağlamaktadır [41]. Rastgele bölmeler, Bemis-Murcko yapı iskelesi ayırma, Butina kümeleme ve parmak izi farklılığı tabanlı algoritma gibi DeepChem'de yer alan çeşitli yöntemlere yardımcı olmaktadır [63].

Artan veri boyutu ve bilgisayar gücüyle yapay zekanın ilaç tasarımındaki tarihsel ilerlemesi Şekil 4'te verilmektedir.



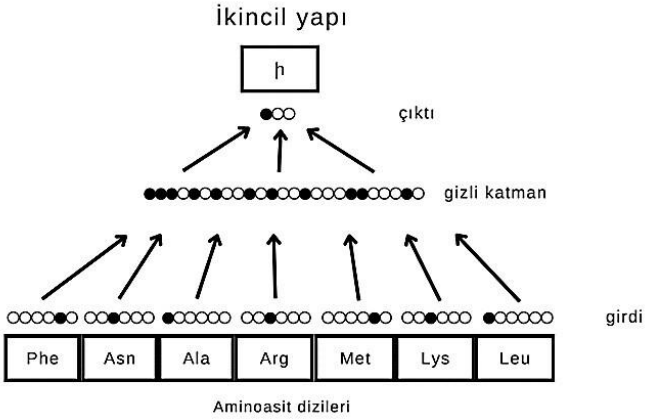
Şekil 4. Artan veri boyutu ve bilgisayar gücüyle yapay zekanın ilaç tasarımındaki tarihsel ilerlemesi [59]

Protein Katlanmasının Tahmininde Yapay Zeka Uygulamaları

Hastalıkların çoğu, protein fonksiyonlarının düzensizliği ile ilişkilidir. Protein yapısı temelinde, protein hedefleri üzerinde bulunan küçük aktif bölgelerin keşfedilmesi ve bu bölgelerle etkileşebilen aktif moleküllerin geliştirilmesi için çeşitli ilaç tasarım yaklaşımları kullanılabilmektedir. Ancak,

proteinlerin üç boyutlu (3D) yapısının deneysel olarak aydınlatılması çok maliyetli ve zaman alıcı bir işlemdir [41]. Bu nedenle bilgisayar destekli teknikler, proteinlerin 3 boyutlu yapısının değerlendirilmesinde önemli rol oynamakla birlikte, 3 boyutlu yapısının netliği tartışmalıdır [66]. Bu durumun proteinlerin çok sayıda uzaysal konumunun olmasından kaynaklandığı öne sürülmüştür. Bu nedenle, protein yapısı tahmin edilirken genellikle, tek boyutlu (1D) yapısal niteliklerin dizileri, protein ikincil (2°) yapısı, solventle erişilebilen yüzey alanı, iskelet bükülme açısı ve benzeri gibi bileşene ayrılarak incelenmektedir [67].

Büyük protein dizilerinde veri setlerinin bulunması nedeniyle, AI tabanlı teknoloji, proteinlerin yapısal özelliklerini öngörmek için kapsamlı bir şekilde kullanılmıştır. İlk olarak, Qian ve ark. (1988) proteinlerin ikincil yapısını öngörmek için doğrusal olmayan sinir ağı algoritmasını kullanmışlardır. Kullanılan algoritmanın diyagramı Şekil 5'te verilmektedir [68].



Şekil 5. Ağ algoritmasının bir diyagramı [68]; Standart ağ 13 bitişik amino asitlik bir diziyi temsil eden 21 birim/grup ile 13 girdi grubuna sahiptir (yalnızca 7 girdi grubu ve 7 birim/grup gösterilmektedir). Giriş katmanından gelen bilgi, merkezi amino asit için ikincil yapı öngörüsünü temsil eden 3 çıkış biriminde bir aktivite paterni üretmek için "gizli katman" birimlerin bir ara katmanı tarafından dönüştürülmektedir

Kullanılan algoritma %64.3 başarı oranı ile diğer algoritmalardan daha başarılı bir sonuç vermiştir. Proteinlerin 3 boyutlu yapısını tam olarak öngörmek hala uzak bir durum olsa da DL algoritması, bu alanın ilerlemesinde önemli bir gelişim göstermiştir. Son zamanlarda DL yaklaşımları, proteinlerin 2° yapısını, solventle erişilebilen yüzey alanlarını, α-karbon atomları dihedral açısını ve iskelet bükülme açılarını öngörmek için kullanılmıştır [68-71].

Qi ve ark. DNN'leri çok görevli bölgesel protein yapısı tahmini geliştirmek için bir farklılaştırıcı olarak kullanmış ve protein çözücü erişilebilirliğini ve 2° yapılarını öngörmek ve evrimsel özellikleri kullanmak için tek bir sinir ağını yönetmişlerdir. Wang ve ark. koşullu rastgele feld (CRF) ile birlikte kolay sinir ağını birleştirmiş ve proteinlerin 2° yapısını öngörmek için bir DeepCNF tekniği geliştirmişlerdir. Bu teknikle, tahmin hassasiyeti %84'e yükseltilerek çözücü erişimi, temas numarası ve düzensiz alanlar dahil olmak üzere proteinlerin yapısal özelliklerini öngörmek için kullanılabilir hale gelmiştir. Ayrıca, Jo ve ark. protein yapı tanımlama performansını oldukça artıran bir derin öğrenme ağı tekniği (DN katlama) oluşturmuştur [69,71,72]. Bu teknik, belirli bir protein çiftinin karşılıklı gelen kısımlarının aynı yapısal katla ilişkili olup olmadığını tam olarak öngörebilmektedir. Protein yapı tahmini, bir protein ana yapısının fonksiyonlarını belirlediğinden, amino asit dizisinden bir proteinin 3D şeklini belirlemek için kullanılabilmektedir [72-74].

2018 yılında DeepMind, protein yapısını tahmin etmek için AlphaFold'u kullanmıştır. AlphaFold, bilinmeyen kıvrımları doğru bir şekilde tahmin etme yeteneğine sahiptir. AlphaFold'un daha yüksek doğruluk oranı, proteinlerin yapıları arasındaki mesafe tahminlerinin doğruluğundan kaynaklanmaktadır [75,76]. 2020 yılına gelindiğinde yayınlanan bir diğer çalışma olan AlphaFold 2 ise, önceki çalışmadan

farklı olarak daha gelişmiş bir yapay zekâ algoritması kullanmıştır. AlphaFold 2, proteinlerin üç boyutlu yapılarını daha doğru bir şekilde tahmin edebilmekte ve önceki çalışmada karşılaşılan bazı zorlukları aşabilmektedir. AlphaFold 2'nin başarısı, daha önce elde edilen diğer yapısal verilerle karşılaştırıldığında %25 oranında daha doğru sonuçlar vermesiyle ölçülmüştür. Bu teknoloji, ilaç tasarımı alanda büyük bir potansiyel sunmaktadır ve biyolojik sistemlerdeki protein yapısının anlaşılmasına önemli katkılar sağlamaktadır [77].

Protein-Protein Etkileşimlerinin Tahmininde Yapay Zeka Uygulaması

Protein-protein etkileşimleri (PPIs) biyofiziksel kimyanın çekirdeğidir. Biyokimyasal süreçleri içeren biyolojik fonksiyonların çoğu, PPIs tarafından yakından kontrol edilmektedir [78-81]. PPIs pek çok biyolojik aktivitede önemli olmakla birlikte, aynı zamanda çok sayıda hastalıkla da doğrudan ilişkilidir [82]. Konvansiyonel hedeflerle (örneğin, nükleer reseptörler, G-protein eşli reseptörler (GPCR'ler), iyon kanalları, kinaz vb.), hedef alanı genişletmek ve küçük moleküllü ilaçların geliştirilmesine yardımcı olmak için PPIs'den yararlanılmaktadır. Bu nedenle, PPIs arayüz bölgesinin ayrıntılı bir şekilde anlaşılması, protein fonksiyonunun açıklanmasına katkıda bulunmasının yanı sıra, protein-protein kompleks yapılarına ve ilgili hastalık tedavisine dayalı ilaç tasarımı için de kritik önem taşımaktadır [83]. Bununla birlikte, PPIs için mevcut deneysel ölçüm yöntemlerinin yüksek maliyet, uzun zaman gereksinimleri, veri setinde yüksek yanlış pozitif ve negatif oranlar gibi dezavantajları nedeniyle PPIs hakkında bilgi çok sınırlı olmaktadır [84]. Bu nedenle, PPI arayüz tahmini için birçok hesaplama yöntemi oluşturulmuştur [85,86].

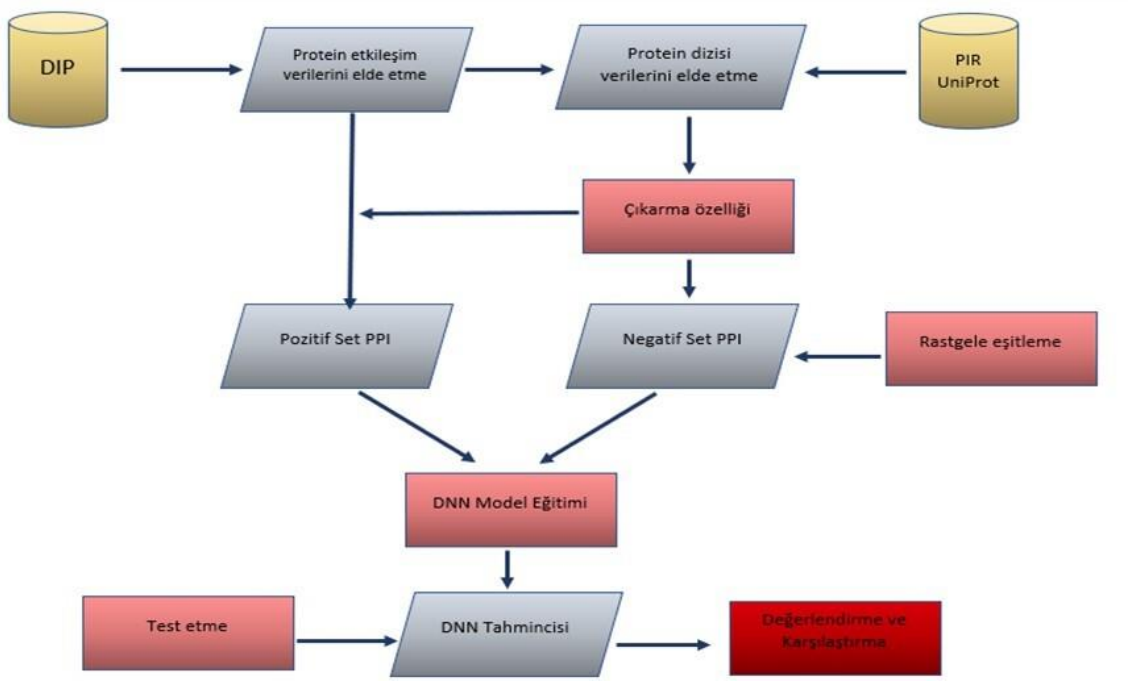
Mevcut PPI tahmini, temel olarak yapılara ve dizilere dayalı iki kategori içermektedir. Bunların arasında, çoğu PPI arabirimi korunaklı olduğundan, protein şablon yapısına dayalı yöntem daha basit ve daha güvenilir sonuç vermektedir. Örneğin, Maheshwari ve ark., zayıf homolog şablon yapısından PPI kalıntılarını tanımlamak için kullanılabilen, şablon yapısına dayalı eFindSitePPI tahmin yöntemini geliştirmişlerdir [87]. Bu yöntem hem deneysel protein yapısında hem de silico ile üretilen protein yapısında yüksek tahmin doğruluğuna sahiptir. Etkileşen iki proteinin 3D yapıları bilindiğinde, PPI arayüzü, tamamlayıcı prensibe dayalı protein-protein kenetleme yöntemleri kullanılarak tahmin edilebilmektedir [41,88].

Yapı tabanlı tahmin algoritmaları dizi tabanlı yöntemlerden daha iyi performans gösterse de bu yaklaşım bilinen protein yapılarının sayısı ve kalitesiyle sınırlıdır. Örneğin, şu anda bilinen bakteri, maya veya insandaki PPI'lerin %80'i hakkında çok az yapısal bilgi bulunmaktadır [89]. Protein dizisi verilerindeki logaritmik büyüme ile AI, dizi tabanlı yöntemler kullanarak PPI'leri tahmin etmede önemli ilerleme kaydetmiştir. 2016 yılında Du ve ark., protein dizilerinden Fisher fraksiyonu özelliklerini çıkarmak için etkileşimli profil gizli Markov modellerini (ipHMM'ler) kullanmışlardır. Bununla birlikte, ilk kez proteinlerin kalıntı-kalıntı temas matrisini tahmin etmek amacıyla bir DNN modeli oluşturmak için, seyrek otomatik kodlayıcı (SAE) kullanılmıştır. DL modelinin genel tahmin doğruluğu, geleneksel ML modelinden %15 daha yüksek olarak %80,82 olarak bulunmuştur [85]. Bu gelişmelerin ardından, Du ve ark. PPI'leri tahmin etmek için DeepPPI adlı bir DNN yöntemi tasarlamışlardır. Bu yöntemin işleyişi Şekil 6'da verilmektedir. Bu yöntemle katman katman soyut yöntemle protein dizilerinden yararlı özellikleri otomatik olarak belirleyerek, protein dizilerinin özgüllüğü öğrenilir hale gelmiştir. Sonuç olarak bu yöntemle, test veri setindeki doğruluk %92,50'ye kadar çıkmıştır [90].

Daha sonra Zeng ve ark. onaylanmış protein komplekslerini öngörmek için dizi yöntemini kullanan 'Complex Contact' web sunucusunu (http://raptorx2.uchicago.edu/ComplexContact/) geliştirmiştir. Sunucu önce proteinler arasındaki dizi homolojisini araştırmakta ve ardından iki adede kadar çoklu dizi hizalaması (MSA) oluşturmaktadır. Bundan sonra, proteinler arası teması tahmin etmek için derin evrişimli sinir ağı (ResNet) ve bununla birlikte evrimsel analiz yaklaşımları kullanılmaktadır [91]. Bu yöntem, protein dizisi homolojisi gereksinimini azaltmakta ve tahmin doğruluğunu önemli ölçüde artırmaktadır.

Son zamanlarda, Xie ve ark., PPI bölgesini tahmin etmek için bir CNN önermiş ve pozitif örnekleri iyileştirmek için kalıntı bağlama eğilimi kullanmıştır. Bu yöntem ile iyileştirilmiş veri setinde elde edilen sonuçlar %91.2 başarı oranını göstermiştir. Ayrıca bu yöntem, büyük bağlanma eğilimi olan numunelerde çok daha iyi sonuçlar vermiştir. Bu yöntem aynı zamanda, kalıntı atomları arasındaki

mesafe ile belirlenen pozitif örneklerde, oldukça büyük bir oranda yanlış pozitif PPI bölgelerinin varlığını ortaya koymuştur [92].



Şekil 6. Protein-protein etkileşimleri tahmini için derin sinir ağlarının çerçevesi [90]

Yapay Zeka ile Sanal Tarama Uygulamaları

Sanal tarama (VS), ilaç hedeflerine (genellikle proteinler) bağlanan aktif küçük molekülleri tanımlamak amacıyla yapılan hesaplamalı ilaç tasarımının ana yöntemlerinden biridir [93]. Bu amaçla, VS kaçınılmaz olarak bilgisayar programları kullanarak bileşiklerin geniş kapsamlı veri tabanlarını değerlendirmektedir [94]. İlaç geliştirmenin ilk aşamalarında uygun olmayan iskeletler içeren bileşikleri filtrelemek ve yeni hedefler bulmak için etkili bir yöntem olarak kullanılabildiğinden, yüksek maliyetli ve düşük başarı oranı gibi problemleri olan yüksek verimli taramaya (HTS) yardımcı olmak için önemli bir araç haline gelmiştir [49].

Sanal taramada ligand bazlı sanal tarama (LBVS) ve yapı bazlı sanal tarama (SBVS) olmak üzere başlıca iki yöntem kullanılmaktadır. LBVS, yüksek biyoaktivitelere sahip diğer ligandları tahmin etmek ve tanımlamak için aktif ligandlar arasındaki kimyasal ve uzaysal benzerlikleri ve fizikokimyasal analizi kullanan aktif ve inaktif ligandların deneysel verilerine dayanmaktadır. LBVS, 3D protein yapısal bilgisine dayanmadığından, bu yöntem öncelikle hedef yapı eksik olduğunda veya elde edilen yapısal doğruluk düşük olduğunda aktif ligandların tahmini için kullanılmaktadır [95].

Geleneksel makine öğrenimi yöntemlerinden olan RF, SVM, DT, KNN ve NB gibi ML yöntemleri, LBVS'de yaygın olarak kullanılmaktadır. Bu yöntemler, öngörülen hedef oranını başarılı bir şekilde iyileştirmekte ve yanlış hedef oranını azaltmaktadır [96]. Bununla birlikte, kimyasal alandaki aktif bileşiklerin seyrekliği (10^{60} teorik bileşik) ve sınırlı eğitim seti, geleneksel ML'lerin LBVS'de istenen başarıya ulaşma noktasında bazı kısıtlamalara sahip olmasına neden olmaktadır. Otomatik çıkarma ve katman katman öğrenme özelliklerine sahip DL'nin ortaya çıkışı, LBVS'nin daha da geliştirilmesine olanak sağlamıştır [97].

Xiao ve ark. açık kaynaklı TensorFlow programı ile büyük verilerin bir DNN prototipini oluşturmuş ve bunu kapsamlı bileşik kitaplıklarını taramak için bir LBVS cihazı olarak kullanmışlardır. 2015'ten önce PubChem'de 95 milyon antikanser hedef inhibitörü tarayan model, 2015'ten sonra inhibitörlerin %50'sini yalnızca %0.01-%0.09'luk bir yanlış pozitif oranıyla tanımlamış ve bu da DNN'lerin LBVS'deki uygulama potansiyelini tam olarak göstermiştir [98].

SBVS genel olarak, hedefin üç boyutlu yapısının hesaplamalı veya deneysel yöntemlerle aydınlatılmasından sonra kullanılır. Bu yöntem esas olarak olası aktif ligandlar ve bağlanma yeri kalıntıları arasındaki etkileşimleri araştırmak için kullanılır ve genellikle LBVS yöntemlerinden daha iyi tahmin performansı gösterir [99]. Bununla birlikte, SBVS tabanlı yöntem, protein yapı sayısında üstel büyüme ve son derece karmaşık protein konformasyonları sorunuyla karşı karşıyadır. Problemi çözmenin anahtarı, ligandlar ve hedefler arasındaki ilişkiyi doğru bir şekilde tanımlamaktır. Protein-ligand bağlanmasındaki ve yapısal verilerdeki artışla birlikte, SBVS'nin daha da geliştirilmesi için yeni araçlar sağlayan AI teknolojisi kullanılarak protein-ligand etkileşimlerini tanımlamak mümkündür [100]. SVM, Boosting ve RF gibi geleneksel makine öğrenimi yöntemleri, ligandlar ve hedefler arasındaki moleküler etkileşimlerin doğrusal olmayan etkileşimlerini açıklayabilmiş ve SBVS'nin sonuçlarını iyileştirmek için başarıyla kullanılmıştır. Bir dizi protein-ligand bağlanma afinite sınıflandırıcısı da oluşturulmuştur [101].

Bu özelliklerin yanı sıra, geleneksel makine öğrenimi yöntemleri, büyük ölçekli uygulamaların elde edilmesini zorlaştıran ve hatta özellik çıkarma sürecinde ilgili bilgilerin kaybolmasına yol açan oldukça zahmetli manuel tanıma ve özellik çıkarma sorununa sahiptir. DL yöntemlerinin ortaya çıkmasıyla bu sorun başarılı bir şekilde çözülmüştür [102]

Pereira ve ark. ilk olarak 2016 yılında SBVS'nin performansını iyileştirmek için derin öğrenmeyi kullanmış ve DeepVS adı verilen geliştirilmiş bir SBVS yöntemi oluşturmak için derin evrişimli sinir ağları (DCNN) modelini kullanmışlardır. Bu yöntem, moleküler yerleştirmenin sonucunu DCNN'nin girdisi olarak almakta ve temel verilerden bileşik atom tipi, atomik kısmi yük ve atomik mesafe gibi ilgili özellikleri otomatik olarak öğrenebilmekte ve çıkarabilmektedir [103].

Ardından Ferrero ve ekibi, Open Targets platformundan elde edilen gen-hastalık ilişkisi verilerine dayanarak terapötik hedeflerin belirlenmesi üzerine araştırma yapmıştır. Bu çalışmada, belirli bir genin bir ilaç hedefi olma ihtimalini tahmin etmek için makine öğrenimi modelleri, RF, SVM, NN ve Gradyan Artırma Makinesi gibi yöntemler kullanılmıştır [104].

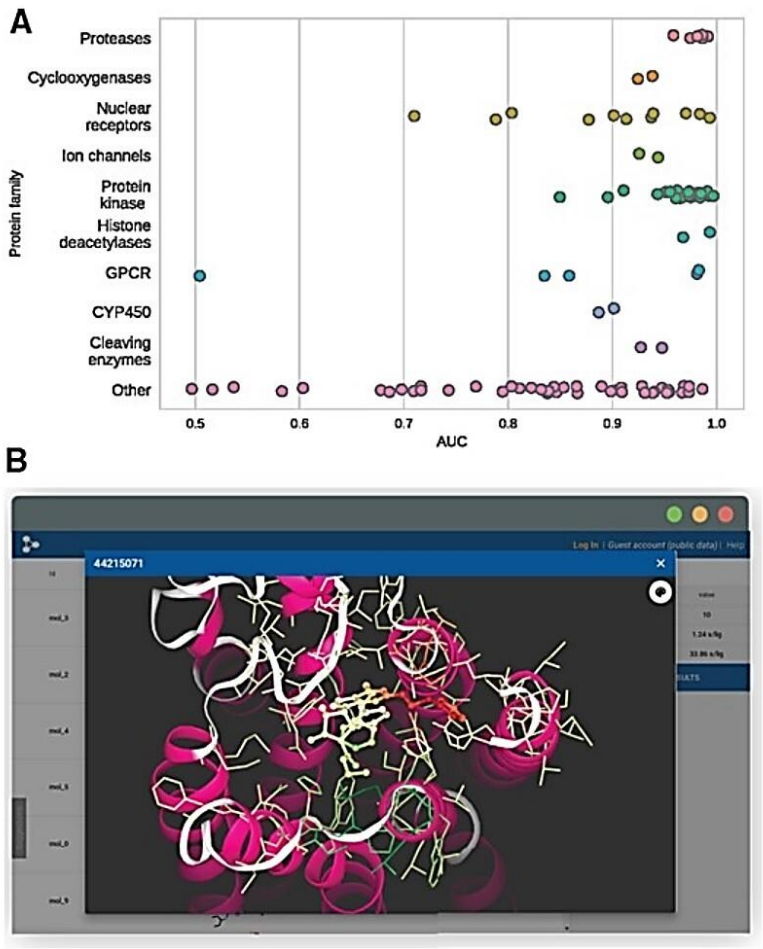
Donner ve ekibi ise, gen ekspresyon verilerinden oluşan moleküllerdeki fonksiyonel grupların yapısını taşıyan ve bu moleküllere en benzer bileşikleri tespit etmek için bir yöntem geliştirmişlerdir. Bu yöntem, derin öğrenme ağlarının avantajlarını kullanarak ve verilerin bütünlüğünü koruyarak bileşiklerin karşılaştırılmasını sağlamaktadır. Bu yöntem, bileşiklerin yapısal olarak farklı olmasına rağmen, paylaşılan terapötik ve biyolojik hedefleri olan ilaçları belirleyebilmekte ve böylece bileşikler arasında daha önce bildirilmemiş fonksiyonel ilişkileri ortaya çıkarabilmektedir. DeepCodex adlı bir çevrimiçi platformda kullanıma sunulmuş olan bu yöntem, farklı yapısal özelliklere sahip bileşikler arasında bile paylaşılan biyolojik hedefleri tespit ederek, bileşikler arasında önceden bilinmeyen fonksiyonel ilişkileri ortaya çıkarabilmektedir [105,106].

Entegre Ağ Tabanlı Hücresel İmzalar Kitaplığı (LINCS, Library of Integrated Network-Based Cellular Signatures), gen ekspresyonu, protein aktivasyonu ve kimyasal bileşiklerin hücreler üzerindeki etkileri gibi biyolojik süreçlerin geniş bir yelpazesini kapsayan büyük ölçekli bir veritabanıdır. Bu veritabanı, ilaç tasarımı ve hastalık mekanizmalarının anlaşılması gibi birçok farklı araştırma alanında kullanılmaktadır [107]. Xie ve ark., LINCS'nın L1000 veritabanındaki transkriptom verilerini kullanarak ilaç-hedef etkileşimlerini öngörmek için bir derin öğrenme yöntemi ile bir çalışma gerçekleştirmişlerdir. Elde edilen sonuçlar, yöntemin doğruluğunun %98 oranında olduğunu bildirmişlerdir. Ayrıca bu çalışma, önceden toplanan verilerin derin öğrenme yaklaşımlarıyla yeniden kullanılmasının ilaçların tasarlanmasındaki önemini ortaya koymuştur [108,109].

Ayrıca, Skalic ve ark. büyük ölçekli aktif ve inaktif bileşikleri sınıflandırabilen ve GPU hızlandırmasını gerçekleştirebilen BindScope adlı bir web uygulaması oluşturmak için DCNN modelini kullanmaktadır. Program, kullanıcıların aynı anda yüzlerce bileşiği görüntülemesine ve sonuçları etkileşimli olarak görselleştirmesine olanak tanımaktadır. BindScop uygulamasıyla ilgili örnek bir çalışma Şekil 7'de verilmektedir [110].

Son zamanlarda, Mendolia ve ark. IC_{50} değerlerini kullanarak Cyclin-Dependent Kinaz 1 (CDK1) protein hedefine karşı aday bileşiklerin biyolojik aktivitesini tahmin etmek için moleküler parmak izleri üzerinde eğitilmiş yeni bir CNN yöntemini sunmuş ve bu çalışma için teknik geliştirmişlerdir. Bunlar; tek parmak izleri sınıflandırıcıları, önemli birleştirmeler için 1D ve 2D CNN sınıflandırıcıları, 1D CNN

sınıflandırıcılarına dayalı bir sınıflandırma ve VS'deki her farklı parmak izi tipinin çıktısını derin sinir ağına dayalı olarak sınıflandıran tekniklerdir [111].

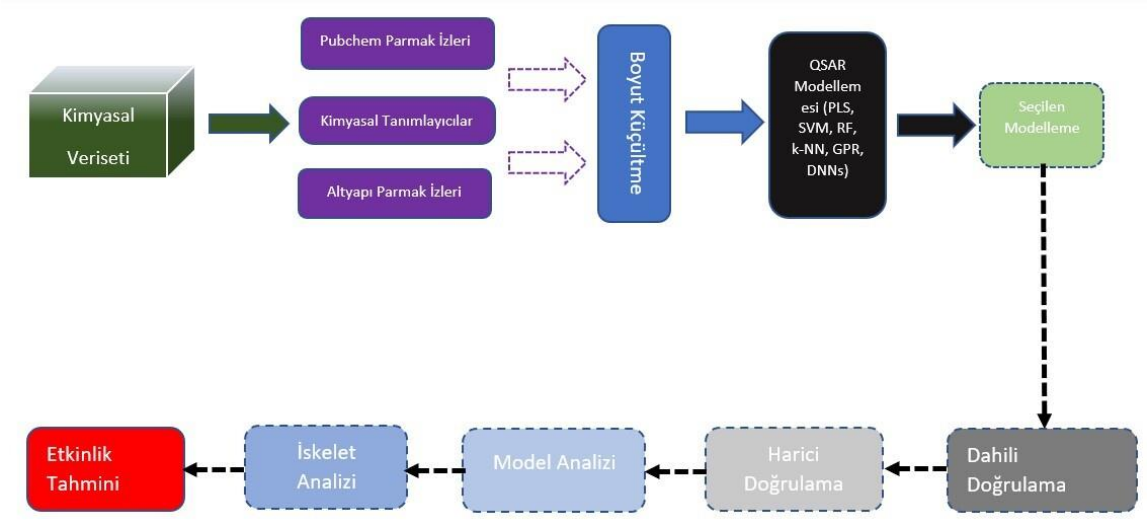


Şekil 7. BindScope web uygulamasındaki örnek çalışma [110]; A. Reseptör başına AUC değeri 5 kat çapraz validasyon kullanılarak değerlendirilmesi. B. Kenetlenmiş pozun, hangi ligand alanlarının olumlu katkıda bulunduğunu gösteren karşılık gelen ayrımcı lokalizasyon haritası ile görselleştirilmesi

Kantitatif Yapı-Aktivite İlişkisinde Yapay Zeka Uygulaması

Kantitatif yapı-aktivite ilişkisi (QSAR), fizikokimyasal özellikler, kimyasal yapı ve biyolojik aktiviteler arasındaki kantitatif haritalama ilişkisini oluşturmak için matematiksel yöntemler kullanmaktadır [112-114]. Bu ilişkiler kurulduktan sonra, yapısal olarak çeşitli moleküler veri tabanını otomatik olarak taramak ve ardından laboratuvarda sentez ve analiz için en umut verici bileşikleri seçmek kolaylaşmaktadır. Böylece, deneysel kaynaklardan büyük ölçüde tasarruf edilebilmekte, deneyin başarısızlık oranı düşürülebilmekte, istenen özelliklerde yeni moleküllerin geliştirme süreci de hızlandırılabilmektedir. QSAR yöntemi temel olarak veri toplama ve ön işleme, moleküler tanımlayıcıların üretimi ve seçimi, matematiksel bir modelin oluşturulması, model değerlendirme ve yorumlama ile model uygulamasını içermektedir. Bu uygulamanın iş akışı Şekil 8'de verilmektedir.

Yapay zekâ, kimyasal yapı ile biyolojik aktivite arasındaki ilişkinin sağlam bir modelini etkili bir şekilde oluşturabildiğinden, QSAR araştırmasının önemli bir parçası haline gelmiştir. 1990 gibi erken bir tarihte, Aoyama ve ark. sinir ağlarını (NN'ler) QSAR analizinde kullanılmıştır [115]. Daha sonra, RF, Boosting, GP, KNN, DL, Cubist ve SVM gibi çeşitli geleneksel ML yöntemleri de QSAR modellerini oluşturmak için yaygın olarak kullanılmıştır [116,117].



Şekil 8. QSAR modellemesinin iş akışı

Veri kümelerindeki sürekli artışla birlikte, QSAR modeli giderek daha karmaşık hale gelmekte ve geleneksel makine öğreniminde kullanılan sığ sinir ağı yönteminin büyük veri kümelerinin ihtiyaçlarını karşılaması zorlaşmıştır. DL'nin ortaya çıkışı, büyük veri kümeleri sorununu çözmek için yeni bir yol sağlamıştır. 2012'de Dahl'ın ekibi, çok görevli DNN'ler, Gauss süreci regresyonu (GPR) ve gradyan destekli makinelerden (GBM'ler) oluşan entegre bir model kullanarak 2012 Merck Kaggle Molecular Activity Challenge'ı kazanmıştır [61]. Bu çalışmayla, bileşik aktiviteyi tahmin etmede yeni bir dönem başlatan büyük bir veri setinde QSAR problemini çözmek için DL ilk kez kullanılmıştır. 2014 yılında Dahl ve ark., bir bileşiğin biyolojik ve kimyasal özelliklerini moleküler yapısından doğrudan tahmin edebilen çok görevli bir DNN geliştirmiştir [118]. Bu çalışmalara kadar Merck, DNN'leri ve RF'yi karşılaştırmak için birçok QSAR veri seti kullanmış ve 15 adet veri setinen 11 veri seti, DNN'lerde rastgele ormana göre daha iyi performans gösterirken, ikinci değerlendirmede, zamana dayalı bölme stratejisiyle 15 veri setinin 13'ü DNN'lerde rastgele ormana göre daha iyi performans göstermiştir [61].

Çok görevli QSAR modelleri, bileşikleri biyolojik etkilerinin yanı sıra deneysel bilgilere dayalı olarak kategorize edebilmektedir. Tenorio-Borroto ve ark., YSA temelinde çok görevli bir QSAR modeli geliştirmişlerdir. Bu modelleme, çoğullama deneylerinden elde edilen veri setini %92'lik bir hassasiyetle sınıflandırmıştır [119].

İlerleyen süreçte, kimyasalların immünotoksisitesini öngörmek amacıyla, yeni çok görevli QSAR-ANN modellerini oluşturmak için birkaç tanımlayıcının kullanılmasıyla ilgili çalışmalar yürütülmüştür [120]. Ayrıca bu modelleme yöntemiyle, TMC-207, Sudoterb, Delamanid gibi güvenli antibakteriyel ilaçların bulunmasına yönelik ilk çalışma Speck-Planche ve ark. tarafından yapılmıştır. Bu modelleme ile elde edilen ilaçlar, *Mycobacterium spp.* üzerindeki antibakteriyel aktiviteleri umut vericidir [121]. QSAR-ANN modeli hem eğitim hem de öngörü setleri için kesinliği yüksek öngörü sonuçları elde etmiştir. Aynı program ve kavramsal süreç takip edilerek, değişik elverişli çoklu görev QSAR modelleri geliştirilmiştir [119,121,122].

Ramsundar ve ark. çoklu görev sınıflandırma ikilemindeki görev sayısının etkisine odaklanmıştır. Yaptıkları çalışmada, 200'den fazla hedef (ağırlıklı olarak proteinler) ve 1.6 milyon bileşik için genişletilmiş bağlantı parmak izleri (ECFP) olarak tanımlanan 37.8 milyon deneysel veri noktası bir araya getirilmiştir [123]. Böylelikle, çoklu görev ağı, RF, lojistik regresyon ve tek görevli sinir ağlarını geride bırakmıştır.

Daha sonra Xu ve ark. çok görevli bir DNN'ye aktarılan bir görevin, eğitim süreci sırasında ek QSAR görevlerinden bilgileri "ödünç alabileceğini" gözlemlemiştir. Çeşitli çalışmalar, yüksek yorumlamalı QSAR modelleri geliştirmede DNN'lerin geleneksel makine öğrenimi tekniklerine göre geliştirilmiş performansını göstermiştir [124].

Yakın zamanda ise Zhao ve ark., modelleri ayrı ayrı oluşturmak yerine, çok sayıda benzer biyolojik hedefi bir arada düşünerek QSAR modellerini oluşturmuştur. Ayrıca (çok görevli öğrenme) MTL tabanlı modelin (tek görevli öğrenme) STL tabanlı modellerden daha yüksek performans gösterdiği sonucuna ulaşmışlardır. Birden çok görev üzerinde paylaşılan bilgilerin kullanılmasına bağlı olarak, MTL modelleri, temel modellerin düşük doğruluğa sahip olduğu durumlarda daha da belirgin başarı performansına sahip olmuştur. MTL modellerinin üstünlüğü, %5'lik anlamlılık düzeyi ile Student's T testi tarafından da desteklenmiştir [125].

ADME/T Farmakokinetik Özelliklerin Tahmininde Yapay Zeka Uygulaması

İlaç tasarlama ve geliştirme sürecinde, hedef veya öncü bileşikler elde edildikten sonra, bu bileşiklerin farmakokinetik özellikleri (absorpsiyon, dağılım, metabolizma ve atılım) ve toksisitesi (ADME/T) üzerinde bir dizi test ve değerlendirmeler yapılmaktadır [126].

Son yıllarda, milyonlarca aktif bileşik keşfedilmiş olmasına rağmen, FDA tarafından onaylanan yeni ilaç molekülü sayısı çok kısıtlı kalmaktadır. Bunun başlıca nedeni, bu aktif bileşiklerin ADME/T özelliklerinin ilaç olmak için gereken standartları karşılamamasıdır [127,128]. Raporlara göre, yan etkilerin ve düşük etkinin yanı sıra; bazı ticari nedenlerle birlikte farmakokinetik özelliklerin yetersizliği (%39) ve klinik öncesi toksisite (%11) ilaç geliştirme başarısızlığının ana nedenlerindendir [129]. Bu nedenle, öncü bileşiklerin ADME/T özelliklerinin erken değerlendirilmesi ve optimizasyonu, üretim verimliliğini ve ilaç geliştirmenin başarı oranını artırmada önemli bir rol oynamaktadır. Bununla birlikte, ilaçların neden olduğu yan etkileri inceleyen *in vivo* toksikolojik deneyler en önemli standartları oluşturmasına rağmen, uzun zaman alması, birçok hayvan kullanılması ve yüksek maliyet gibi nedenlerden dolayı bilgisayar destekli ADME/T tahmini, zamanla ilaç tasarımı için tercih edilen bir yöntem olmuştur [130]. Süreç içerisinde, ADME/T özelliklerini tahmin etmek için çok sayıda model tanımlanmıştır [131]. Bu modellerle birlikte bilgisayar tabanlı araçların büyük ölçekli kullanımının ilaç geliştirme maliyetini %50 azaltabileceği öne sürülmüştür [132].

ADME/T için 1930'lu yıllardan 1990 ve hatta 2000'li yılların başlarına kadar aktif olarak basit çoklu doğrusal regrasyon yöntemleri, LFER (linear free energy relationship), QSPR (Quantitative structure property relationship), HTS, GSE (general solubility equation) yöntem ve hesaplamaları kullanılırken günümüzde daha kesin sonuçlar veren programların gelişmesiyle yerini daha gelişmiş yöntemlere bırakmıştır [127].

Bu çalışmalardan biri olan ALOGPS, ilaç tasarımı (ADME/T ve HTS) ve çevre kimyası çalışmalarında kullanılan bir programdır ve bileşiklerin logP, suda çözünürlük ve pKa değerleri hakkında öngörüler sağlamaktadır. ALOGPS 2.1 sürümü, özellikle ilaç tasarımı çalışmalarında kullanılmak üzere tasarlanmıştır ve 1-oktanol/su içindeki partisyon katsayıları, logP ve sulu çözünürlük gibi özellikleri tahmin etmektedir. ALOGPS'un öne çıkan önemli özelliği, kullanıcı tarafından sağlanan yeni verileri birleştirme yeteneğine sahip olmasıdır. Bu özellik, ANN'nın kendi kendine öğrenme özelliği sayesinde mümkün olmaktadır. Bu nedenle, ALOGPS, ilaç tasarımı ve çevre kimyası çalışmalarında önemli bir araçtır ve yeni verilerin eklenmesiyle daha doğru öngörüler sağlayabilmektedir [133].

ADME/T tahmin modelini oluşturmak için SVM, GP, RF ve NB dahil olmak üzere bazı geleneksel ML algoritmaları kullanılmıştır [134-137] Ardından, güçlü genelleme ve özellik çıkarma yeteneği nedeniyle DL, ADME/T tahmin modelleri oluşturmak için kullanılmıştır. 2013 yılında, Lusci ve ark. ilk önce moleküler yapıyı kodlamak için yeni bir yönlendirilmemiş grafik tekrarlayan sinir ağı (UG-RNN) yöntemini kullanmış ve bu yöntemle kimyasal yapılar grafiğe dönüştürülmüştür. Bu yöntem, otomatik öğrenmeyi gerçekleştiren DL ağ yapısına dayalı bileşiklerin suda çözünürlüğünü etkili bir şekilde tahmin etmek için kullanmışlardır [138].

Amerika Birleşik Devletleri federal kurumları (Çevre Koruma Ajansı (EPA), FDA ve Ulusal Sağlık Enstitüleri (NIH)) tarafından başlatılan veri yarışmasında, 21. Yüzyılda Toksisite Testi (Tox21) girişimi, en büyük çalışma olarak kabul edilmiştir. Tox21 girişiminde, kimyasalların insan sağlığı üzerindeki toksisite etkisini tahmin etmek, *in vivo* ve *in vitro* deneylerin sayısını azaltmak için daha etkili ve zaman kazandıran yöntemler geliştirilmesi amaçlanmıştır [139]. 2014 yılında yapılan Tox21 yarışmasında, Mayr ve ark., DL teknolojisini bileşiklerin toksisite tahminine uygulamış ve DeepTox

adlı, diğer yarışmacılardan daha başarılı bulunan, çok görevli bir DNN modeli geliştirmiştir. Sonuç, DL'nin toksisite tahmini açısından geleneksel yöntemlerden üstün olduğunu göstermiştir [62].

2015 yılına gelindiğinde ise Clark ve ark., ML tarafından oluşturulan, ADME/T tahmin modellerinin kullanışlılığını geliştirmek için NB'yi kullanan bir yazılım modülü oluşturulmuştur. Modül, Kimyasal Geliştirme Kiti (CDK) projesinde açık kaynaklı bir bileşen olarak piyasaya sürülmüş ve ADME/T, *in vivo* ve *in vitro* biyolojik aktiviteler, fizikokimyasal özelikler ve diğer amaçlar için bir dizi NB modeli oluşturabilen CDD Vault (Costumer Due Diligence) ve çeşitli mobil uygulamalarda uygulanmıştır [140].

Yine bu yılda en yeni derin öğrenme çerçevesi olan Yinelemeli İyileştirme Uzun Kısa Süreli Bellek (IterRefLSTM), grafik evrişimli bir sinir ağıyla entegre edilmiştir ve bu prototipleri, ilişkili ancak ayrı görevler arasında bilgi aktararak eğitmek için geliştirilmiştir. Bu çalışma, Tox21 ve veri tarama ve değerlendirme için kullanılan SIDER koleksiyonlarının aralıklı alt kümelerinde eğitilen prototiplerin performansını önemli ölçüde artırmış ve sonuç olarak; genellikle daha az girdi verisiyle kaybedilen bilgileri geri kazandırmıştır [141].

Ardından, Hughes ve ark. 702 adet epoksidasyon reaksiyon verisi ve DCNN'leri kullanarak epoksidasyon bölgesini doğru bir şekilde tahmin etmek için bir model oluşturmuştur. Model, yüzlerce epoksidasyon reaksiyonunu kantitatif olarak birleştirmiş ve epokside edilmiş ve epokside olmayan moleküller için sırasıyla %94.9'a kadar bir AUC ve %79.3'e kadar bir AUC ile atomik ve moleküler seviyelerde epoksidasyon bölgesi (SOE) tahminlerine ulaşılmıştır. Epokside metabolitler, genellikle ilaç toksisitesinin önde gelen nedeni olduğundan, epoksidasyon bölgesinin (SOE) doğru tahmini, daha güvenli ilaçlar elde etmek için metabolit oluşumu riskini etkili bir şekilde azaltabilmektedir [142].

Ayrıca, Xu ve ark. 475 adet ilaç bazında DL, DILI (Drug Induced Liver Injury) olarak adlandırılan, ilaca bağlı bir karaciğer hasarı tahmini modeli oluşturmak için UG-RNN moleküler kodlama yöntemini dikotomi ile birleştirmiştir. Sonuçlar, UG-RNN moleküler kodlamayı diktomi ile birleştirmenin, ilaçların DILI riskini tahmin etmek için daha doğru sonuçlar verdiğini göstermiştir. Bu çalışma, ilaçların DILI riskini tahmin etmek için yeni bir yöntem sunmakla birlikte ilaç geliştirme sürecinde önemli bir rol oynamaktadır [143].

Iorio ve ark. tarafından yapılan çalışmada da, kanser hücrelerindeki gen mutasyon profilleri ve ekspresyon seviyeleri gibi faktörler göz önünde bulundurularak, ilaç moleküllerinin IC_{50}'sini tahmin etmek için elastik ağ modelleri kullanılmıştır. Bu çalışmada, elde edilen verilerin pratikteki sonuçlarla doğrulandığı bildirilmiştir [144]. Corte´s-Ciriano ve ark. da aynı veri setini RF modellemelerini, ilaç moleküllerinin IC50'sini tahmin etmek kullanmışlardır. Bu çalışmada, her bir tahminde istatistiksel güven ölçüsüne bağlı olarak öngörü performansının iyileştirilebileceği gösterilmiştir [145]. Her iki çalışma da ilaçların etkililiğini tahmin etmek için makine öğrenmesi yöntemlerini kullanmıştır. Elastik ağ modelleri ve RF modeli, ilaç geliştirme sürecinde önemli bir rol oynayabilmekte ve ilaçların etkililiğini tahmin etmek için yeni bir yaklaşım sunmaktadır.

Yine NB modelinin bir örneği olarak, PASS Online, farmakolojik etkiler, etki mekanizmaları, toksik ve yan etkiler dahil olmak üzere 4000'den fazla biyolojik aktivite türünü tahmin etmek için kullanılan bir programdır [146]. Bu program, bileşiğin yapısal formülüne dayanarak, farmakolojik etkiler, etki mekanizmaları, toksik ve yan etkiler gibi birçok biyolojik aktiviteyi öngörebilmektedir. DRABAL adlı etiket sınıflandırma yöntemi ise Bayes ağının yapı öğrenmesini kullanarak, 400.000'den fazla bileşiğin 1.4 milyondan fazla etkileşimini analiz edebilmekte ve PubChem BioAssay veri tabanından beş büyük HTS testi arasındaki mevcut ilişkileri inceleyebilmektedir. Bu yöntemler, bilgisayarda tasarlanan bileşiklerin biyolojik aktivitelerinin öngörülmesinde kullanılmakta ve ilaç tasarımı sürecinde önemli bir rol oynamaktadır [147].

Uygun moleküler tanımlayıcıların DL'nin ADME/T veri kümelerinde diğer geleneksel makine öğrenimi yöntemlerine göre herhangi bir iyileştirme sağlayıp sağlamadığını değerlendirmek için Korotcov ve ark., FCFP6 (FingerCode Print 6) parmak izlerini kullanarak farklı hesaplama yöntemlerini karşılaştırmak için farmasötik araştırmayla ilgili karmaşık uç noktalara sahip sekiz farklı ikili sınıflandırma veri seti seçmiştir. Dikkat çekici bir şekilde, farklı ölçümler veya veri kümeleri için sıralanmış puanlara dayalı olarak, DNN en iyi performansı sergilemiştir ve bu da DL'nin karmaşık veri kümeleriyle başa çıkma konusundaki güçlü yeteneğini göstermektedir [148].

Pande ekibi ise, dört farmasötik veri grubu (Kinase, Kaggle, Factors ve UV veri seti koleksiyonları) üzerinde, RF teknikleri üzerindeki çok görevli derin ağlarının üstünlüğünü ve dayanıklılığını da bildirmiştir. Böylelikle toksisiteyi tahmin etmek için derin öğrenmenin başarısı ve diğer ilaç tasarım ve geliştirme girişimi alanlarındaki başarı, büyük ölçüde girdi verilerinin kalitesine ve miktarına bağlanmıştır [149].

Çok fazla veri noktasından anlamlı kimyasal bilgiler çıkarmak için Altae-Tran ve ark., aralıklı veri içeren görevler için iyileştirilmiş tahmin gücü sağlamak için "tek adımda öğrenme" yöntemini geliştirmiştir [150].

Li ve ark. ise insan sitokrom P450 (CYP450) izoformlarının eşzamanlı inhibisyonu için çok görevli bir model geliştirmiş ve çoklu görev modelinin, tek görev modellerinden, daha önce açıklanan sınıflandırıcılardan ve ortalama beş tasarlama görevinde geleneksel makine öğrenimi yöntemlerinden daha iyi öngörü sonuçları sunduğunu göstermişlerdir. Onların çoklu görev DNN modeli, on katlı çapraz doğrulama için %86,4 ve harici test veri setleri için %88.7 ortalama öngörü doğruluğu sunmuştur [151].

İlerleyen zamanlarda, Wenzel ve ark., DNN modellerini kullanarak, metabolik kararsızlık gösteren indol-3-karboksamid veya azaindol bağlı bir seri insan renin inhibitörü bileşiğin deneysel ve tahmin edilen verileri ilişkilendirmiştir. Deneysel ve tahmin edilen veriler arasında iyi bir korelasyon bildirilmiştir. Ayrıca, çoklu görev modeli tek görev modelinden daha iyi sonuçlar vermiştir [152].

Günümüze kadar geliştirilmiş AI destekli bu yöntemlerle hesaplama güçleri geliştirilerek daha kısa sürede sonuç verecek şekilde çalışmalara devam edilmektedir.

İlaçların Yeniden Hedeflendirilmesinde Yapay Zeka Uygulaması

İlacın yeniden konumlandırılması olarak da bilinen ilacın yeniden hedeflendirilmesi, onaylanmış ilaçlardan yeni endikasyonların keşfedilmesi süreci olarak tanımlanmaktadır. Onaylanmış ilaçlar için mevcut bulunuyor oluşu ve bilinen güvenlik nedeniyle yeni endikasyonların incelenmesi, ilacın yeniden kullanımıyla birlikte, ilaç güvenliği ile ilgili sorunların riskini de etkili bir şekilde azaltabilmektedir [153].

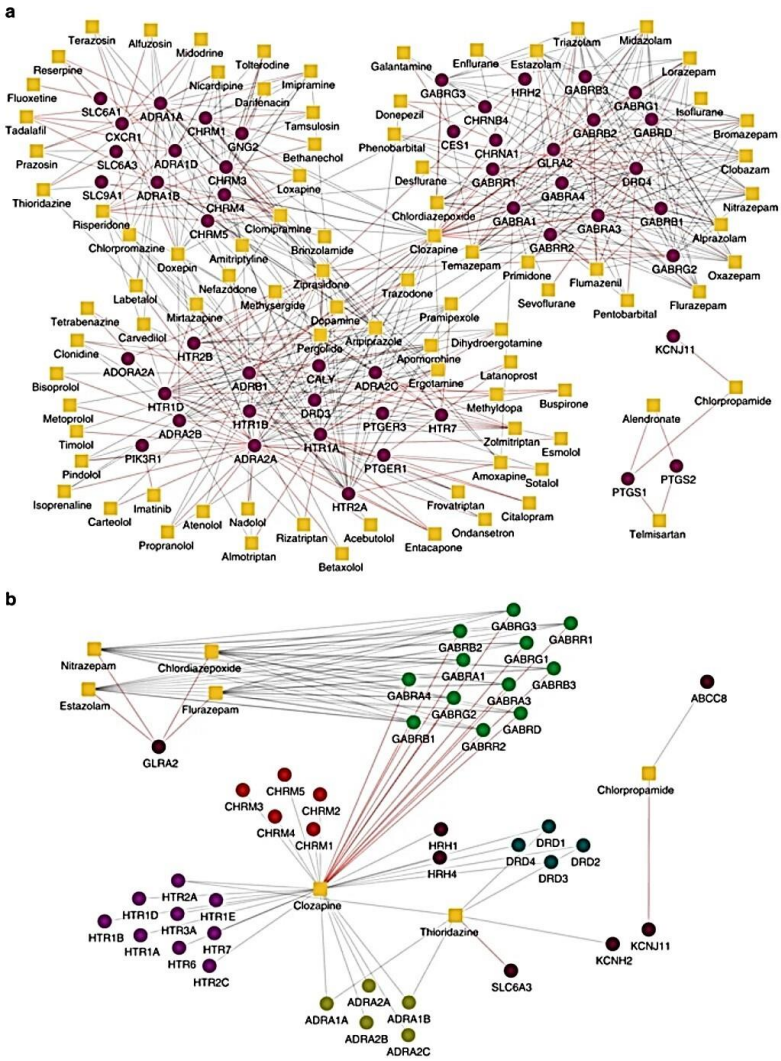
İlaç-hedef etkileşimi (DTI) tanımlaması, ilaç tasarımı ve ilacın yeniden hedeflendirilmesinde önemli bir rol almaktadır. Bununla birlikte, ilacın yeniden kullanım analizine ilişkin çok az veri vardır ve çeşitli biyoanaliz teknikleri, büyük ölçekli DTI'lerin gereksinimlerini karşılayamamaktadır. Böylece, DTI'leri hesaplamalı yöntemlerle tahmin etmek önemli bir araştırma konusu haline gelmiştir. Ligand ve yapı bazlı yöntemler, en yaygın kullanılan iki geleneksel hesaplamalı DTI tahmin yöntemidir [154].

Yapısal olarak benzer moleküllerin benzer biyolojik aktivitelere sahip olduğu varsayımına dayanan ligand bazlı yöntemler, hedef moleküllerin biyolojik aktivitelerini tahmin etmek için QSAR'ı uygulamaktadır [155]. Yapı bazlı yöntemler, hedefin kristal yapısına göre küçük molekülleri taramak için temel olarak moleküler yerleştirmeyi kullanmaktadır [156].

Sınırlı sayıda bilinen hedef aktif molekül ve hedef proteinlerin üç boyutlu yapısı nedeniyle, bu iki geleneksel hesaplama yöntemi büyük ölçüde yetersiz kalmaktadır. Son yıllarda, sürekli deneysel veri birikimi ve AI'nın heterojen verileri işlemedeki üstün performansı yardımıyla, DTI'leri tahmin etmek için birçok ML yöntemi uygulanmıştır [154,157-159]. En sık kullanılan makine öğrenimi modelleri, RF, SVM ve ANN gibi ikili sınıflandırıcılardır. DL yöntemleri de yüksek performansları ve çok soyut veri temsilini öğrenme yetenekleri nedeniyle çok daha fazla dikkat çekmiştir [160-162].

Mayr ve ark., ChEMBL gibi büyük ölçekli bir ilaç tasarım veri seti üzerinde birkaç DL yönteminin performansını değerlendirmiş ve sonuçları, ilaç hedefleri tahmini için diğer geleneksel ML yöntemleri ile karşılaştırmıştır. İleri yönlü DNNs (FNNs) tüm rakip yöntemlerden önemli ölçüde daha iyi performans gösterdiğini ve belirli bir hedefi tahmin etme performansının *in vitro* analizlerle kıyaslanabilen, hatta daha iyi olduğu bulunmuşlardır [65].

Wen ve ark., ilk kez 2017 yılında DL'yi DTI tahminine uygulayan DeepDTIs adlı bir yöntem geliştirmiştir. Hedefleri sınıflandırmadan FDA onaylı ilaçlar ve hedefler arasındaki yeni DTI'ları doğru bir şekilde tahmin etmek için DBN'yi (deep-belief network) kullanmışlardır. Ayrıca DrugBank veri tabanından çıkarılan harici deneysel DTI verilerini test etmiş, uzaydaki tüm olası DTI'ları başarıyla tahmin etmiş ve en olası 10 adet DTI literatürde doğrulanmıştır [163].



Şekil 9. DTINet tarafından öngörülen ilaç-hedef etkileşimlerinin ağ görselleştirmesi (en iyi 150 tahmini içeren genel ilaç-hedef etkileşim ağının görselleştirilmesi) [164]; a. Hedef ve ilaçlar sırasıyla mor daireler ve sarı kutular halinde gösterilmektedir. b. Literatürde bilinen deneysel veya klinik kanıtlarla desteklenebilecek yeni DTI tahminlerinin çeşitli örneklerinin ağ görselleştirmesidir. İlaçlar sarı kutularda gösterilmekte, etkileşime giren hedeflerinin farklı aileleri farklı renklerde daireler halinde gösterilmektedir. İkisinde de, bilinen ilaç-hedef etkileşimleri gri, yeni tahmin edilen etkileşimler ise kırmızı çizgilerle gösterilmektedir

Heterojen veri kaynaklarının (ilaçlar ve hastalıklar arasındaki ilişki gibi) entegre edilmesi de DTI tahmininin doğruluğunun geliştirilmesine katkıda bulunabilmektedir. Bu ilişkiye dayanarak, Luo ve ark., heterojen ağlardan yeni DTI'ları tahmin etmek için DTINet adlı bir hesaplama süreci oluşturmuştur. DTINet tarafından öngörülen ilaç-hedef etkileşimlerinin ağ görselleştirmesi Şekil 9'da verilmektedir. DTINet tarafından tahmin edilen üç ilaç ile siklo-oksijenaz (COX) proteini arasındaki yeni etkileşim, DTI'lerin nispeten güçlü bir tahmin performansı gösteren deneylerle de doğrulanmıştır [164].

CRISPR/Cas9, gen inaktivasyonu, aktivasyonu, mutasyonu ve kromozom yeniden düzenlemeleri gibi birçok genomik mühendislik işlemi için kullanılabilen çok yönlü bir araçtır [165]. Biyoteknoloji uzmanı Christof Fellmann, CRISPR-Cas'ın hedef molekülleri tanımlamaya yardımcı olmasının, ilaç tasarımında büyük bir etkiye sahip olabileceğini belirtmiştir. Bu teknoloji, hastalıklara neden olan veya engelleyen genleri ve proteinleri tanımlayarak, potansiyel ilaçlar için hedeflerin belirlenmesine yardımcı

olabilmektedir. Ayrıca, CRISPR-Cas, hastalıkları taklit eden hücresel ve hayvan modellerinin oluşturulmasını kolaylaştırarak, ilaçların güvenliği ve etkinliği hakkında daha doğru sonuçlar elde edilmesine olanak sağlamaktadır. Bu sayede, klinik çalışmalarda daha iyi öngörücüler oluşturulabilmekte ve ilaçların geliştirilmesi süreci hızlandırılabilmektedir [166].

Molekül Transformatörü-İlaç Hedef Etkileşimi (MT-DTI) adlı derin öğrenme tabanlı bir ilaç-hedef etkileşim modeli kullanılarak, SARS-CoV-2'nin viral proteinleri üzerinde etkili olabilecek ticari olarak kullanılan mevcut ilaçlar araştırılmıştır. Sonuçlar, HIV tedavisinde kullanılan antiretroviral ilaç olan Atazanavirin'in SARS-CoV-2 3C benzeri proteinaz karşı en etkili kimyasal bileşik olduğunu göstermiştir. Bununla birlikte Atazanavir'in, Remdesivir, Efavirenz, Ritonavir ve Dolutegravir gibi diğer ilaçlardan daha yüksek bir inhibitör potensine sahip olduğu bildirilmiştir [167]. Bu çalışma, farklı bir viral enfeksiyonun tedavisinde kullanılan antiretroviralin başka bir viral enfeksiyondaki etkinliğini ortaya koyması açısından önemlidir ve MT-DTI modelinin ilaç tasarımı sürecinde kullanımının faydalı olabileceğini göstermektedir.

De novo İlaç Tasarımında Yapay Zeka Uygulaması

De novo ilaç tasarımı, reseptörün 3 boyutlu yapısını kullanarak ilgilenilen hedef için beklenen aktiviteye sahip yeni kimyasal yapılar elde etmek için moleküler tasarım ve değerlendirme amacıyla bilgisayarı kullanan algoritmalara dayalı bir yöntemdir. Yeni kimyasal yapıların biyolojik aktivitesini, ilaç metabolizmasını ve farmakokinetik (DMPK) özelliklerini, sentez için uygun koşulları ve sentezin uygulanabilirliğini öngörmesi beklenmektedir [168].

İlk *de novo* ilaç tasarımında, bağlama ceplerini hedeflemek için sterik ve elektronik olarak uygun olan ligandları geliştirmek amacıyla yapı bazlı bir yöntem kullanılmıştır. Bu yöntemle tasarlanan bileşikler genellikle zayıf DMPK özelliklerine sahiptir ve sentezlenmesi zordur [169]. Buna karşılık, ligand bazlı yöntem, kimyasal yapıların büyük bir veri tabanını oluşturmak için kullanılmaktadır. Molekülün kimyasal alanını araştırmak için DMPK özelliklerini, sentez fizibilitesini, biyolojik aktiviteyi ve hedef yapısı benzerliğini dikkate alan bir skor fonksiyonu kullanılmaktadır. Böylece sentetik olarak uygulanabilir birçok molekül elde edilebilmektedir [170].

İkinci yöntemdeki amaç ise, araştırmacıların mesleki bilgilerine dayalı olarak hedef yapısı analogları tasarlamak olmuştur. Yeni moleküller, sentez veya reaksiyon kuralları kullanılarak güvenilir ve verimli bir şekilde tasarlanabilmesine rağmen, bu tasarımlar genellikle laboratuvar ortamındaki uygulanabilirlik ve reaksiyonların zorlu doğası nedeniyle sınırlıdır [171].

Üçüncü yöntemde de amaç, uygun öngörüde aktivite bölgesini, karşılık gelen moleküler yapıya eşlemeyi amaçlayan ters QSAR fikrini benimsemektir. Bu yöntem nispeten zordur, çünkü seçilen moleküler tanımlayıcıların yalnızca bir ileri QSAR tahmin modelinin oluşturulması için değil, aynı zamanda bir moleküler yapıya dönüşüm için de uygulanabilir olmasını gerektirmektedir [172].

Yeni ilaç tasarımının mevcut zorluklarının iyileştirilmesinde, güçlü genelleme ve öğrenme yetenekleriyle DL yaklaşımı, bazı beklenen özelliklere sahip yeni kimyasal yapıları otomatik olarak oluşturmak için kullanılmıştır. Olivecrona ve ark. de novo ilaç tasarımı için dizi bazlı bir optimizasyon yöntemi kullanmış ve öngörülen aktiviteye sahip bileşikler oluşturmak üzere ChEMBL veri tabanında önceden eğitilmiş RNN'de ince ayar yapmak için pekiştirmeli öğrenmeyi (RL) benimsemiştir. Model, dopamin tip 2 reseptörü için aktif bileşiklerin oluşumunu tahmin etmek için kullanıldığında, model tarafından tahmin edilen bileşiklerin %95'inden fazlasının aktif olduğu bildirilmiştir [173].

Kadurin ve ark. druGAN adı verilen, istenen moleküler özelliklere sahip yeni moleküllerin novo tasarım için gelişmiş bir üretken karşıt otokodlayıcı (AAE) modeli önermiştir. Bu yöntem, moleküler parmak izlerinin ayarlanabilirliği açısından varyasyonel otomatik kodlayıcı VAE'den açıkça üstündür ve derin üretken modeli kullanarak spesifik anti kanser özelliklerine sahip yeni moleküller geliştirme yeteneğini ve verimliliğini önemli ölçüde artıran büyük moleküler veri setlerini işleme yeteneğine sahiptir [174].

Gomez-Bombarelli ve ark. ise moleküllerin farklı tasarımını çok boyutlu sürekli bir tasarıma dönüştürmek için bir teknik bildirmiştir. Amaçlanan özelliklere sahip yeni bileşikleri otomatik olarak oluşturmak için çok katmanlı bir algılayıcı (MLP) ve varyasyonel otomatik kodlayıcı (VAE) entegre etmişlerdir. Bu DNN, bir kod çözücü, bir kodlayıcı ve bir tahmin ediciden oluşmaktadır. Kodlayıcı, farklı basitleştirilmiş moleküler giriş hattı giriş sistemi olan SMILES (Simplified Molecular Input Line

Entry System) dizisini gizli uzayda sürekli bir vektöre dönüştürmekte ve kod çözücü bu vektörleri tekrardan farklı SMILES dizisine dönüştürmektedir [175].

Zhavoronkov ve ark. geliştirdiği Generative Tensorial Reinforcement Learning (GENTRL) modeli de novo küçük molekül tasarımı için kullanılan bir derin öğrenme modelidir. Bu model, sentetik fizibilite, yenilik ve biyolojik aktiviteyi optimize ederek kullanılmaktadır. Bu modeli kullanarak araştırmacılar, fibroz ve diğer hastalıklarda rol oynayan Diskoidin Alan Receptor 1 (DDR1) adlı bir kinaz hedefi için güçlü inhibitörler keşfetmiştir. Keşfedilen bileşiklerin biyokimyasal özelliklerini doğrulamak için bileşikleri önce sentezlemiş ve daha sonra hücre bazlı testlerle ölçümlerini yapmışlardır. Bu çalışmada, farelerde farmakokinetiği kanıtlanmış bir öncü aday bileşiği elde etmişlerdir. İki aydan daha kısa sürede sonuçlanan bu araştırma, derin öğrenme yöntemlerinin hızlı ve etkili moleküler tasarım için potansiyelini göstermektedir [176].

Ardından Skalic ve ark. tarafından, yeni moleküllerin üretilmesinde kullanılabilecek, zayıf yapısal çeşitlilik sorununu çözmek için çekirdek bileşiklerinin 3 boyutlu şeklinden ve farmakodinamik özelliklerinden yararlanan bir yöntem önerilmiştir. Bu aynı zamanda öncü bileşik benzeri yeni bir tasarım yürütülmesi için uygulanan ilk yöntemdir. Bu yöntemde, çekirdek bileşiklerinin 3 boyutlu temsilini bozmak için önce bir VAE kullanılmış ve ardından CNN'ler ve RNN'lerden oluşan ağ sistemi tarafından SMILES dizi sembolü üretilmiştir. Son olarak SMILES analiz edilerek yeni moleküller elde edilmiştir. Bu yöntemle tasarlanan yeni iskeleler ve fonksiyonel gruplar, kimyasal alanda henüz keşfedilmemiş ancak öncü bileşik benzeri özelliklere sahip olabilecek alanları kapsayabilmektedir [177].

ADAMs (a Disintegrin and Metalloproteinase) hücre yapışması, hücresel göç, hücresel sinyalleme ve proteoliz gibi fizyolojik işlemlerde önemli transmembran proteinler ailesidir [178,179]. Bu protein ailesinden olan ADAM10, alzheimer, prion hastalığı, alerjik reaksiyonlar, glioblastoma ve pankreas kanseri gibi hastalıkların tedavisi için umut verici bir hedeftir [180-183]. ADAM10 inhibitörlerinin moleküler generatif modelini oluşturmak için, transfer öğrenmesi ile birlikte geçişli tekrarlayan birim (GRU) tabanlı bir derin sinir ağı kullanılmıştır. Bu çalışmada, GRU tabanlı generatif modelin, moleküllerin SMILES dizilerini doğru bir şekilde öğrenebildiği ve yeni potansiyel ADAM10 inhibitörleri üretebildiği gösterilmiştir. Geleneksel ligand bazlı yöntemlerle kıyaslandığında, GRU tabanlı model, kimyasal ligandların yalnızca SMILES bilgilerini gerektirmekte ve etkili geniş bir seri potansiyel yeni bileşikler oluşturabilmektedir [184].

Green ve ekibi ise 2020 yılında, kimyasal yapı oluşturma, deneysel tasarım, aktif öğrenme ve kemoinformatik araçlarını entegre eden otomatik moleküler tasarım için Biyolojik Tepki Analizi ve Tasarım Sistemi (BRADSHAW) adlı bir sistem geliştirmişlerdir. Bu sistem, tasarla-yap-test et-analiz et (DMTA) döngüsünde otomatik moleküler tasarım yapmayı mümkün kılmaktadır. Kolay kullanılan arayüzü sayesinde, büyük ölçekli otomatik tasarım erişimi kolaylaştırılmıştır ve yazılım geliştirme süreci en aza indirgenmiştir. Sistem geleneksel kemoinformatiklerin ve modern makine öğrenimi algoritmalarının kullanımını içermektedir. Geriye dönük bir durum çalışmalarında da, BRADSHAW'ın MMP12 inhibitörlerinin tasarımı için öncü bileşik optimizasyonunda başarıyla kullanılabildiği gösterilmiştir [185].

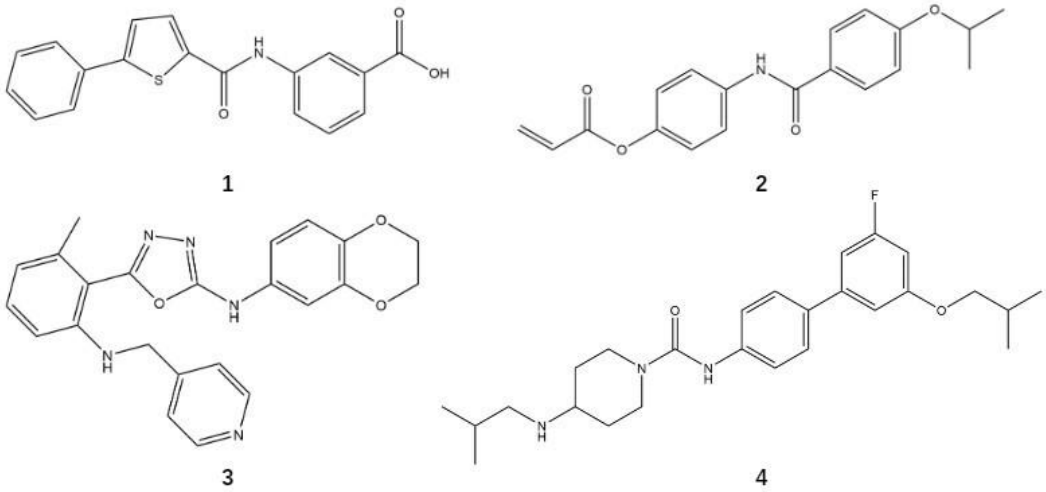
Yapay Zekâ Teknolojisiyle Geliştirilen İlaç Molekülleri ve Son Gelişmeler

Günümüzde her geçen gün yeni ilaç moleküllerine duyulan ihtiyaç artmaktadır. Yapay zekâ teknolojilerindeki gelişmeler de göz önüne alındığında yapay zekanın ilaç tasarımında kullanılmasıyla geliştirilen moleküller daha çok gündeme gelmektedir.

Al sistemleri *de novo* tasarım modelleriyle de uyum içinde aktif olarak kullanılabilmektedir [186-188]. Bu sistemler, denetimli ileri beslemeli ağların, denetimli ve yarı denetimli varyasyonel otomatik kodlayıcıların, tekrarlayan sinir ağlarının ve üretken çekişmeli ağların derin geri dönüşümünü (tersine çevirme) içermektedir [189-191]. Son zamanlarda, generatif AI'ya dayanan yenilikçi bir de novo moleküler tasarım kavramı önerilmiştir. Bu durum, bilinen biyoaktif bileşiklerden öğrenmenin bir yolu olarak umut vaat etmekte ve kalıtsal biyoaktivite ve sentezlenebilirliğe sahip yeni bileşikleri kendi kendine tasarlamaktadır [192].

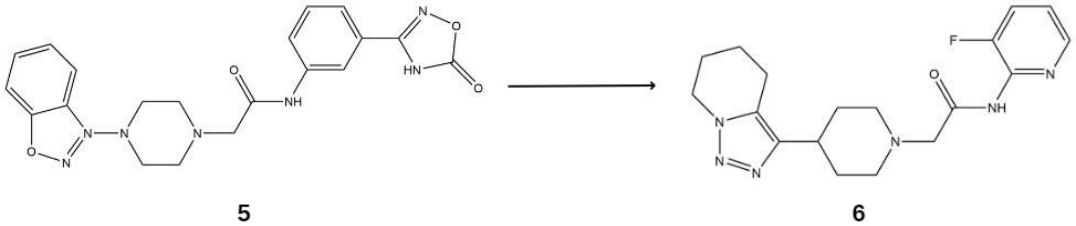
De novo kaynaklı bileşiklerin ilk başarılı dört sentezi (Şekil 10), generatif moleküler tasarımın ilaç tasarımına uygulanabilirliğini doğrulamaktadır. Derin sinir ağlarıyla üretilen RXRγ agonistleri

bileşik 1 ve 2 öncü prospektif tasarımlar olmuştur. Bu derin sinir ağları, ChEMBL'den SMILES biyoaktif bileşik dizileriyle önceden eğitilmiş ve transfer öğrenimi kullanılarak nükleer hormon reseptör hedefleri üzerinde ince ayar yapılmıştır. VEGFR2 kinaz inhibitörü olarak kabul gören bileşik 3, bilgisayar tarafından üretilen moleküllerin biyoaktivitesinin ligand-reseptör yerleştirme kullanılarak tahmin edildiği, ilgili bir SMILES tabanlı yaklaşımla oluşturulmuştur. Kısmi 5-HT2B antagonisti olarak bildirilen bileşik 4 ise sanal ileri sentez için bir nöral ağ ile de novo yapı tasarımıyla üretilmiştir [190,192-194].



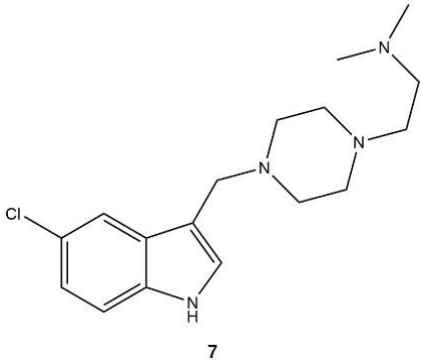
Şekil 10. *De novo* yöntemiyle elde edilen ilk başarılı bileşik örnekleri [190,192-194]

Bunun yanı sıra bir kimyasal serinin çok amaçlı optimizasyonu, potansiyel bir preklinik ilaç adayının arzu edilen tüm özelliklerini aynı anda karşılayan bileşikleri tanımlamayı amaçlamaktadır. Bu amaçla son zamanlarda, IKTOS ve Servier'deki bilim adamları, 11 adet hedef üzerinde çalışılarak (fenotipik aktivite, 5-HT2A, 5-HT2B, α1 ve D1 reseptörlerine karşı seçicilik, NaV 1.2 ve hERG iyon kanalları, sıçan ve insanda karaciğer mikrozomal stabilitesi ve Caco-2 Fab'lar ve Efflux) açıklanmayan başka bir hedef için ileri aşama bir öncü optimizasyon bağlamında bileşiklerin ligand tabanlı optimizasyonu için derin öğrenmenin kullanımını açıklamışlardır. 880 molekül içeren ilk veri setinde hiçbir molekül, bu 11 amaçtan herhangi birini karşılayamamıştır. Proje veri setinden QSAR modelleri geliştirilmiş ve bu modeller derin öğrenme uzun kısa süreli bellek (LSTM) modellerine dayanan tescilli bir SMILES tarafından oluşturulan yeni sanal yapıları puanlamak için kullanılmıştır. Ardından 11 hedef için oluşturulan yapılar üzerinde QSAR puanlaması kullanılmıştır. Son olarak, tüm hedefleri karşılayan 150 sanal yapı tahmini yapılmıştır. Çok büyük çoğunluğu ilk veri setinde bulunmayan bu sanal yapılardan 20 tane bileşik sentez için seçilmiş ve 11'i başarıyla sentezlenmiştir. Detaylı çalışmaların ardından 3 bileşik, belirtilmiş 11 hedef kriterinin hepsini karşılamıştır. 11 hedeften 9'unu karşılayan Şekil 11'de verilen bileşik 5, 11 hedefin tümünü karşılayan bileşik 6'nın tasarımına, sentezine ve tahliline yol açan QSAR eğitim setinin bir parçası olmuştur [195].



Şekil 11. Sentezi yapılan etkin bileşik 5 ve bileşik 6 molekülleri [195]

Daha önceki bir çalışmada, ETH Zurich ve Novartis'ten araştırmacılar, moleküler yapı için uyarlanabilir bir algoritma (MAntA olarak bilinen Moleküler Karınca Algoritması) kullanmışlardır [196]. Bu algoritma diğer 5-HT reseptörü alt tiplerine veya hERG potasyum kanalı da dahil olmak üzere 18 başka protein hedefinden oluşan bir panele bağlanmadan 5-HT2B reseptörünün seçici antagonistleri olan küçük, sentetik olarak erişilebilir de novo tasarımları otomatik olarak üretmek için kullanılmıştır [197]. Bu yöntem sonucunda istenen seçiciliği sergileyen ve MAntA tarafından önerilen sentetik yolla uyumlu olarak, bir indirgeyici aminasyon adımında ticari olarak mevcut başlangıç malzemelerinden sentezlenen bileşiğin tasarımı gerçekleştirilmiştir. Sentezlenen bu bileşik 7, Şekil 12'de verilmektedir.



7

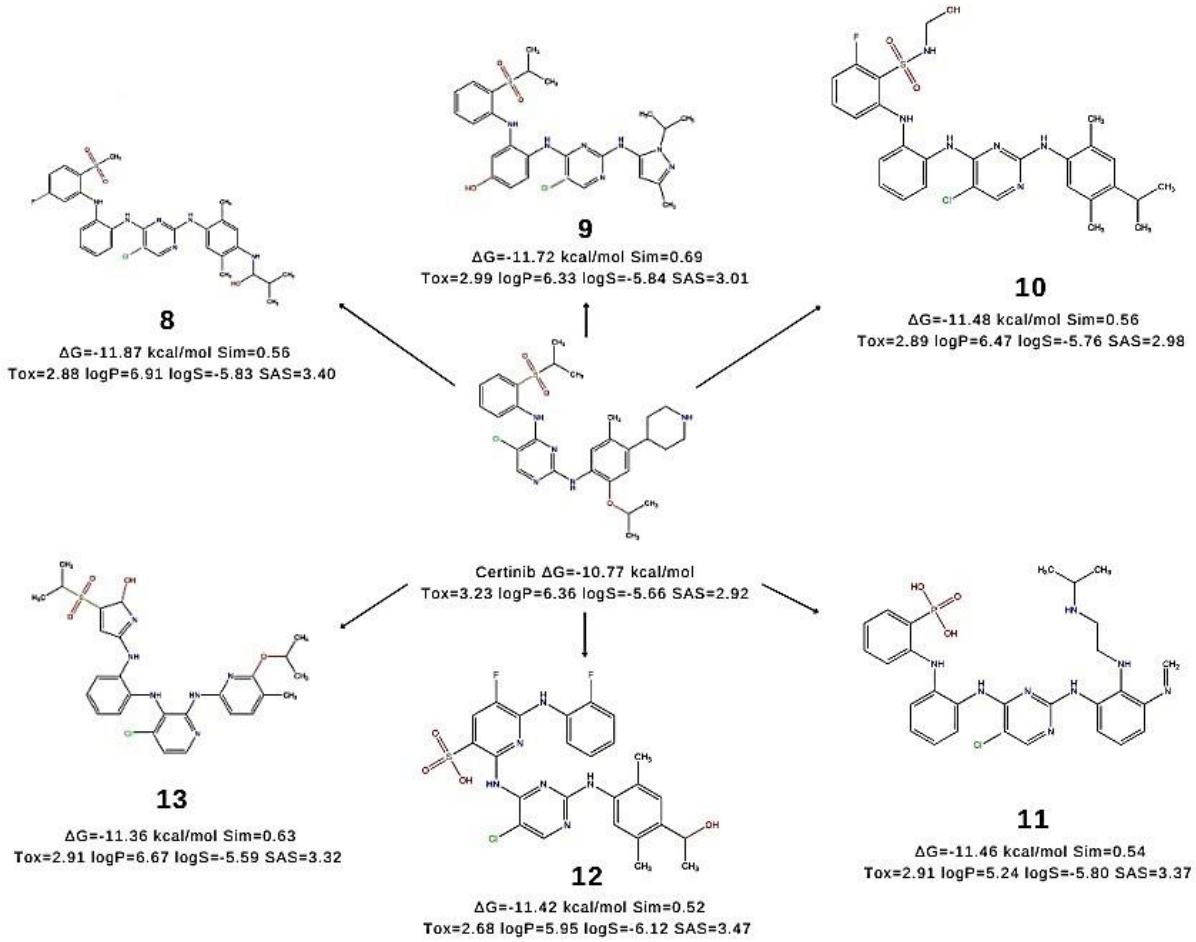
Şekil 12. MAntA ile tasarımı yapılan bileşik [197]

Yukarıda belirtilen molekül tasarımlarının yanı sıra, piyasadaki mevcut moleküllerin optimizasyonuyla da daha etkili, güvenli ve daha ucuz bir şekilde elde edilebilen molekül tasarımları yapılabilmektedir.

Bu amaçla, otomatik olarak yeni ilaç benzeri moleküller üretmek için çoklu özellik optimizasyonuna dayalı bir üretken ağ kompleksi (GNC) geliştirilmiştir. GNC temelde, SMILES dizilerini, DNN tabanlı molekül üreticileri, önceden eğitilmiş kodlayıcıları ve kod çözücülerini kullanmaktadır [198]. Geliştirilen GNC ile alternatif ilaç adaylarını tasarlamak amacıyla son yıllardaki kritik hastalıklar ve çeşitli kanserleri tedavi etmek için FDA onaylı, tek ve çok hedefli ilaçlar üzerinde çalışmalar yapılmıştır.

Tek hedefli ilaç tasarımında 'Cerinitib' üzerinde çalışılmıştır. Novartis tarafından geliştirilmiş ve akciğer kanseri türlerini tedavi etmek için Nisan 2014'te FDA tarafından onaylanmıştır. Ceritinib son derece pahalıdır ve ABD'de Ceritinib bazlı tedavinin aylık maliyeti günümüzde yaklaşık 11.428 dolardır. Ceritinib, ALK (anaplastik lenfoma kinaz) tirozin kinaz reseptörünü inhibe etmektedir. Hedef ALK'ya potensiyel inhibitörler tasarlamak için eğitilen GNC, ceritinib molekülünü referans molekül olarak kullanarak alternatif moleküller tasarlamıştır. Tasarlanan moleküllerin enerjileri (ΔG), ilaca benzerlik puanları (Sim), hesaplanan toksisite (Tox), log P, log S değerleri ve sentetik erişilebilirlik puanları (SAS'lar) referans molekülle kıyaslandığında eşdeğer ve hatta daha başarılı bağlanma afinitesi gösterebilen 6 başarılı moleküle (bileşik 8-13) Şekil 13'te yer verilmiştir [198].

Çift hedefli ilaç tasarlamada ise, 'Ribociclib' üzerinde çalışılmıştır. Ribociclib (ChEMBL ID: CHEMBL3545110) Novartis ve Astex Pharmaceuticals tarafından geliştirilmiş ve bazı meme kanseri türlerini tedavi etmek için 2017 yılında FDA tarafından onaylanmıştır. ABD'de Ribociclib tedavisinin aylık maliyeti yaklaşık 10.950 dolardır. Ribosiklib, CDK4 ve CDK6 olmak üzere iki farklı hedefi inhibe etmektedir. Alternatif ribociclib molekülü tasarımlarında CDK4 ve CDK6'ya bağlanma afiniteleri öncelikli olarak göz önünde bulundurulup en başarılı bulunan 6 molekül (bileşik 14-19) Şekil 14'te gösterilmiştir. Tasarlanan moleküllerin çoğu, referans ilaç Ribociclib'den daha iyi bağlanma afinitelerine sahiptir. Örneğin CDK4'e birinci ve dördüncü bileşiklerin afinitelerinin Ribociclib'inkinden daha yüksek olduğu ve diğer üçünün Ribociclib ile benzer afinitelere sahip olduğu saptanmış, CDK6'ya olan bağlanma afiniteleri ise tüm bileşiklerde daha iyi olduğu bildirilmiştir [198].



Şekil 13. Ceritinib molekülü ve alternatif olarak üretilen ilk altı molekül (hedef ALK için öngörülen ΔG'leri, ilaca benzerlik puanları (Sim), hesaplanan toksisite (Tox), log P, log S değerleri ve sentetik erişilebilirlik puanları (SAS'lar)) [198]

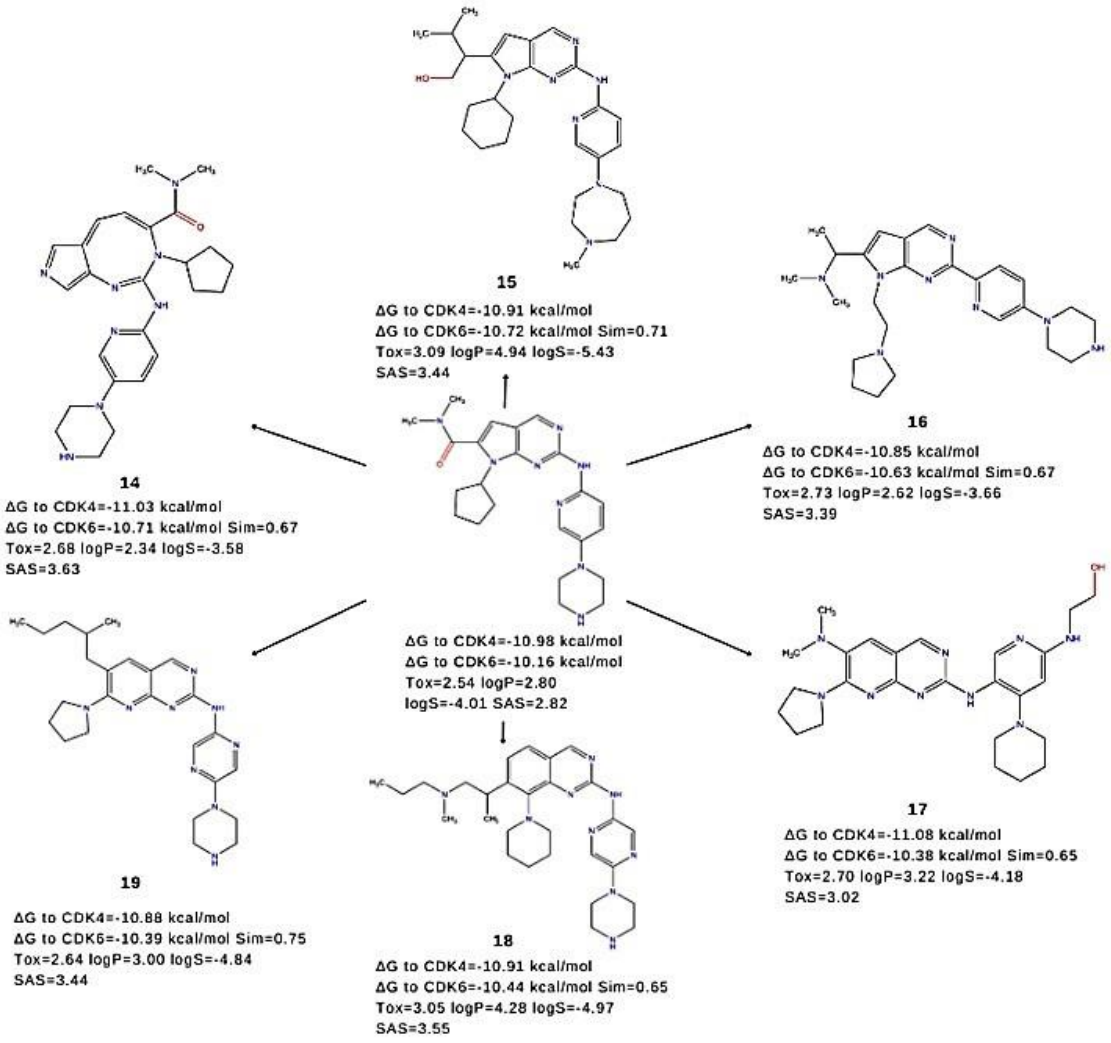
Geliştirilen bu yöntemle Cerinitib ve Ribociclib dışında Acalabrutinib, İdelasidib, Dabrafenib gibi piyasada bulunan birkaç ilaç molekülüne alternatif ilaç adayı oluşturmak için çalışmalar devam etmektedir.

Bu çalışmaların yanı sıra, entegre DMTA platformlarının uygulamaları, ilaç tasarımı ve optimizasyonuna uygulanabilmektedir [199]. Bu amaçla, AbbVie'deki araştırmacılar, küçük bileşik kitaplıkların otomatik sentezi için, esas olarak ticari olarak temin edilebilen bileşenlerden oluşturulan entegre bir robotik platform geliştirmişlerdir [200]. Ardından seçici hepsin inhibitörlerinin üzerinde çalışılmasıyla birlikte, otomatik DMTA döngülerinde bilinen hepsin inhibitör bileşiğinden Şekil 15'te verilen bileşik 20'den yeni bir bileşik olan bileşik 21 tasarlanmıştır. Tasarım sonucu, hepsin'e karşı yarı maksimum inhibitör konsantrasyon (IC_{50}) değeri 1μM'den 33 nM'ye yükseltilmiş ve ürokinaz tipi plazminojen aktivatörüne göre seçicilik 30 kattan 100 kata çıkarılmıştır. Tüm bu çalışmaların yaklaşık 1.5 saat gibi kısa bir sürede bittiği gözlemlenmiştir.

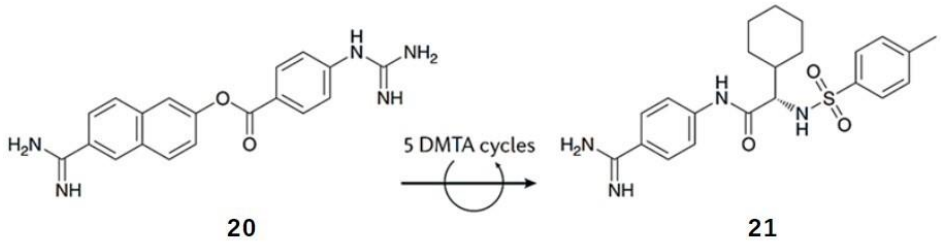
Birçok yeni ilaç molekülüne ihtiyaç duyulduğu gibi antibiyotiğe dirençli bakterilerin hızla ortaya çıkması nedeniyle, yeni bir antibiyotik tasarlama ihtiyacı da artmaktadır. Ne yazık ki, yeni antibiyotiklerin tasarımı giderek zorlaşmaktadır, çünkü doğal ürün keşfi, artık aynı moleküllerin tekrar tekrar keşfedildiği dereplikasyon sorunuyla boğuşmaktadır [201].

Makine öğrenimindeki son gelişmeler göz önüne alındığında, artık yeni yapısal antibiyotik sınıflarını tanımlamak amacıyla moleküler özellik tahmininde algoritmik çözümler başarıyla uygulanmaktadır. Erken dönem ilaç tasarımının büyük ölçüde in silicoda gerçekleştirilmesine izin veren

metodolojilerin benimsenmesi, mevcut deneysel yaklaşımların ulaşamayacağı geniş kimyasal alanların keşfedilmesini sağlamaktadır [202].



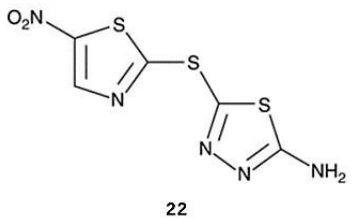
Şekil 14. Ribociclib molekülü ve alternatif olarak üretilen ilk altı molekül (CDK4 ve CDK6 hedeflerine yönelik öngörülen ΔG'leri, ilaca benzerlik puanları (Sim), hesaplanan toksisite (Tox), log P, log S değerleri ve sentetik erişilebilirlik puanları (SAS'lar)) [198]



Şekil 15. Hepsin inhibitörü (20) ve tasarlanan yeni bileşik (21) [200]

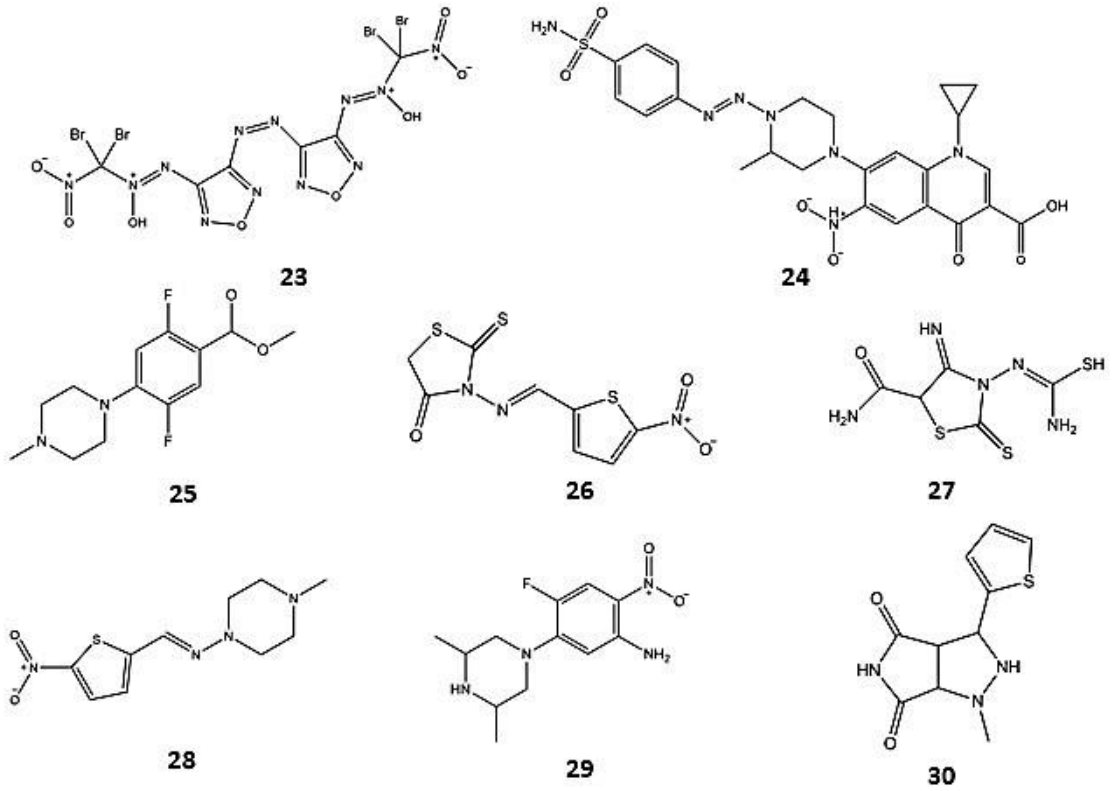
Sinir ağı modelleri, parmak izi vektörleri, otomatik tanımlayıcılar gibi yaklaşımların kullanılmasıyla yeni antibakteriyel aktiviteye sahip molekülleri tasarlayabilen derin bir sinir ağı eğitilmiştir. Birden fazla kimyasal veri setleri üzerinde tahminler yapılmış ve Drug Repurposing

Hub'dan, geleneksel antibiyotiklerden yapısal olarak farklı olan ve *Mycobacterium tuberculosis* ve karbapenem dirençli *Enterobacteriaceae* dahil olmak üzere geniş bir filogenetik patojen spektrumuna karşı bakterisidal aktivite gösteren bir molekül olan c-Jun N-terminal kinaz inhibitörü SU3327 (Halicin) tasarlanmıştır. Ayrıca Şekil 18'de gösterilen bileşik 22, Halicin molekülü, fare türlerinde *Clostridioides difficile* ve pan ilaç rezistanlı *Acinetobacter baumannii* enfeksiyonlarını etkili bir şekilde tedavi etmiştir [203,204].



Şekil 16. Halicin molekülü

Halicin'e ek olarak, ZINC15 veritabanında bulunan 107 milyon molekülden deneysel olarak test edilmiş 23 farklı molekül seti tasarlanmış ve oluşan bu setten, bilinen antibiyotiklerden yapısal olarak uzak Şekil 17'de verilen 8 ek antibakteriyel bileşik keşfedilmiştir. Bu çalışmada, bu moleküllerden bileşik 23 ve bileşik 24, dikkate değer bir şekilde güçlü geniş spektrumlu aktivite sergilemiş ve *E. coli'*deki bir dizi antibiyotik direnci belirleyicisinin üstesinden geldiği bildirilmiştir. Bu çalışma, makine öğreniminin, öncü bileşik tanımlamasının doğruluk oranını aynı anda artırarak ve tarama çalışmalarının maliyetini azaltarak antibiyotik tasarlama çabaları üzerinde sahip olabileceği önemli etkiyi vurgulamaktadır [204].



Şekil 17. Keşfedilen antibakteriyel etkili bileşikler [204]

Tüm bu gelişmelerle birlikte, yakın zaman önce Al ile ilaç tasarımında kilometre taşı sayılabilecek gelişmeler, 30 Ocak 2020'de Exscientia'nın, Faz 1 Klinik denemesine girmek için Al tarafından tasarlanan ilk molekülü DSP-1181'i duyurmasıyla devam etmiştir. DSP-1181 bileşiği ile ilgili çalışmalar, işbirlikçi Sumitomo Dainippon Pharma tarafından uzun etkili ve güçlü bir serotonin 5-HT1A reseptör agonisti olarak, obsesif-kompulsif bozukluğunu (OKB) tedavi etmek için faz I klinik çalışmalarına kadar ilerletilmiştir. Normalde on yıla kadar sürebilen bu süreç 12 ayda sonuçlanmıştır. Yapısal analizler sonucunda, iddia edilen molekülün, FDA'nın 1967'de onayladığı sık kullanılan birinci nesil bir antipsikotik ajan olan Haloperidol ile benzerliği ortaya konmuştur [205-207].

Ardından Aralık 2020'de, Exscientia'nın en gelişmiş, adenozin A2a reseptör antagonisti kabul edilen, öncü ilaç adayı EXS21546, Birleşik Krallık'ta çeşitli tümör türleri için immüno-onkoloji tedavisi amacıyla Faz 1 klinik çalışmasına başlamıştır. EXS21546 üzerindeki çalışmalar, yapay zeka firması Exscientia ve ilaç firması Evotec arasındaki işbirliğinin bir parçası olarak devam etmektedir [207].

DSP-0038 ise Exscientia ve Sumitomo Dainippon Pharma arasındaki iş birliğinin bir parçası olarak keşfedilen çift hedefli bir 5-HT1a reseptör agonisti ve 5-HT2a reseptör antagonisti olarak kabul edilmektedir. Mayıs 2021'e gelindiğinde, Amerika Birleşik Devletleri'nde, DSP-0038'in klinik çalışmasının başlatıldığı açıklanmıştır. Şu anda Alzheimer hastalığı psikozu tedavisi için araştırılmaktadır [207].

SONUÇ VE TARTIŞMA

Yapay zekâ ve makinelerde öğrenme kavramı, bir fikir olarak ortaya çıkmasından günümüze kadar sürekli gelişerek hayatımızda çığır açmaya devam etmektedir. Yaşanan gelişmeler matematik, istatistik, mühendislik ve tıp gibi alanlara yön vermeye devam ederken, ilaç tasarımında da yapay zekâ ve bilgisayar destekli teknolojilerden faydalanılmaktadır.

İlaç tasarımında yapay zekâ çalışmaları ilkel algoritmalarla başlamış olup süreç içinde makine öğreniminin gelişmesiyle yerini ML tekniklerine ve makine öğreniminin de daha gelişmiş alt dalı olan DL'e bırakmıştır. Geliştirilen yapay zekâ uygulamaları, ilaç tasarımının önemli aşamaları olan proteinlerin katlanmasının tahmininde, protein-protein etkileşimlerinin tahmininde, sanal taramada, kantitatif yapı-aktivite ilişkilerinin incelenmesinde, ADME/T farmakokinetik özelliklerinin tahmininde, ilaçların yeniden hedeflendirilmesinde ve de novo ilaç tasarımında aktif olarak kullanılmakta ve çok önemli rol almaktadır. Özellikle derin sinir ağları ve tekrarlayan sinir ağları gibi yapay sinir ağları, ilaç tasarımında yapay zekanın kullanımına büyük ölçüde yön vermiştir.

Yapay zekânın kullanımının yaygınlaşmasıyla birlikte ilk çalışmalar QSAR çalışmaları ile başlamış olup başarılı sonuçlar elde edilmiştir. İlerleyen çalışmalar doğrultusunda DL algoritmalarının ML algoritmalarından daha başarılı sonuçlar verdiği gözlemlenmiştir.

İlaç tasarımı için büyük önem taşıyan protein-protein etkileşimleri ve protein yapısının deneysel olarak belirlenmesi zor, zaman alıcı ve maliyetli bir süreç olsa da yaşanan bu gelişmelerle birlikte PPI ve protein yapısının tahmini üzerinde DL'nin önemi açıkça görülmektedir.

Yine sanal tarama çalışmalarında uygulanan ML ve DL uygulamaları ilaç-hedef etkileşimlerini öngörmede ve terapötik hedefleri belirlemede başarılı bir şekilde kullanılmış ve aktif olarak kullanılmaya devam edilmektedir. ADME/T üzerinde AI uygulamalarının ardından öncü bileşiğin erken değerlendirilmesi ve optimizasyonu konularında zaman ve üretim verimliği açısından ciddi bir başarı gözlenmiştir. Bu ilerlemelerinin yanı sıra, ilaçları yeniden hedeflendirmek amacıyla yapılan çalışmalarda biyoteknoloji alanındaki ilerleme büyük ölçüde hızlanmış ve yakın zaman önce yaşanan SARS-CoV-2 pandemisi için Atazanavir gibi alternatif ilaçlar tespit edilmiştir.

İlaç endüstrisinde uygulanan yapay zekâ ve ML algoritma teknolojilerindeki gelişmelere rağmen, bu teknolojilerin özel olarak ilaç tasarım sürecine ve genel olarak ilaç endüstrisine uygulanması ve entegrasyonu ile ilgili birçok zorluk bulunmaktadır. Bu sorunlardan biri verimsiz veri entegrasyonudur. Bu sorun, ham veri kümelerini içerebilecek veriler, işlenmiş veriler, meta veriler veya aday veriler gibi veri kümeleri arasında var olan çeşitlilikten kaynaklanmaktadır. Verimli analiz için bu veri kümeleri toplanmalı ve harmanlanmalıdır, ancak şu anda bunu yapmanın pratik bir yöntemi yoktur. Bu, ilaç tasarım süreci başlamadan önce yapılmalıdır, çünkü uygun şekilde biçimlendirilmiş veriler olmadan

makine öğrenimi algoritmalarının çıktıları hatalı olmaktadır. Bu nedenle, ilaç tasarım süreci başlamadan önce mevcut verileri veri bankalarına entegre etmek için daha verimli yöntemler gerekmektedir.

Diğer bir sorun ise mesleki beceri ve tecrübe yetersizliğidir. Şu anda ilaç endüstrisinde çalışan birçok kişi, yapay zekâ sistemlerini çalıştırmak için gerekli becerilere veya niteliklere sahip değildir. Aynı şekilde yapay zekâ veri bilimindeki birçok kişi de moleküler kimya ve biyolojide yetkin değildir, uygun algoritmalar oluşturmak için kimya ve biyoloji bilgisiyle birlikte yapay zekâ alanında da uzmanlık gereklidir. Ancak çok azı her ikisinde de uzmandır ve yapay zekayı farmasötik bağlamda uygulamak için doğru beceri kombinasyonuna sahiptir.

Bunlarla birlikte 'kara kutu' fenomeni olarak bilinen, model mekanizmasının belirsiz olmasıyla şeffaflık ve yorumlanabilirlik çoğu modelde yetersiz kalmaktadır ve sonuçları açıklamak için sınırlı yöntemlere sahiptir. Ayrıca, ilaç şirketleri tarafından üretilen büyük hacimli biyomedikal veriler genellikle halktan gizlenmekte ve pahalı özel ticari veriler olarak görüldüğünden bazı büyük veri setleri kolayca elde edilememektedir. Bu sebeplerden dolayı üretilen sonuçlara güven eksikliği oluşmaktadır ve ilaç endüstrisinde makine öğrenimi ve yapay zekâya şüpheyle yaklaşılmaktadır. Yapay zekâya olan bu güvensizlik ilaç endüstrisinde yapay zekânın gelişimi için finansman eksikliğine sebep olmaktadır. Bu durum, potansiyeline kıyasla daha yavaş, daha az verimli araştırma ve geliştirmeye yol açarak ilaç endüstrisinde yapay zekâ ile ilgili ilerlemelerde azalmaya yol açabilmektedir. Bunlar, gerçek gelişimin önünde duran farklı engellerdir ve yapay zekânın ilaç tasarımına entegre edilmesi için aşılması gereken zorluklardır.

Tüm bu zorluklara rağmen, birçok molekül tasarımı ve sentez aşamalarını ön görmesi bakımından ilaç tasarımında AI'nın kullanımının ciddi bir yer kapladığı görülmektedir. Son zamanlarda, OKB tedavisi için DSP-1181'in ve hiçbir veri setinde bulunmayan tamamen özgün bir antibiyotik molekülü olan Halicin'in tasarımı buna en güzel örneklerdir. DSP-1181 ile ilgili çalışmalar faz aşamalarına kadar başarılı bir şekilde ilerlemiş ancak faz 1 aşamalarında yetersiz bulunmuştur. Yine de bu çalışma, normalde onlarca yılı bulabilecekken sadece 12 ayda sonuçlanması büyük bir başarı olarak kabul edilmektedir. Geleneksel antibiyotiklerden yapısal olarak farklı olan Halicin ise *Mycobacterium tuberculosis* ve karbapenem dirençli *Enterobacteriaceae* dahil olmak üzere geniş bir patojen spektrumuna karşı bakterisidal aktivite gösteren bir molekül olarak tasarlanmıştır. Halicin *Clostridioides difficile* ve pan ilaç rezistanlı *Acinetobacter baumannii* enfeksiyonlarını etkili bir şekilde tedavi etmesinin görülmesinden sonra henüz deneysel aşamada olsa da kullanılabilecek en güçlü antibiyotik molekülü özelliği taşımaktadır.

Bu örneklerde görüldüğü gibi ilaç tasarımında yapay zekâ teknolojilerinin kullanımı hem zamandan hem maliyetten önemli ölçüde tasarruf sağlayarak bu süreci ciddi bir şekilde destekleyip hızlandırmıştır. Bununla birlikte ilaç şirketleri ile yapay zekâ şirketlerinin iş birliği değer kazanmış olup ortak yürüttükleri çalışmaların gün geçtikçe arttığı açıkça görülmektedir. Bu durum ilaç tasarımına yeni bir bakış açısı ve canlılık kazandırmıştır. Yakın gelecekte tıbbi verilerin daha fazla birikmesi ve yapay zekâ algoritmalarının geliştirilmesiyle birlikte, yapay zekâ teknolojisinin yeni ilaç tasarımı ve geliştirmenin tüm alanlarını kapsaması ve ilaç tasarımında en çok kullanılan yöntem haline gelmesi beklenmektedir.

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

KLİNİKTE ÖNEMLİ OLAN KATEKOLAMİN VE TÜREVLERİNİN YAPILARININ İNCELENMESİ

INVESTIGATION OF THE STRUCTURES OF CLINICALLY IMPORTANT CATECHOLAMINES AND THEIR DERIVATIVES

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ÖZ

Amaç: *Katekolaminler olarak adlandırılan monoamin yapısındaki 3,4-dihidroksifeniletilamin türevi dopamin, epinefrin ve norepinefrin, çok önemli biyolojik rolleri olan endojen bileşiklerdir. Katekol yapısı taşıyan bu biyomoleküllerin, kendine özgü reseptörleri uyararak, organizmadaki pek çok sistemi kontrol ettiği bilinmektedir. Özellikle bu endojen bileşiklerin, adrenerjik ve dopaminerjik sistem üzerinden uyarıcı etkilerinin olduğu görülmektedir. Pek çok biyolojik süreçte hormon veya nörotransmitter olarak yer alan bu bileşikler, terapötik önemleri nedeniyle sentetik olarak da elde edilerek klinikte kullanılmaktadır. Ayrıca, endojen katekolaminlerin farmakolojik ve farmasötik özelliklerini iyileştirmek amacıyla, kimyasal modifikasyonlar ile yeni pek çok türevi geliştirilmiştir. Klinikteki kullanımlarının geniş ve önemli olması, bu bileşikleri araştırmacılar için değerli kılmaktadır. Katekolamin ve türevi bileşiklerin aktivitelerinin incelenmesi kadar kimyasal yapılarının anlaşılması ve sentez yöntemlerinin araştırılması da yeni türevlerin geliştirilmesi açısından çok önemlidir.*

Sonuç ve Tartışma: *Bu nedenle bu çalışmada klinik önemleri olan katekolamin türevlerinin yapıları ve özellikleri araştırılmıştır. Çalışma sonucunda katekolaminlerin kimyasal özellikleri, biyosentezleri ve sentetik olarak elde edilme yöntemleri ile biyolojik aktiviteleri ve klinikteki kullanımları ortaya konulmuştur.*

Anahtar Kelimeler: *Adrenalin, dopamin, katekolaminler, noradrenalin*

ABSTRACT

Objective: *Dopamine, epinephrine, and norepinephrine which are derivatives of 3,4-dihydroxyphenylethylamine are endogenous compounds with very important biological roles called catecholamines, in monoamine structure. It's known that these biomolecules carrying the catechol structure control many systems in the organism by stimulating their specific receptors. In particular, it is observed the stimulating effects of these endogenous compounds on the adrenergic and dopaminergic systems. These compounds, which are involved in many biological processes as hormones or neurotransmitters, are also obtained synthetically due to their therapeutic importance and use in the clinical. In addition, many new derivatives have been developed with chemical modifications in order to improve the pharmacological and pharmaceutical properties of endogenous catecholamines. The wide and important clinical use of these compounds makes them valuable for researchers. It's very important with regard to the development of new derivatives, to research the activities of catecholamines and derivative compounds used in the clinical, to understand their chemical structures, and to investigate the methods of obtaining them.*

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Result and Discussion: *Therefore in this study, the structures and properties of catecholamine derivatives with clinical significance were investigated. As a result of this study, the chemical properties, biosynthesis, and synthetically obtainment methods of catecholamines, their biological activities, and use clinically were revealed.*
Keywords: *Adrenaline, catecholamines, dopamine, noradrenaline*

GİRİŞ

Dopamin, epinefrin (adrenalin) ve norepinefrin (noradrenalin), katekolaminler olarak adlandırılan önemli biyolojik rollere sahip monoamin yapısında endojen bileşiklerdir [1]. Katekolaminler, kimyasal olarak 3,4-dihidroksifeniletilamin türevleridir [2]. İnsan vücudunda pek çok biyolojik yolakta mediyatör olarak yer alan önemli biyomoleküller olan bu yapılar, aynı zamanda Parkinson, şizofreni gibi birçok hastalığın patolojisinde de önemli rol oynamaktadır [3,4]. Bazı dokularda daha yoğun bulunan katekolaminler, özellikle beyin, adrenal medulla ve sempatik sinir hücrelerinde sentezlenmektedir [5].

Endojen katekolaminlerden dopamin, düşük dozlarda dopaminerjik reseptörleri uyarmakta ve kalpteki beta-1 (β1) reseptörler üzerinden kalbin kasılma gücünü arttırmakta, yüksek konsantrasyonlarda ise, alfa (α) reseptörler üzerinden vazokonstriksiyona neden olmaktadır [6]. Epinefrin, yine α reseptörleri üzerinden koroner vazodilatasyon yaparken, genel bir vazokonstriksiyona sebep olmaktadır. Ayrıca, β1 reseptörlerini uyararak kalbin kasılma gücünü ve atış hızını artırmakta, akciğerlerde β2 reseptörler üzerinden, güçlü bronkodilatör etki ile solunumu etkilemektedir [6]. Norepinefrin ise, daha çok β1 reseptörler üzerinden kalpte pozitif inotropik etki oluşturmakta ve sistemik vazokonstriksiyona neden olmaktadır [7].

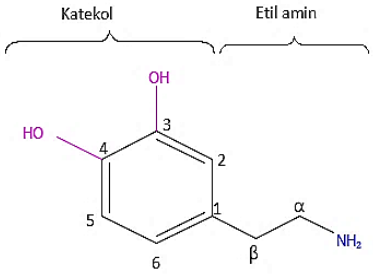
Katekolaminler, özellikle ilaç araştırma-geliştirme çalışmalarında yaygın olarak kullanılan ve ilaç endüstrisi için önemli olan feniletanolamin veya feniletilamin yapılarında biyolojik bileşiklerdir. Feniletilamin türevi olan endojen katekolaminlerden dopamin, kalp ve kan damarları üzerine etkili olduğu için, kalp krizi, kan zehirlenmesi ve şok tedavisinde kullanılmaktadır [8]. Feniletanolamin yapısındaki epinefrin, özellikle akciğerler üzerine olan etkilerinden dolayı, astım ve anafilaktik reaksiyonlarda kullanılmaktadır [9]. Bir diğer feniletanolamin yapısı taşıyan norepinefrin ise, vazokonstriktif etkisi nedeniyle, akut hipotansiyon durumlarında kan basıncını yükseltmek amacıyla kullanılmaktadır [10].

Endojen katekolaminler, yapılarında taşıdıkları katekol halka sisteminden dolayı dayanıksız olduklarından oral olarak kullanımları mümkün olamamaktadır. Bu nedenle, yerlerine kullanılabilecek daha iyi farmasötik özelliklere sahip olan ve oral yolla kullanılabilen analogları ile birlikte, etkilerini inhibe etmek amacıyla da antagonist türevleri geliştirilmiştir. Örneğin, sentetik katekolaminlerden izoproterenol (izoprenalin), feniletanolamin türevi bir bileşik olup, β1 ile β2 reseptörleri üzerinde agonist etkilidir. Bradikardi tedavisinde, kalp bloğunda ve nadiren astımda kullanılmaktadır [11]. Diğer feniletanolamin türevi olan fenilefrin ise, α-1 adrenerjik reseptör agonisti olduğundan dolayı, hipertansif, midriyatik veya dekonjestan olarak kullanılmaktadır [12-14]. Salbutamol başka bir feniletanolamin türevidir ve β2 seçici adrenerjik agonist etkili olmasından kaynaklı astım ve kronik obstrüktif akciğer hastalığında (KOAH) kullanılmaktadır [15]. Yine aynı türevlerden terbutalin, aynı seçicilikte olmasa da salbutamol ile benzer etkiye sahip olduğundan bronşiyal astım tedavisinde etkilidir [16]. Aynı grupta bulunan efedrin, α ve β reseptörleri üzerine etkili olup, genellikle şiddetli olmayan kronik astımın kontrol edilmesi için ve hipotansiyonda kullanılırken, izomerlerinden biri olan psödoefedrin ise, daha düşük toksisitesi nedeniyle çocuklarda soğuk algınlığının tedavisinde ve nazal dekonjestan olarak sıklıkla tercih edilen sempatomimetik bir bileşiktir [17-19].

Bu bilgiler, endojen katekolaminlerin ve analoglarının biyolojik aktiviteleri nedeniyle klinikteki önemlerini göstermektedir. Farmasötik özellikleri iyileştirilmiş daha dayanıklı, etki süresi uzun ve daha etkin yeni katekolaminlerin geliştirilmesi ve klinik kullanıma girebilmesi için, etkin olan bileşiklerin incelenerek yapılarının ve sentez yöntemlerinin araştırılması büyük önem arz etmektedir. Bu nedenle bu çalışma kapsamında, katekolaminlerin kimyasal özelliklerinin, elde edilme yöntemlerinin, biyolojik aktivitelerinin ve klinikteki kullanımlarının ortaya konulması hedeflenmiştir.

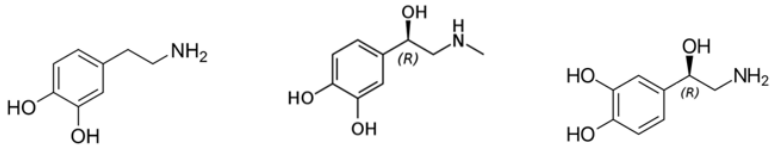
Katekolaminlerinin Kimyasal Özellikleri

Katekolaminler olarak adlandırılan ve biyojenik amin grubunda olan monoamin yapısındaki bileşikler dopamin, epinefrin ve norepinefrin, kimyasal olarak 3,4-dihidroksifeniletilamin türevleridir [1,2]. Endojen katekolaminler yapı iskeletinde, üçüncü ve dördüncü konumunda hidroksil grubu bulunan benzen halkası üzerinde etilamin yan zinciri taşımaktadır. Bu temel kimyasal yapı Şekil 1'de gösterilmektedir [20].



Şekil 1. Katekolaminlerin temel kimyasal yapısı

Bu endojen aminlerin katekolaminler olarak adlandırılmasının sebebi de iki komşu hidroksil grubu taşıyan benzen halkasına "katekol" adı verilmesinden kaynaklanmaktadır [2]. Dopamin ve norepinefrin primer, epinefrin ise sekonder amin taşımaktadır (Şekil 2) [1].



Şekil 2. Sırasıyla dopamin, epinefrin ve norepinefrinin kimyasal yapısı

Dopamin, sistematik olarak '4-(2-aminoetil)benzen-1,2-diol' şeklinde adlandırılan, $C_8H_{11}NO_2$ kapalı formülüne ve 153.18 molekül ağırlığına sahip olan bir bileşiktir. Hava ile teması çok hızlı bozunmasına neden olmaktadır. Dopamin, oksijenle doğrudan reaksiyona girebildiğinden oksidasyona karşı oldukça hassastır ve özellikle bazik ortamda kendiliğinden oksitlenmektedir. Ayrıca, oldukça hidrofilik yapıda olduğundan (LogP değeri;-0.98) yağda çözünürlüğü çok düşüktür ve kan beyin bariyerini geçememektedir [21].

Epinefrinin kimyasal adlandırması '(*R*)-4-(1-hidroksi-2-(metilamino)etil)benzen-1,2-diol' şeklinde olup, yapısında bulunan kiral merkezden dolayı iki enantiyomeri bulunmaktadır. Organizmada sentezlenen doğal epinefrin, L-formundadır. Kapalı formülü $C_9H_{13}NO_3$ ve molekül ağırlığı 183,20 olan epinefrinin, erime noktası 211.5°C'dir. Yine benzer şekilde düşük yağda çözünürlüğü (LogP değeri;-1.2) etkisini ve beyine geçişini etkilemektedir [22].

'4-[(1*R*)-2-amino-1-hidroksietil]benzen-1,2-diol' olarak isimlendirilen norepinefrin ise, $C_8H_{11}NO_3$ kapalı formülüne ve 169.18 olan molekül ağırlığına sahiptir. Aynı şekilde oksijenden ve ışıklı ortamdan etkilenmektedir. Asidik pH'larda daha kararlı olup en kararlı olduğu pH 4'tür. Epinefrine çok yakın partisyon katsayısına sahiptir (LogP değeri;-1.24) ve benzer çözünürlük özellikleri bulunmaktadır [23].

Endojen Katekolaminlerin Biyosentezi

Hormon ve nörotransmitter olarak görev yapan katekolaminlerin biyosentezleri beyin, adrenal medulla ve sempatik sinir uçlarında gerçekleşmektedir [3-5].

Yüksek fizyolojik aktiviteye sahip olan katekolaminlerin biyosentezi fenilalanin ve tirozinden hareketle yapılmaktadır. Bu bileşiklerden dopamin ve norepinefrin, başlıca sinir sisteminde nöronlarda

ve epinefrin ise, adrenal medullada kromaffin hücrelerde (az miktarda norepinefrin ve dopamin de üretilir) sentezlenmektedir.

Biyosentezleri için önemli bir enzim olan ve cAMP'ye bağlı fosforilasyon ile aktive edilen tirozin hidroksilaz, dopamin ve norepinefrin tarafından inhibe edilerek katekolaminlerin seviyesi negatif feed-back mekanizması ile kontrol edilmektedir.

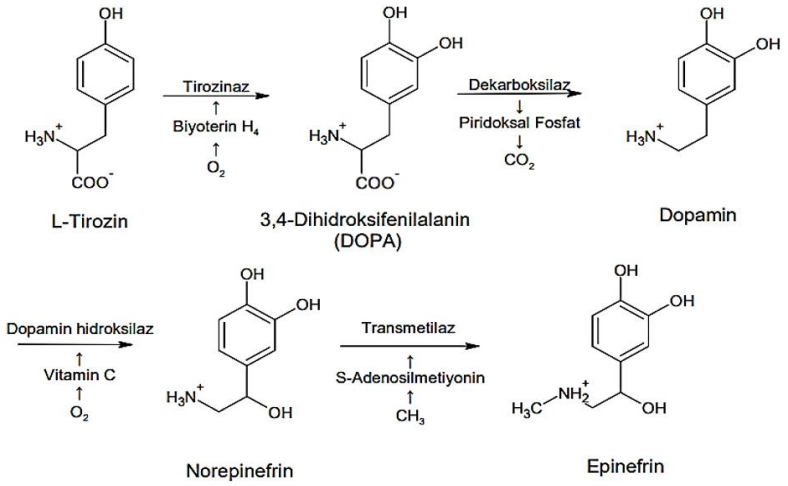
Tirozinden hareketle yapılan katekolaminlerin biyosentezi Şekil 3'te verilmiş olup, reaksiyon aşamaları şu şekildedir [24];

1. Dopa olarak bilinen 3,4-dihidroksifenilalanin biyosentezinde; tirozin hidroksilaz enzimi, L-tirozinin benzen halkasının 3 numaralı konumuna ikinci bir hidroksil grubu eklenmesini sağlar.

2. Dopamin biyosentezi basamağında, dopa dekarboksilaz, bu biyojenik aminin oluşumunu katalize etmek için koenzim olarak piridoksal fosfatı kullanır. Enzim, aromatik amino asitler ve türevleri üzerinde etkili olan geniş bir substrat spesifikliğine sahiptir. Merkezi sinir sisteminin bazı nöronlarında metabolik yol, dopamin üretimiyle sonlanırken, birçok nöronda, norepinefrin üretmek üzere yolak devam etmektedir.

3. Norepinefrin biyosentezinde, dopamin, norepinefrine dönüşmek üzere dopamin-β-hidroksilaz tarafından hidroksillenmektedir. Reaksiyon için moleküler oksijen, askorbik asit ve bakır gerekmektedir. Vücutta sentezlenen norepinefrinin çoğu sempatik sinir sisteminde nöronlarda üretilmektedir.

4. Epinefrin biyosentezi; norepinefrin, transmetilasyon yoluyla epinefrine dönüştürülmektedir. Reaksiyon, kortizol hormonu tarafından adrenal korteksten sentezi indüklenen feniletanolamin-*N*-metiltransferaz (PNMT) ile katalizlenirken, metil donörü olarak da SAM (*S*-adenozil metiyonin) kullanılır. Bu enzim en çok adrenal medullada bulunmaktadır. Bu nedenle epinefrinin temel sentez yeri adrenal medulladır ve ayrıca bu bezde sentezlenen ana katekolamindir.



Şekil 3. Katekolaminlerin biyosentezi

Katekolamin Türevlerinin Elde Edilme Yöntemleri

Katekolaminler, önemli biyoaktivitelerinden dolayı özellikle klinik kullanımları için çeşitli yöntemlerle sentetik olarak elde edilebilmektedir.

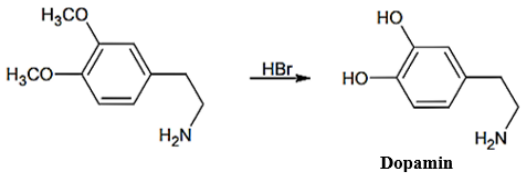
Dopamin, Şekil 4'te gösterildiği gibi 2-(3,4-dimetoksifenil)etan-1-amin'in hidrojen bromür ile reaksiyonuyla elde edilebilmektedir [25].

Ayrıca dopaminin, veratrolden hareketle klorometilasyon yoluyla ve sırasıyla siyanasyon, katalitik hidrojenasyon ve demetilasyon aşamaları sonucu elde edilebildiği bildirilmiştir [26].

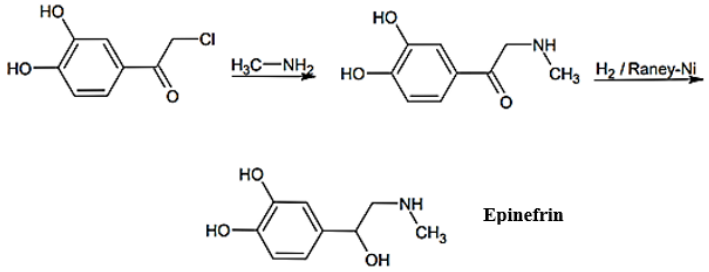
Epinefrin sentezi için kullanılan yöntemlerden birisi, Şekil 5'te gösterildiği gibi 2-kloro-1-(3,4-dihidroksifenil)etan-1-on ve metilaminin tepkimesi ile yürütülmektedir [25,27].

Başka bir yöntemde epinefrin, Şekil 6'da gösterildiği gibi katekol ile metilaminoasetaldehit

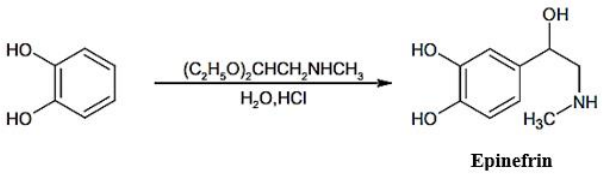
dietilasetali ile reaksiyonu sonucu elde edilebilmektedir [25].



Şekil 4. Dopamin sentezi

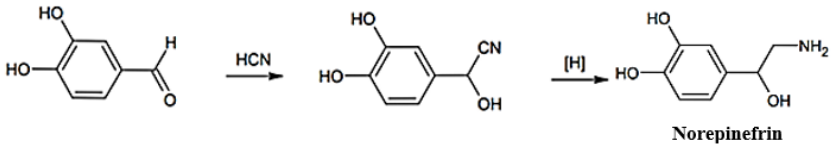


Şekil 5. Epinefrin sentezi



Şekil 6. Epinefrinin katekolden hareketle sentezi

Norepinefrinin eldesi için en çok kullanılan yöntemlerden birisi Şekil 7'de gösterildiği gibi 3,4-dihidroksibenzaldehit'in hidrojen siyanür ile reaksiyonudur [25].



Şekil 7. Norepinefrin sentezi

Norepinefrin ayrıca, Şekil 8'de gösterildiği gibi dimetil 4-formilbenzen-1,2-dikarboksilat'ın metilnitrit ile tepkimesinden hareketle de elde edilebilmektedir [25].

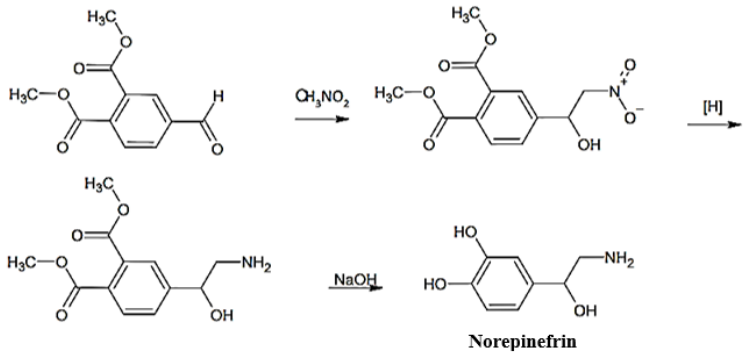
Bir başka yöntem ise, Sandow ve ark tarafından, kloroasetik asit ve katekolden hareketle 4-kloroasetil katekol üzerinden Norepinefrin elde edildiği bildirilmiştir [28].

Ayrıca katekolamin türevlerinin eldesi için de pek çok metot geliştirilmiştir. Feniletanolamin türevi olan efedrin ((-)-*eritro*-2-(metilamino)-1-fenilpropanol), karma etkili bir sempatomimetiktir. Şekil 9'da gösterildiği gibi benzaldehitin etilnitrit ile tepkimesinden başlanarak sentezlenebilmektedir [25].

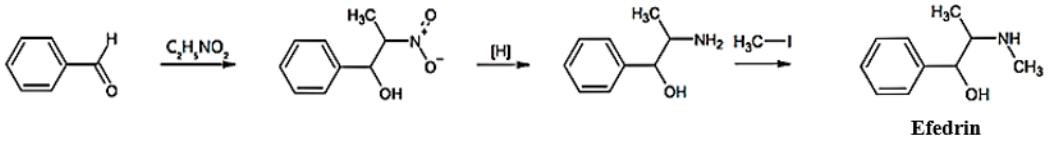
Yine benzaldehit üzerinden metilamin ile mikrobiyal fermentasyon yoluyla da efedrinin elde edildiği bildirilmiştir [29].

Efedrin gibi pek çok feniletanolamin yapısında sempatomimetik bileşik bulunmaktadır. Kimyasal yapısı 1-(3,4-dihidroksifenil)-2-izopropilaminoetanol olan izoproterenol, Şekil 10'da gösterildiği gibi

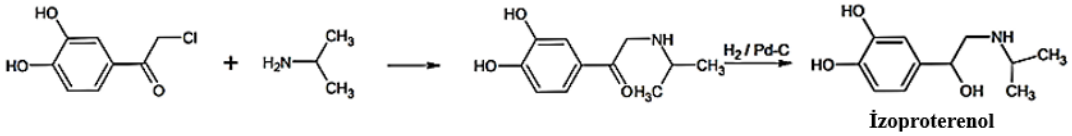
2-kloro-1-(3,4-dihidroksifenil)etan-1-on'un propan-2-amin ile tepkimesinden hareketle sentezlenmektedir [25].



Şekil 8. Norepinefrin nitrometan ile sentezi

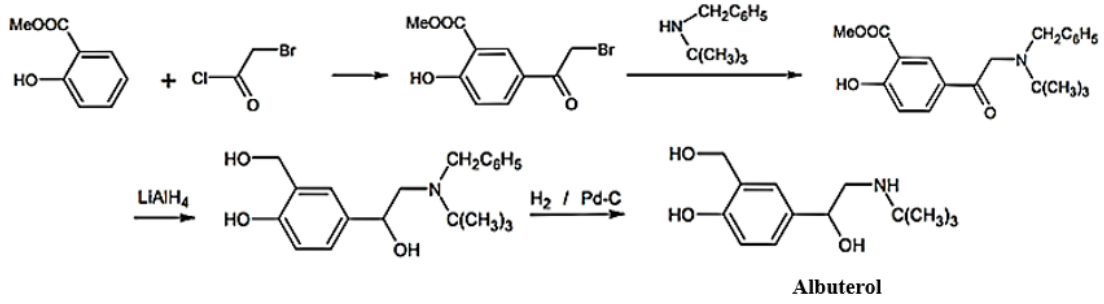


Şekil 9. Efedrin sentezi



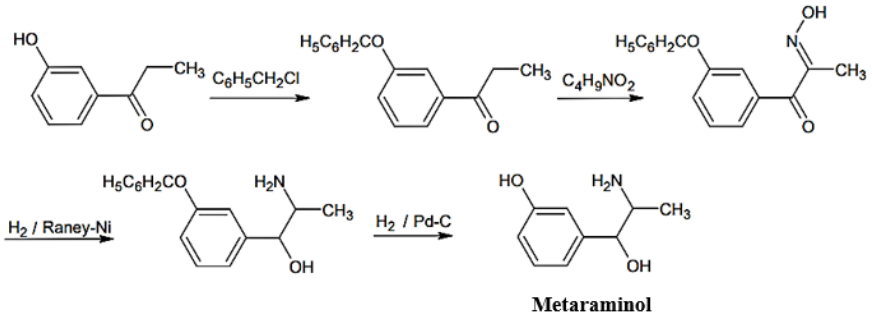
Şekil 10. İzoproterenol sentezi

Benzer yapıdaki diğer önemli bileşik albuterol (salbutamol) (2-(ter-butilamino)-1-(4-hidroksimetilfenil)etanol), astımda kullanılan β2 seçici adrenerjik etkili bir bileşiktir. Reaksiyon şeması Şekil 11'de gösterildiği gibi metil ester salisilik asidin bromoasetil klorür ile reaksiyonuyla başlanarak sentezlenebilmektedir [25]. Ayrıca aktif levo formunun izole edilmesiyle ilgili çalışmalar da mevcuttur [30].



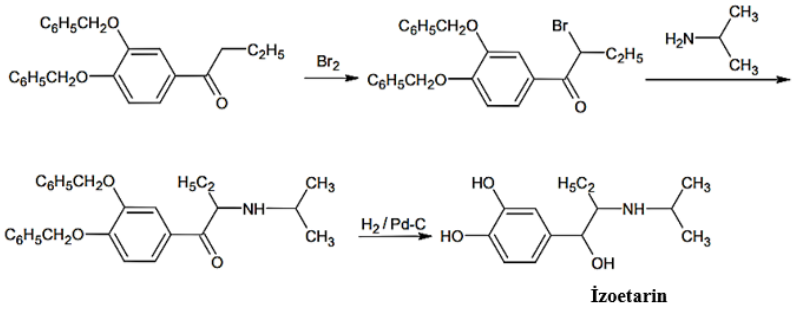
Şekil 11. Albuterol sentezi

Metaraminol (1-(3-hidroksifenil)-2-aminopropanol), feniletonalamin türevi olan α ve ß reseptörlerini uyarıcı etkili diğer bir bileşiktir. Şekil 12'de gösterildiği gibi 1-(3-hidroksifenil)propan-1-on ile benzil klorürün tepkimesinden hareketle elde edilmektedir [25].



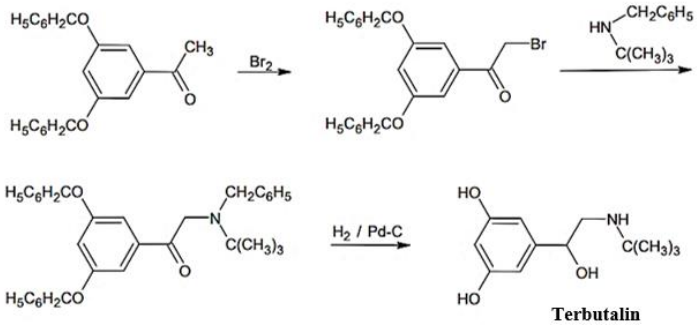
Şekil 12. Metaraminol sentezi

İzoetarin (1-(3,4-dihidroksifenil)-2-izopropilaminobutanol) de yine feniletanolamin türevlerinden olan β2 seçici adrenerjik agonisttir. Şekil 13'de gösterildiği gibi 1-[3,4-bis(benziloksi)fenil]bütan-1-on'un brom ile reaksiyonuyla başlanarak sentezlenmektedir [25].



Şekil 13. İzoetarin sentezi

Adrenerjik agonistlerden olan terbutalin (1-(3,5-dihidroksifenil)-2-(ter-butilamino)etanol) ise, Şekil 14'de gösterildiği gibi 1-[3,5-bis(benziloksi)fenil]etan-1-on ile bromun tepkimesinden hareketle elde edilmektedir [25]. Yine aktif R izomerinin saf olarak eldesi ile ilgili çalışmalar bulunmaktadır [31].

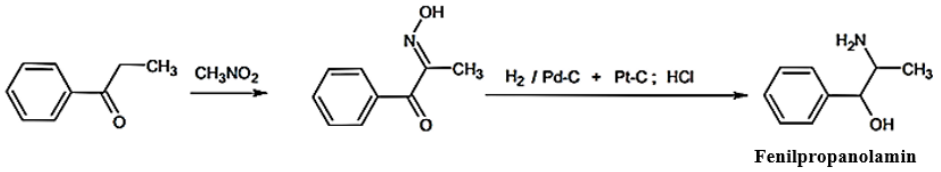


Şekil 14. Terbutalin sentezi

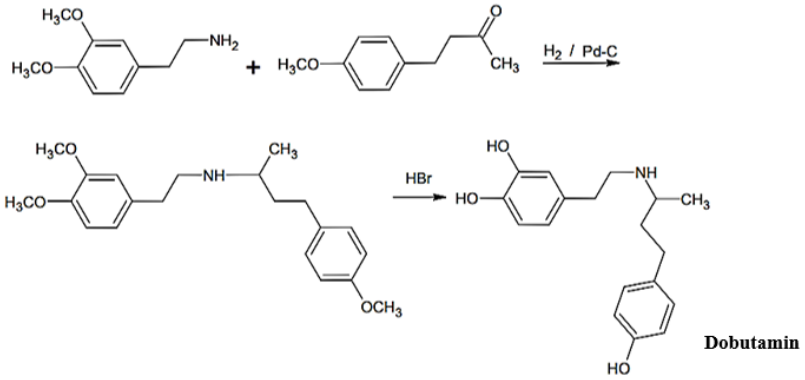
Feniletanolamin türevlerinden fenilpropanolamin (1-fenil-2-aminopropanol), α-1 reseptörleri üzerine etkilidir. Şekil 15'te gösterildiği gibi 1-fenilpropan-1-on ve metilnitrit tepkimesinden başlanarak sentezlenebilmektedir [25].

Katekolamin türevlerinden dobutamin (3,4-dihidroksi-*N*-[3-(4-hidroksifenil)-1-metilpropil]-ß-feniletilamin), özellikle kalpte β1 reseptörler üzerinde etkili olan feniletilamin yapısında bir bileşiktir.

Şekil 16'da gösterildiği gibi 2-(3,4-dimetoksifenil)etan-1-amin'in 4-(4-metoksifenil)bütan-2-on ile reaksiyonundan hareketle elde edilmektedir [25].

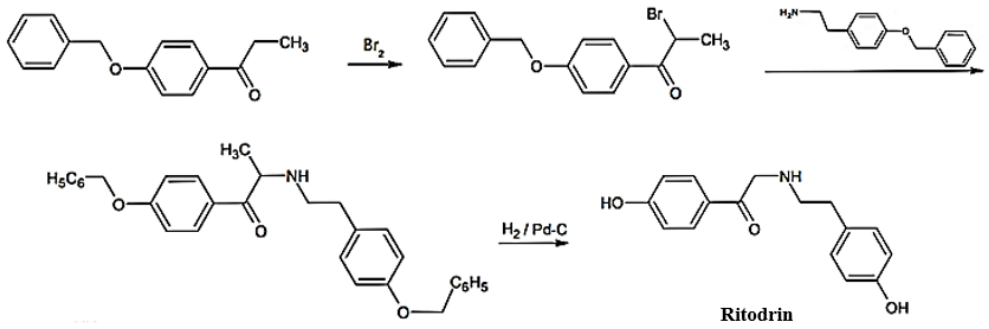


Şekil 15. Fenilpropanolamin sentezi



Şekil 16. Dobutamin sentezi

Yine feniletilamin yapısı taşıyan β2 adrenerjik agonist etkili olan ritodrin (1-(4-hidroksifenil)-2-[2-(4-hidroksifenil)etilamino]propanol), Şekil 17'de gösterildiği gibi 1-[4-(benziloksi)fenil]propan-1-on ile bromun tepkimesinden başlanarak elde edilebilmektedir [25].



Şekil 17. Ritodrin sentezi

Endojen Katekolaminlerin Biyoaktiviteleri

Endojen katekolaminler epinefrin, norepinefrin ve dopamin, önemli fizyolojik olaylara eşlik etmektedir. Vücudun kronik ve akut strese adaptasyonunda önemli rol oynarlar. Ruhsal ve fiziksel strese karşı özel hassasiyet gösteren sempatik medullar sisteme bağlıdırlar [32]. Örneğin, soğuğa maruz kalma sonucu oluşan periferik vazokonstriksiyon ile ısı kaybını azaltmak ve ısı üretimini artırmak için yağ asidi metabolizmasını uyaran, sempatik sinir sisteminin aktive olmasıyla katekolaminlerin ani salınımı görülmektedir [33]. Katekolaminlerin birçok metabolik işlemlerin kontrolünde de önemli görevlerinin olduğu bilinmektedir. Aslında katekolaminlerin, bağışıklık sistemi dahil olmak üzere; streste, duygusal

proseslerde, öğrenmede, uykuda, psikomotor aktivitelerde ve hafızada düzenleyici rolleri bulunmaktadır [20]. Katekolaminlerin uyarılması ile farklı reseptörler üzerinden etkileşimleri sonucu çeşitli fizyolojik belirtiler ortaya çıkmaktadır [33].

Dopaminin kardiyovasküler etkilerine, afinitesine göre değişen birkaç farklı reseptör tipi aracılık etmektedir. Düşük konsantrasyonlarda dopaminin birincil etkileşimi, özellikle renal, mezenterik ve koroner yataklarda vasküler D1 reseptörleri ile olmaktadır. Bu şekilde, adenil siklazı aktive ederek cAMP'nin hücre içi konsantrasyonlarını yükseltmekte ve vazodilatasyona yol açmaktadır. Glomerüler filtrasyon hızında, renal kan akımında ve Na^+ atılımında artışa neden olmaktadır. Renal tübüler hücrelerde D1 reseptörlerinin aktivasyonu, cAMP'ye bağımlı ve cAMP'den bağımsız mekanizmalarla sodyum taşınmasını azaltmaktadır. Proksimal tübüler hücrelerde ve henle kulpunun çıkan kolunun medüller kısmında artan cAMP üretimi, Na^+-H^+ ve Na^+, K^+-ATPaz pompasını inhibe etmektedir. Dopaminin, renal kan akışı ve glomerüler filtrasyon hızındaki artışa sebep olması ile natriürez gibi renal tübüler etkileri ortaya çıkmaktadır. Daha yüksek konsantrasyonlarda ise β1 adrenerjik reseptörler üzerine de etki ederek, miyokard üzerinde pozitif inotropik etki oluşturmaktadır. Dopaminin, ayrıca sinir uçlarından norepinefrin salınımına da neden olması, kalp üzerindeki etkilerine katkıda bulunmaktadır [8].

Dopamin tarafından hipofiz ara lobundaki D2 reseptörlerinin uyarılması ile cAMP sentezi, plazma membranında spontan rejeneratif elektriksel aktivite ve melanosit stimüle edici hormon benzeri etki ile peptit salınımı azalmaktadır [34].

Dopaminerjik D3 reseptörleri ise, presinaptik yerleşim gösteren otoreseptörlerdir ve dopamin sentez ve salınımını negatif feed-back mekanizması ile kontrol altında tutmaktadır. Dopaminin aşırı salınımı ile otoreseptörlerin uyarılması sonucu, sinir ucunda hiperpolarizasyon meydana gelerek salınımı inhibe edilmekte, ayrıca tirozin hidroksilaz enziminin etkinliğinin azaltılmasıyla dopamin sentezi yavaşlamaktadır [34].

Epinefrin, α ve β reseptörlerine etki edebilen, en güçlü α reseptör aktivatörüdür. Epinefrin, α-1 reseptörü üzerinden güçlü vazokonstriktör etkiye sahiptir ve sistolik kan basıncını arttırmaktadır. Ayrıca, böbreklerde damarları daraltarak, böbrek kan akımını ve idrar debisini azaltmaktadır. Benzer şekilde, deriye giden kan akımını da azaltmaktadır [35].

Kardiyovasküler sistem üzerinde güçlü β1 agonistik etki ile (+) kronotrop ve (+) inotrop etki meydana getirmektedir. Koroner arterlerde ve bronşiyal damarlarda vazodilatasyona, venlerde vazokontriksiyona neden olmaktadır [35].

Ayrıca epinefrin, β2 reseptörler üzerinden bronşiyal kasları gevşeterek solunumu etkileyen güçlü bir bronkodilatördür. En çok bronşiyal astımda olduğu gibi çeşitli hastalıklar, ilaçlar ve otakoidler nedeniyle bronşiyal kasların kasılmasıyla salınımı uyarılmaktadır. Bu durumda epinefrin, bronkokonstriksiyona neden olan maddelere karşı fizyolojik bir antagonist olarak davranmaktadır. Mast hücrelerinden antijen kaynaklı inflamatuar mediyatörlerin salınımını inhibe etmesi ve az da olsa bronşiyal sekresyonları azaltması da astımda ve anafilaksi sırasında oluşabilecek bronkospazmda faydalı olmaktadır. Mast hücre sekresyonunun inhibisyonuna β2 reseptörleri aracılık ederken, mukoza üzerindeki etkilerine α reseptörleri aracılık etmektedir. Bunların dışında epinefrinin, karaciğerde glikojenolizi uyararak kan şekerini artırdığı da bilinmektedir [9,36].

Norepinefrin, hem α-1 hem de α-2 adrenerjik reseptörlerine etki ederek sistemik vazokonstriksiyona neden olmaktadır. Ancak asıl etkisi kalpte β1 reseptörleri üzerinden, kalbin inotropik olarak uyarılması ve koroner arterleri dilate etmesidir. Genel olarak intestinal kaslar ve akciğerler üzerine etkisi zayıftır [36].

Norepinefrin ve epinefrinin farmakolojik etkileri, *in vivo* ve *in vitro* olarak kapsamlı karşılaştırıldığında, ikisi de efektör hücreler üzerinde doğrudan agonist olmakla birlikte α ve β reseptörlerine olan afinite farklılıkları, fizyolojik etkilerinin güçlerini değiştirmekte, bu durum klinik kullanımlarını etkilemektedir.

Katekolamin ve Türevlerinin Klinikteki Önemi

Katekolamin ve türevlerinin biyolojik aktiviteleri nedeniyle klinikte önemli kullanımları bulunmaktadır.

Epinefrin

Epinefrin, hem hipotansiyon hem de şiddetli akut anafilaktik reaksiyonlarda ilk tercih edilen ilaçtır [9]. Anafilaktik şok sırasında gereken dolaşım ve kan basıncı desteği için hızlı volüm resüsitasyonu ile birlikte yapılan vazopresör tedavisinde etkilidir. İlaçlara, serumlara, böcek sokmalarına, yiyeceklere veya diğer alerjenlere karşı gelişen reaksiyonlardan kaynaklı, ürtiker, kaşıntı, anjiyoödem ile dudakların, göz kapaklarının ve dilin şişmesi gibi semptomları da hafifletmektedir [37]. Katekol yapısı oral kullanıma izin vermemektedir. Parenteral veya az da olsa lokal kullanımı mevcuttur.

Norepinefrin

Yeterli sıvı hacmi replasmanından sonra devam eden şok tedavisinde hemodinamik dengesizlikleri düzeltmeye yardımcı olarak vazokonstriksiyon ve kardiyak stimülasyon için kullanılmaktadır. Kimyasal yapısından dolayı oral yoldan biyoyararlanımı çok düşüktür. En çok intravenöz yolla uygulanmaktadır [38].

Dopamin

Dopamin, genellikle sistolik kan basıncını arttırırken, diastolik kan basıncını etkilememektedir. Düşük dozlarda dopamin verildiğinde, mezenterik ve renal gibi bazı vasküler yataklarda bölgesel arteriyel direnci azaltması, diğerlerinde ise sadece küçük artışlara neden olmasından dolayı, toplam periferik direnç genellikle değişmemektedir. Yüksek konsantrasyonlarında ise, vasküler α-1 reseptörlerini aktive ederek daha genel vazokonstriksiyona yol açtığından dolayı şok tedavisinde kullanılmaktadır [8].

Dobutamin

Dobutamin kardiyak β1 reseptörlere seçiciliği yüksek bir agonisttir. Bu nedenle, şiddetli kardiyak dekompansasyon sırasında miyokardın kasılmalarını geçici olarak güçlendirmenin gerekli olduğu durumlarda ve özellikle kalbe cerrahi müdahale ile ilişkili kardiyak aktivitenin dekompansasyonu sırasında kullanılmaktadır [25].

İzoproterenol (İzoprenalin)

İzoprenalin, kalpteki β1 reseptörleri uyararak kalp verimini arttırırken, solunum yolunda β2 reseptörleri üzerinden bronkodilatasyona yol açmaktadır. Elektrik şoku veya kalp pili gerektirmeyen hafif, geçici veya ciddi kalp bloğu ataklarını, ventriküler taşikardi veya fibrilasyonun neden olmadığı Adams-Stokes ataklarını ve anestezi sırasındaki bronkospazmı tedavi etmek için kullanılmaktadır. İzoprenalin ayrıca klinikte, hipovolemik şok, septik şok, hipoperfüzyon, konjestif kalp yetmezliği ve kardiyojenik şokta, sıvı ve elektrolit replasman tedavisine destekleyici olarak verilmektedir [11].

Fenilefrin

Katekol yapısı taşımayan, feniletanolamin türevi olan fenilefrin, α-1 adrenerjik reseptör agonistidir. Parenteral olarak intravenöz yolla, şok tedavisinde veya anestezinin neden olduğu hipotansiyonu önlemek veya ortadan kaldırmak için kullanılmaktadır. Göz bebeklerini genişletmek ve vazokonstriksiyonu indüklemek için bir oftalmik formülasyonu da bulunmaktadır. Burun tıkanıklığını tedavi etmek için intranazal formülasyonu, hemoroidleri tedavi etmek için ise topikal formülasyonu geliştirilmiştir [12-14].

Salbutamol (Albuterol)

Katekol yapısının 3 numaralı konumunda bulunan hidroksilin, hidroksimetil grubuyla değiştirilmesiyle elde edilmiş olan bir feniletanolamindir. Yapıdaki bu değişiklik ile önemli derecede bronkodilatör etkili ve düşük kardiyak stimülasyon yapan, seçici bir β2 adrenerjik agonist geliştirilmiştir. Bronşiyal astım, kronik bronşit, geri dönüşümlü obstrüktif hava yolu hastalığı ve diğer kronik bronkopulmoner bozukluklara bağlı bronkospazmın profilaksisi ve semptomatik tedavisinde inhalasyon yoluyla kullanılmaktadır [15].

Terbutalin

Terbutalin de yine seçici etki elde etmek üzere geliştirilmiş, yapısında katekol yerine rezorsinol halka sistemi taşıyan diğer β2 seçici adrenerjik agonisttir. Seçiciliği salbutamol kadar olmasa da, bu etkisinden kaynaklı bronşiyal astımda kullanılmaktadır. Katekol yapısının olmaması kateşol *O*-metil transferaz (COMT) ile metabolizasyonu önleyerek etki süresini uzatmaktadır. Bronşit ve amfizem ile ilişkili astım ve bronkospazmı olan hastalarda bronkospazmın önlenmesi ve tedavisi için kullanılmaktadır. Oral ve inhalasyon yoluyla uygulanabilmektedir [16].

Efedrin

Feniletanolamin yapısı taşıyan efedrin, kan basıncını artıran, lokal vazokonstriktör etkisi bulunan bir bileşiktir. Bu nedenle, anestezi altında hipotansiyonu önlemek için intravenöz yolla ve bronşiyal astım gibi alerjik durumları tedavi etmek için farklı yollarla kullanılmaktadır. Lokal olarak nazal mukoza üzerine dekonjestan amacıyla da kullanılmaktadır [17,18].

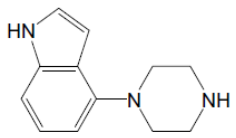
Psödoefedrin

Psödoefedrin, efedrinin treo formu olan etkin izomeridir. Benzer etkilere ve kullanım alanlarına sahip olmakla birlikte düşük toksisitesi nedeniyle, akut nezle, akut östaki salpenjit, östaki borusu tıkanıklığı olan seröz otitis media, vazomotor rinit ve aerotit (barotit) media ile ilişkili tıkanıklığın geçici olarak giderilmesi için daha çok tercih edilmektedir. Psödoefedrin ayrıca alerjik rinit, krup, akut ve subakut sinüzit, akut otitis media ve akut trakeobronşit tedavisinde en iyi sonucu elde etmek için analjezik, antihistaminik, antibiyotik, antitüssif veya ekspektoranlarla birlikte kullanılmaktadır [19].

Yeni Gelişmeler

Katekolaminlerin klinik kullanımları göz önüne alındığında, terapötik olarak etkin ilaçların geliştirilmesinin önemi de artmaktadır. Bu amaçla, katekolaminlerin spesifik reseptörleri ile doğrudan etkileşerek, onların etkilerini taklit eden veya inhibe eden etkin bileşikler üzerinde çalışmalar devam etmektedir.

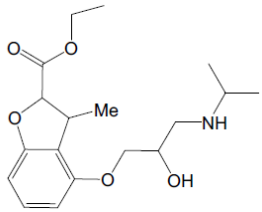
Hedef reseptörlerin yapılarının daha iyi açıklanmasıyla daha etkin bileşiklerin gelişimi üzerine yapılan çalışmalar da hız kazanmıştır. İlk adrenerjik reseptör olarak β2 reseptörünün 2007 yılında protein veri bankasına kaydolmasından sonra, reseptörün aktif ve inaktif bölgeleri daha sonraki çalışmalarda keşfedilerek ortaya konulmuştur [39-45]. Benzer şekilde 2008 yılında, β1 reseptörünün kristalize yapısı bildirilmiştir [46]. Bu çalışmalarla her iki tip reseptörün agonist ve antagonist bağlanma bölgeleri ortaya konularak, reseptörler arası farklılıklar da aydınlatılmıştır. Bu şekilde daha seçici etkili bileşiklerin geliştirilmesi için önemli veriler elde edilmiştir. Yapılan çalışmalar sonucunda pek çok farklı yapıda bileşik tasarlanabilmiştir. Bu bileşiklerin yapıları incelendiğinde ise birçoğunun katekol yapısı taşımamasına rağmen etkin bir şekilde bağlanma bölgelerine bağlanabildiği gösterilmiştir. Örneğin, Christopher ve ark yaptıkları bir çalışmada, Şekil 18'de verilen indol yapısındaki bileşiğin β1 reseptörlerine yüksek affinite ile bağlandığı bildirilmiştir [47].



Şekil 18. β1 reseptör agonisti

Kolbe ve ark ise, 2009 yılında yaptıkları çalışma sonucunda Şekil 19'da verilen benzofuran yapısındaki bileşiğin en yüksek afiniteyle β2 reseptörüne bağlandığını açıklamışlardır [48].

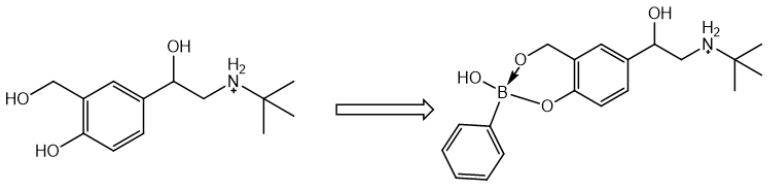
Kooistra ve ark tarafından 2016 yılında yapılan bir başka çalışmada ise, taranan 34 bileşikten 18'inin β2 reseptör üzerinde aktivitesi bulunmuştur [49]. Bu bileşiklerden aktif olan yeni yapılardan ikisi Şekil 20'de verilmiştir.



Şekil 19. β2 reseptör agonisti

Şekil 20. β-2 reseptör agonisti etkin bileşikler

Salbutamol bileşiği üzerinde de pek çok modifikasyon yapılarak farklı ilaç molekülleri geliştirilmeye çalışılmaktadır. Salbutamol üzerinden geliştirilen β2-adrenerjik agonist bileşikler üzerinde yapılan bir çalışmada, boronterol isimli (*R*)-4-(2-(ter-butilamino)-1-hidroksietil)-2-(hidroksimetil)fenilhidrojen fenilboronat yapısındaki bileşiğin (Şekil 21) düz kas gevşetici bir ilaç olarak salbutamolden en az sekiz kat daha güçlü olduğu bildirilmiştir [50].



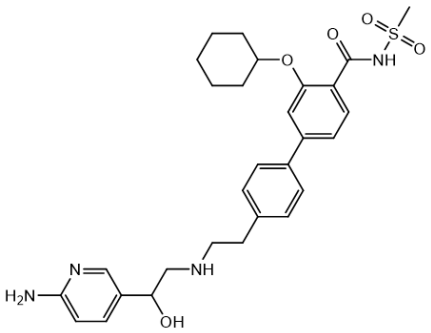
Şekil 21. Salbumatol ve boronterol bileşiği

Bu çalışmalardan bir diğeri ise, salbutamolün intraoküler basıncı düşürme amaçlı oküler kullanımı için ön ilaç türevleri olarak geliştirilen bileşiklerdir [51]. Bu türevlerde, penetrasyonu artırmak amaçlı salbutamolün 3 adet hidroksil grubu üzerinden asetil, isobutiril and pivalil triesterleri kullanılmıştır. Çalışma sonucunda, lipofilite ile ilişkili olarak aktif forma dönüşme hız sabitleri asetil > izobütiril > pivalil esteri şeklinde bulunmuştur. Üç ön ilaç türevinin de oküler etkisinin, salbutamolden daha yüksek olduğu bildirilmiştir. Özellikle 5 saat sonra tripivalil esterin diğer iki esterden daha aktif olduğu ve 8 saat sonra ise, triizobütiril esterle birlikte triasetattan önemli ölçüde daha aktif olduğu bulunmuştur. Bu bulgular ile ön ilaç lipofilikliğinin transkorneal penetrasyon üzerindeki etkisinin önemi doğrulanmıştır.

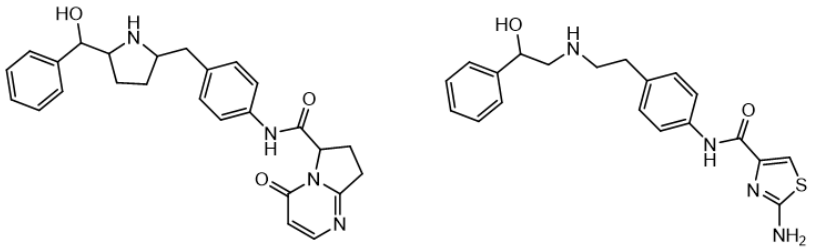
Adrenerjik sistem üzerinde klinik anlamı olan önemli bir diğer hedef ise β3-adrenerjik reseptörlerdir. Bu reseptörün kristal yapısı aydınlatılmaya başladıkça, bu reseptöre özgü etkili bileşikler de geliştirilmeye başlanmıştır. Özellikle obezite ve tip-2 diyabet hastalığının tedavisi için bu reseptörler üzerinde seçici etkili olan (kardiyovasküler yan etkileri olmayan) molleküller üzerine çalışmalar yapılmaktadır [52,53]. Reseptör aktivasyonunu anlamaya yönelik ve moleküler yerleştirme çalışmalarına yol gösterecek hipotezler ortaya konulmuştur [54,55]. Özellikle reseptör yapısındaki aktif bölgenin dar ve uzun olması dikkat çekmektedir [56]. Yapılan çalışmalarda bu reseptör üzerinden etkili olan bileşiklerin yapıları da bunu doğrulamaktadır. Hattori ve ark, bir çalışmalarında bifenilasilsülfonamid analoglarının β3 reseptörüne bağlanmalarını araştırmışlardır [57]. Elde ettikleri serideki en aktif bileşiğin ise Şekil 22'de verilen molekül olduğunu bildirmişlerdir.

Ayrıca β3-adrenerjik reseptörlerin, özellikle mesanede yoğun olarak bulunduğu görülmüştür. Bu nedenle aktif mesane sendromunun tedavisinde önemli bir ilaç hedefi haline gelmiştir. 2022 yılında yapılan bir başka çalışmada, aktif mesane sendromunda etkinliği bilinen β3 reseptörü agonistleri

vibegron ve mirabegron moleküllerinin (Şekil 23) seçicilikleri araştırılmıştır [58]. Her iki molekül de önemli ölçüde seçicilik göstermekle birlikte, mirabegronun β1 ve β2 reseptörleri üzerine düşük aktivite gösterdiği, vibegron molekülünün β2 reseptör aktivitesinin çok düşük olduğu ve β1 üzerine ise aktivite göstermediği bildirilmiştir.



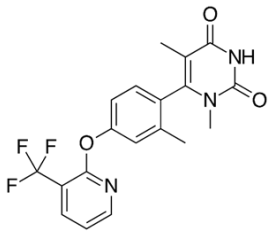
Şekil 22. β3 reseptör agonisti



Şekil 23. β3 reseptör agonistleri vibegron ve mirabegron molekülleri

Parkinson hastalığının tedavisinde kullanılmak üzere yeni dopaminerjik bileşik araştırma çalışmaları da devam etmektedir. Bu amaçla dopamin biyosentezi için ara ürün olan levodopayı tek başına içeren inhalasyon preparatları ile ilgili çalışmalar bulunmaktadır [59,60].

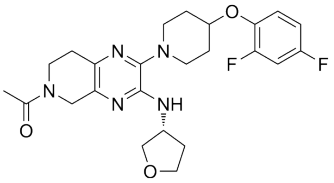
Yine dopaminerjik reseptörler üzerinden dopaminin etkilerini taklit eden antiparkinson etkili yeni bileşiklere örnek olarak Tavapadon bileşiği (Şekil 24) verilebilir [61]. Bu bileşik spesifik olarak D2/D3 reseptörleri yerine D1/D5 reseptörlerini hedefleyen kısmi bir dopamin agonistidir ve faz 3 klinik denemeleri devam etmektedir [62].



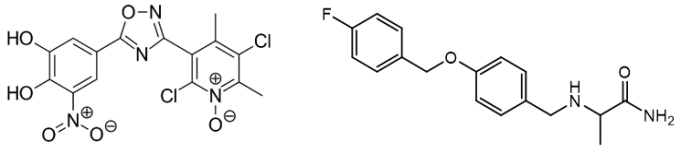
Şekil 24. Tavapadon bileşiği

Benzer etkili bir diğer bileşik ise faz 2 klinik deneme aşamasında olan CVN424 kodlu moleküldür (Şekil 25). Bu molekülün de dopaminerjik reseptörler üzerine invers agonist etkili olduğu bildirilmiştir [63].

Parkinson hastalığı için yapılan çalışmalarda elde edilen opikapon ve safinamid gibi katekolamin yapısındaki enzim inhibitörleri de tedaviye yardımcı olarak kullanılmak üzere geliştirilmiştir (Şekil 26). Bu bileşiklerden opicapone, COMT inhibitörü olarak 2020 yılında FDA onayı almıştır [64]. Safinamid ise yine FDA onaylı MAO-B (monoamin oksidaz) enzim inhibitörü bir ilaçtır [65].



Şekil 25. CVN424 bileşiği



Şekil 26. Opikapon ve safinamid bileşikleri

SONUÇ VE TARTIŞMA

Biyojenik amin grubunda olan dopamin, epinefrin ve norepinefrin bileşiklerinin, yapılarında monoamin grubu dışında taşıdıkları, birbirine komşu iki hidroksil grubu bulunan benzen halkasının (katekol) isminden kaynaklı olarak "endojen katekolaminler" olarak tanımlanmaktadır. Vücutta hormon ve nörotransmitter olarak görev alan bu bileşiklerin biyolojik aktiviteleri çok önemlidir. Başta otonom sinir sistemi ve kardiyovasküler sistem olmakla birlikte, hemen hemen tüm organizmayı etkileyen vital işlevleri bulunmaktadır. Bu nedenle belirli kan seviyelerinin sağlanması, düzenli biyosentezinin yapılarak depolanmalarını da gerektirmektedir. Bu katekolaminlerin biyosentezinin, özellikle sinir sistemi ve adrenal medullada, fenilalanin ve tirozin aminoasitlerinden hareketle gerçekleştiği bilinmektedir. Nöronlarda biyosentezlerinin, tirozin hidroksilaz aracılı dopa eldesiyle başlayarak, dekarboksilaz ile dopamin oluşumu ve hidroksilaz enzimi ile norepinefrin biyosentezi şeklinde sonlandığı görülmektedir. Adrenal medullada ise reaksiyon devam ederek transmetilaz enzimi aracılığıyla epinefrin üretilebilmektedir.

Organizmada her bir katekolamin için özel afiniteye sahip reseptörler bulunmakta, bu da aktivite farklılıklarını doğurmaktadır. Endojen katekolaminlerin spesifik olarak etkili olduğu bölgelerin ve aktivitelerinin farklılıklarından dolayı terapötik kullanım alanları da değişmektedir. Dopamin, daha çok kalp ve kan damarları üzerine etkili olduğu için, kalp krizi, kan zehirlenmesi ve şok tedavisinde kullanılırken epinefrin, α ve β reseptörler üzerinden gösterdiği etkilerinden dolayı, bronkodilatör olarak astım ve anafilaktik reaksiyonlarda kullanılmaktadır. Norepinefrin ise, vazokonstriktif etkisi nedeniyle, akut hipotansiyon durumlarında kan basıncını yükseltmek amacıyla tercih edilmektedir.

Bu bileşiklerin önemli klinik kullanımları, sentetik olarak elde edilmesini zorunlu kılmıştır. Ayrıca, endojen katekolaminlerin dayanıksız yapıları gereği oral yoldan kullanılamadığından ve etki sürelerinin çok kısa olmasından dolayı, farmasötik özellikleri iyileştirilmiş daha etkin türevlerin de geliştirilmesine ihtiyaç duyulmuştur. Uzun etki süreli ve daha etkin olan yeni sentetik türevler üzerinde yapılan araştırmalar, katekolamin ve analoglarının eldesi ile ilgili birçok sentetik yöntemin de gelişmesine sebep olmuştur. Bu şekilde daha kolay, daha yüksek verimle ve düşük maliyetle üretimler gerçekleştirilebilmiştir. Sentetik yöntemler uygulanarak sadece katekolamin analogları değil aynı zamanda antagonist türevleri de geliştirilmiştir. Bu çalışmalarda, daha seçici etkili türevler de geliştirilerek yan etkileri azaltılmış analoglar elde edilmiş ve klinikte pek çok alanda faydalı olan bileşikler tedaviye sunulmuştur.

Terbutalin ve albuterol örneklerinde görüldüğü gibi, kateşol yapısındaki değişikliklerin etkide doğurduğu farklılıklar çok açıktır. Kateşol yapısı taşıyan izoproterenol bileşiğinin bronşiyal astımda kullanımı kardiyolojik yan etkilere neden olurken, rezorsinol yapısı taşıyan terbutalin veya hidroksillerden birinin hidroksimetil grubu ile değiştirildiği albuterol bileşiklerinde β2 reseptörlere seçiciliğin artması ile (özellikle albuterol için), β1 aracılı kardiyolojik bu yan etkiler ortadan kaldırılabilmiştir. Aynı zamanda kateşol yapısının değiştirilmesi, bu bileşiklerin metabolizasyon

yolaklarından COMT enzimi aracılı metilasyonlarını önleyerek, etki sürelerinde artışa da sebep olmuştur. Benzer şekilde kateşol yapısı taşımasına rağmen, izoetarin bileşiğinde olduğu gibi yan zincirde amine komşu dallanma ile yine β2 seçici agonistik etki elde edilmiştir. Yan zincirde olan dallanmalar da yine bu bileşiklerin önemli metabolizma yolaklarından MAO enzimi ile oksidasyonlarını sterik olarak engellemektedir. Ayrıca, yapılan modifikasyon çalışmaları sonucu, dobutamin bileşiğinde olduğu gibi, feniletilamin türevlerinde amin üzerine hacimli yapıların sübstitüe edilmesinin β reseptörlere afiniteyi ve kardiyoselektiviteyi artırdığı da görülmektedir. Yine antagonist etkili türevlerin geliştirilmesi için de yapılan pek çok çalışmada, kateşol yapısı taşımayan ama benzer iskelet üzerinde modifikasyonların yapıldığı bilinmektedir.

Bu çalışma kapsamında elde edilen veriler, katekolamin ve türevlerinin klinik önemlerini ve bununla birlikte yapılarının aktivite üzerindeki rollerini açıkça göstermektedir. Sunulan temel bilgiler ışığında, katekolaminlerin kimyasal özelliklerinden, biyosentezleri ve sentetik olarak elde edilme yöntemlerine, biyolojik aktivitelerinden, klinikteki kullanımlarına kadar pek çok veriye ulaşılabilmektedir. Yeni etkin türevlerin geliştirilmesi üzerine yapılacak olan çalışmalar için, bu temel bilgilerin ve yapıların anlaşılması araştırmalar açısından önemli bir kaynak olmaktadır.

YAZAR KATKILARI

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DERLEME MAKALE / REVIEW ARTICLE

TABLET DOZAJ FORMLARININ TASARIMINDA VE GELİŞTİRİLMESİNDE ÜÇ-BOYUTLU BASKI TEKNOLOJİSİ

THREE-DIMENSIONAL PRINTING TECHNOLOGY IN TABLET DOSAGE FORM DESIGN AND DEVELOPMENT

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ÖZ

Amaç: *Eklemeli üretim olarak da bilinen üç boyutlu (3B) baskı, mühendislik, mimarlık ve uzay bilimleri gibi çeşitli endüstriyel alanlarda devrim yaratmıştır. Son yıllarda eczacılık alanında da umut vadeden bir teknoloji olarak 3B baskı, kişiselleştirilmiş katı dozaj formları, implantlar ve tıbbi cihazları yüksek hassasiyet ve doğrulukla üretmek için avantajlı bir yaklaşım olarak karşımıza çıkmaktadır. Bu teknoloji, geleneksel farmasötik üretim proseslerinin bazı sınırlamalarının üstesinden gelme potansiyeline sahiptir ve gelişmiş dozaj formu tasarımı, artan hasta uyuncu ve azaltılmış üretim maliyetleri gibi avantajlar sunmaktadır. Bu derleme kapsamında 3B baskılama ile tablet üretim yöntemleri incelenmiştir.*

Sonuç ve Tartışma: *Dozaj formlarının uygulanmasına yönelik farklı yollar arasında oral yol, hasta uyuncu açısından en çok tercih edilen veriliş yoludur. Oral yolla veriliş için tabletlerin üretimi, işlenmesi ve depolanması diğer dozaj formlarına göre daha kolay ve ekonomiktir. 3B baskı, farklı geometrik şekillerde ve farklı etkin madde salım profillerine sahip tabletlerin formülasyonu ve üretimine imkân sağlamaktadır. Bu baskılama teknolojisi temel olarak aynı baskı aşamalardan oluşsa da kendi içinde oldukça çeşitlenmiş ve Amerikan Test ve Malzemeler Derneği tarafından yedi farklı yönteme ayrılmıştır. Bu çeşitli yöntemler arasında tablet üretiminde oldukça başarı sağlayan eriyik birikim modelleme yöntemi pek çok çalışmaya konu olmuştur. Bununla birlikte tüm 3B baskılama yöntemleri ile tabletler ve kapletler üretilebilmektedir. Ancak kişiselleştirilmiş ilaçlar için kalite ve emniyeti gösterecek belirlenmiş bir çerçeve bulunmamaktadır ve de 3B teknolojinin sahte ilaç, yasal olmayan ilaçların yaygınlaşması ve yanlış etiketleme gibi riskleri de olabilecektir. 3B üretim için iyi imalat uygulamalarının (GMP) benimsenmesi, standart işlem prosedürlerinin oluşturulması, tüm üretim hattında ve sonrasında bitmiş üründe kalite kontrolün (örneğin tabletlerde içerik tektürlülüğü, kütle tektürlülüğü, çözünme testi) yürütülmesi gereklidir.*

Anahtar Kelimeler: *3B baskı, baskılama yöntemleri, fotopolimerizasyon, mürekkep püskürtme, toz yatağı füzyon*

ABSTRACT

Objective: *Three-dimensional (3D) printing, also known as additive manufacturing, has revolutionized various industrial fields such as engineering, architecture and space sciences. As a promising technology in the field of pharmacy in recent years, 3D printing appears as an advantageous approach to produce personalized solid dosage forms, implants and medical devices with high precision and accuracy. This technology has the potential to overcome some of the*

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limitations of traditional pharmaceutical manufacturing processes and offers benefits such as improved dosage form design, increased patient compliance, and reduced manufacturing costs. Within the scope of this review, tablet production methods with 3D printing were examined.
Result and Discussion: *Among the different routes for administering dosage forms, the oral route is the most preferred route of administration regarding patient compliance. The production, processing and storage of tablets for oral administration is easier and more economical than other dosage forms. 3D printing enables the formulation and production of tablets with different geometric shapes and different active ingredient release profiles. Although this printing technology basically consists of the same printing stages, it is quite diversified within itself and is divided into seven different methods by the American Society for Testing and Materials. Among these various methods, the melt deposition modeling method, which is very successful in tablet production, has been the subject of many studies. However, tablets and caplets can be produced with all 3D printing methods. However, there is no established framework to demonstrate quality and safety for personalized medicines, and 3D technology may have risks such as counterfeit medicine, proliferation of illegal drugs and mislabeling. For 3D production, it is necessary to adopt good manufacturing practices (GMP), establish standard operating procedures, and conduct quality control (e.g. content uniformity, mass uniformity, dissolution testing in tablets) throughout the entire production line and subsequently on the finished product.*
Keywords: *3D printing, inkjet, photopolimerization, powder bed fusion, printing methods*

GİRİŞ

Son on yıla bakıldığında piyasada bulunan ilaçlarla tedavi yerine bireyselleştirilmiş ilaçlarla hasta odaklı tedavi büyük bir ilgi görmeye başlamıştır. Bu tedavi şeklinde, standart ilaç tedavisine kıyasla ilk olarak hastalığa karşı uygun etkin madde/ler belirlenmekte ve hastaya göre bireyselleştirilmiş bir ilaç hazırlanmaktadır. Böylece hazırlanan ilaçtan maksimum terapötik etki ve minimum yan etki sağlanarak hastanın tedavi süreci hızlandırılmış olmaktadır.

Bireysel ilaçların üretiminde son yıllarda gelişen ve çeşitlenen üç boyutlu (3B) baskılama teknolojisi sıklıkla kullanılmaktadır. Elde edilen birçok başarılı sonuç, ilaç üretim teknolojilerine "eklemeli üretim" ya da "üç boyutlu baskılama" kavramlarını kazandırmıştır. Bu yöntem, temel olarak bilgisayar ortamında hazırlanan sanal bir tasarımın baskı kafası ve püskürtme ucu kullanılarak katman katman yazdırılmasına dayanmaktadır. Bu teknoloji, hastaya uygun olarak seçilen dozun küçük serilerinin üretiminin hızla gerçekleştirilmesi veya hastanın anatomik ihtiyaçlarını karşılayan özel protezlerin üretimine kadar birçok alanda konvansiyonel ilaç üretiminin önüne geçerek hastaya tedavi sürecinde avantaj sağlamaktadır.

Üç Boyutlu Baskılama Teknolojisi ve Eczacılık

3B baskılama teknolojisi, bilgisayar ortamında hazırlanan 3B tasarımların uygun malzemeler ile art arda yazdırılıp biriktirilmesi esasına dayanmaktadır. Uluslararası Standartlar Teşkilatı (ISO) bu teknolojiyi baskı kafası, püskürtme ucu ve bir yazıcı teknolojisi kullanılarak materyalin katman katman biriktirilmesiyle 3B nesnelerin üretilmesi olarak tanımlamıştır. Ayrıca 3B baskılama teknolojisi, eklemeli üretim olarak da nitelendirilmektedir [1,2].

Eczacılıkta 3B baskılama teknolojisi, 3B baskılama yöntemlerinden faydalanılarak kişiselleştirilmiş çeşitli oral dozaj formlarının üretimi için çekici bir strateji olarak karşımıza çıkmaktadır. Bu teknoloji, optimum ilaç etkinliği ve azaltılmış yan etkiler gibi hedeflerin gerçekleştirilmesi hususunda genomik biliminin desteği ile kişiselleştirilmiş dozaj formlarının geliştirilmesi ve üretilmesi için bir zemin oluşturmaktadır [3]. Bu teknoloji ile birden fazla etkin madde içeren dozaj formları hazırlanabilmekte ve stabilite sorunu giderilebilmekte, hastaya spesifik etkin madde dozu dozaj formuna yüklenebilmekte, yaş grubuna bağlı olarak farklı formlarda baskılanabilmekte ve etkin madde salımı modifiye edilebilmektedir [4,5].

Üç Boyutlu Baskılama Teknolojisinin Keşfi ve Gelişimi

3B baskılama teknolojisi, 1970'lerin başında ortaya çıkmıştır. Pierre A. L. Ciraud direkt metal lazer sinterleme (DMLS) yöntemi olarak adlandırılan bir yöntem kullanarak toz halindeki maddelerin

belirli bir geometriye sahip nesnelerin üretilmesi için lazer ışını gibi yüksek enerjili bir ışın ile katılaştırılması ve bu işlemin tabakalar halinde gerçekleştirilmesi ile 3B bir nesnenin oluşturulabileceği fikrini ortaya atan ilk kişidir [6-8]. Takip eden yıllarda Ross Housholder tabakalar halinde 3B bir nesne oluşturmak üzerine bir patent almıştır. Daha sonra Carl Deckard, toz halindeki malzemenin lazer ışını ile katılaştırılması olarak da bilinen seçici lazer sinterleme (SLS) tekniğini geliştirmiştir.

Charles Hull tarafından 1984 yılında geliştirilen stereolitografi (SLA) yöntemi, 3B baskılama teknolojisi tarihinin en büyük icadı olmuştur. Geliştirilen bu yöntem sıvı reçinenin, ultraviyole (UV) ışık ile fotopolimerize edilerek katılaştırılmasına dayanmaktadır [1]. Aynı bilim insanı tarafından 1986 yılında 3B nesneler üretmek için bilgisayar destekli çizim teknolojisi ve programlama geliştirilmiş ve SLA yöntemiyle entegre edilmiştir [9]. Scott Crump tarafından geliştirilen günümüzde en çok kullanılan yöntemlerden bir diğeri olan eriyik yığma modelleme (FDM) yöntemi, nesnelerin termoplastik malzemeler kullanılarak üst üste biriktirilmesi ile üretimi temeline dayanmaktadır [1].

Bilim insanı Emanuel Sachs ve arkadaşlarının 1990'larin başında geliştirdiği bir yöntem olan bağlayıcı püskürtme, bir toz tabakası üzerine sıvı bir bağlayıcının püskürtülüp katılaştırılmasına dayanmaktadır. Bu buluşla birlikte üretim süresini azaltarak endüstriyel verimliliğin arttırılması amaçlanmıştır. 2005 yılında 'RepRap' denilen kendi kendini kopyalayan hızlı prototipleme sağlayan 3B bir yazıcı tasarlanmıştır. Bu teknik malzemelerin katman katman dizilerek 3B nesnenin oluşturulması için plastik filamentin sarılması, eritilmesi ve akabinde füzyonu temeline dayanmaktadır [10,11].

Günümüzde yüksek işlem verimliliğine sahip 3B baskılama yöntemleri geliştirmek ve bu yöntemlerle üretilebilen nesnelerin çeşitliliğini arttırmak üzere çalışmalar yoğun bir şekilde devam etmektedir.

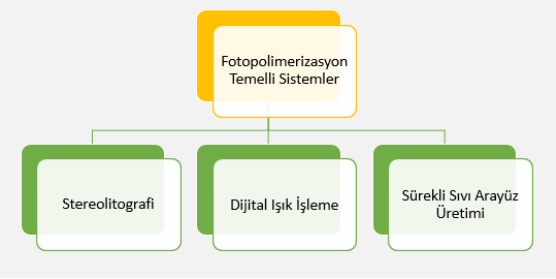
Üç Boyutlu Baskılama Yöntemleri ve Sınıflandırılması

3B baskılama geleneksel üretim yöntemlerinden farklı olarak, ilacın kişiselleştirilebilme ve talep üzerine üretimi gibi işlemleri mümkün kılmaktadır. Günümüzde birbirinden farklı çalışma prensiplerine sahip 3B yazıcılar mevcuttur. Yazıcılar, 3B yazdırma sürecinin ayrılmaz bir parçası olup istenen geometriye ve işlevsel özelliklere sahip nesneler üretmek için temelde püskürtme, ısı uygulaması ve lazer enerjisi gibi harici enerjileri kullanmaktadır [9].

Bu derlemede 3B yazıcıların çalışma prensibini temel alarak bir sınıflandırma yapılmıştır: Fotopolimerizasyon, mürekkep püskürtme, toz yatağı füzyon, malzeme ekstrüzyon ve yönlendirilmiş enerji biriktirme (eczacılıkta kullanımı bulunmadığından bahsedilmeyecektir) olmak üzere beşe ayrılmıştır [9].

Fotopolimerizasyon Temelli Sistemler

Işığa duyarlı maddelerin (fotopolimer) lazer veya UV ışık kaynağına maruziyeti sonucunda polimerizasyon reaksiyonunun indüklenip fotopolimerin sertleşmesiyle birlikte 3B nesnenin üretimi temeline dayanmaktadır. Bu tekniğin en büyük avantajı mevcut yazıcılar arasında en hızlı ve en yüksek çözünürlüklü 3B baskılamayı yapabilmesidir. Buna karşın en büyük dezavantajı dozaj formu üretimi için kullanılabilecek fotopolimerlerin sınırlı olmasıdır. Fotopolimerize edilebilir maddelere örnek olarak hidrojeller, metakrilat bazlı polimerler, tiyol-en ve tiyol-in sistemleri verilebilmektedir [10,12,13]. Bu tekniği kullanan teknolojilere örnek olarak SLA, dijital ışık işleme (DLP) ve sürekli sıvı arayüz üretimi (CLIP) verilebilmektedir (Şekil 1) [12,14-16].



Şekil 1. Fotopolimerizasyon temelli sistemlerin sınıflandırılması

Stereolitografi (SLA)

Bu yöntemde sıvı reçinenin katılaştırılması bilgisayar kontrollü lazer ışını ile gerçekleştirilerek hızlı bir şekilde 3B model oluşturulmaktadır. İlk katman iki boyutlu (2B) olacak şekilde zemine basılmakta, ilk katmanın üzerine sırasıyla inşa edilecek sonraki katmanlar için de aynı işlem tekrarlanarak 3B nesne üretilmektedir (Şekil 2). Temel enerji kaynağı olarak UV ışık kullanılmaktadır [10,17,18]. Bu kapsamda yapılan çalışmalara bakıldığında bu teknikle 3B baskılamanın oldukça yaygın olduğu görülmüştür.

Wang ve ark. [19] bir çalışmada, modifiye salım özelliklerine sahip tabletlerin üretimi için stereolitografi yönteminin uygunluğunu değerlendirmiştir. Etkin madde olarak 4-aminosalisilik asit (4-ASA) ve parasetamol; monomer olarak polietilen glikol diakrilat (PEGDA); foto başlatıcı olarak difenil (2,4,6-trimetilbenzoil) fosfin oksit ve çözücü olarak polietilen glikol 300 (PEG300) kullanılmıştır. Suni gastrointestinal sistem ortamlarında palet yöntemi ile *in vitro* salım deneyi gerçekleştirilmiş, salım profilleri incelendiğinde etkin madde salımının ortam pH'sından etkilenmediği; belirleyici unsurun PEGDA/PEG300 oranının olduğu tespit edilmiştir. Sonuçta uygun bir polimer konsantrasyonu ile kontrollü salım profilinin elde edilebileceği görülmüştür.

Xu ve ark. [20] tarafından yürütülen bir çalışmada, irbesartan, atenolol, hidroklorotiyazid ve amlodipin olmak üzere dört farklı antihipertansif etkin madde içeren çoklu tabletler iki farklı katman sıralaması ile (Tip 1 ve Tip 2) SLA yöntemi kullanılarak üretilmiştir (Şekil 7). Tip 1'de dozu daha yüksek olan etkin maddeler dış tabakalara, tip 2'de ise iç tabakalara baskılanmıştır. *İn vitro* karakterizasyon çalışmaları neticesinde fotopolimer olarak kullanılan PEGDA (M.A. 575 g/mol) ile amlodipin arasında geçimsizlik olduğu tespit edilmiş dolayısıyla amlodipin etkin bir şekilde yüklenememiştir. *İn vitro* salım çalışmaları gastrointestinal sistemin tamamen simüle edildiği ortamlarda palet yöntemi ile yapılmıştır. Atenolol salımının yüzey alanı/hacim oranının daha yüksek olduğu tip 1'de daha yüksek olduğu; hidroklorotiyazid ve irbesartanın ise yüzey alanı/hacim oranından önemli derecede etkilenmediği görülmüştür. Her iki tasarımda da sadece atenololün %100'ü salım ortamına geçmiş, hidroklorotiyazid ve irbesartan için ise bu oran sırasıyla %48 ve %17 olmuştur. Bu durum bu iki etkin maddenin suda düşük çözünürlüğüne bağlanmıştır.

Dijital Işık İşleme (DLP)

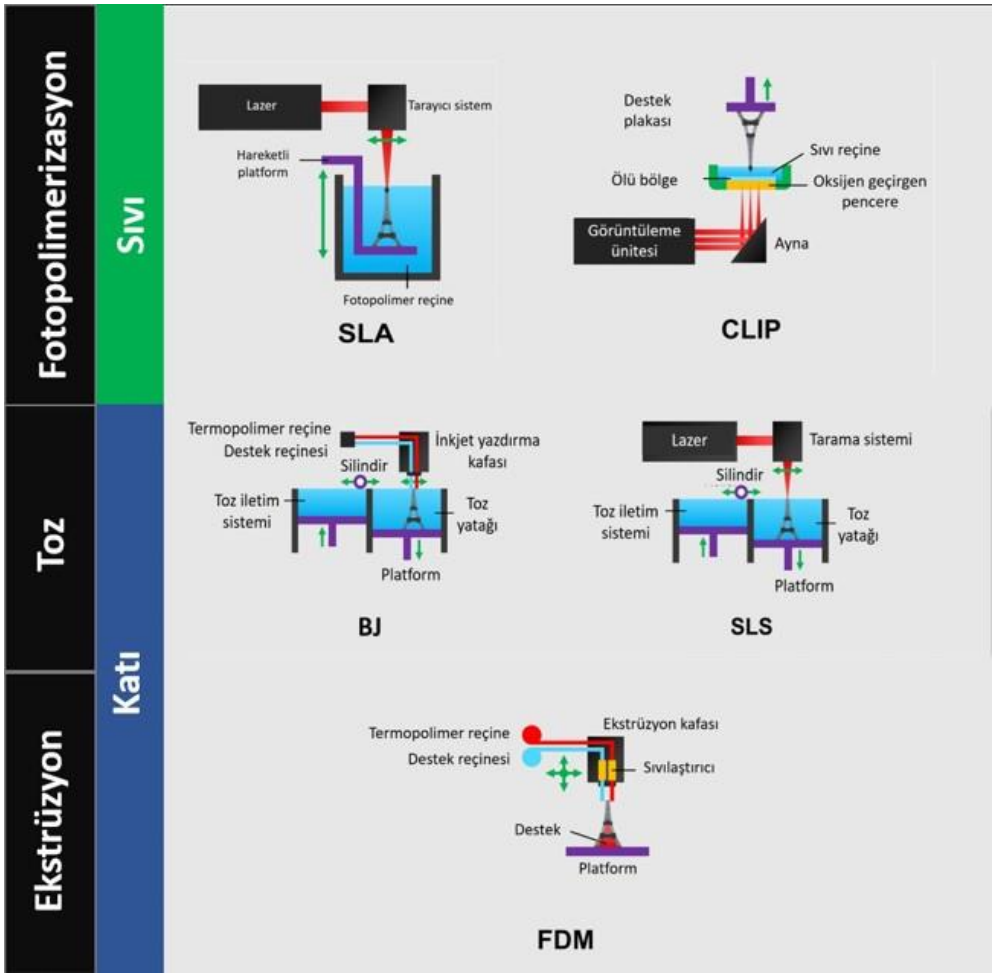
Bu yöntem esasen SLA yöntemine benzer şekilde çalışmaktadır. Ancak buradaki fark, UV ışığını fotoreaktif maddelerin yüzeylerine yansıtan ve odaklayan bir dijital mikro ayna cihazının (DMD) kullanılmasıdır. Baskılama sırasında inşa platformu reçine havuzuna batırılmakta ve reçinenin polimerizasyonu kabın altından gerçekleştirilmektedir. Böylece polimerize edilen katmanın havayla teması kesilmekte, oksijenden kaynaklanacak bir inhibisyon azaltılmaktadır. Mükemmel çözünürlüğe sahip bir teknik olup tek seferde tüm 2B kesiti sertleştirebilmesi sayesinde stereolitografiden daha hızlı bir üretim imkân sunmaktadır. Ayrıca diğer fotopolimerizasyon yöntemlerine göre çok daha az miktarda atık üretmektedir [10,21-23].

Kadry ve ark. [21] bir çalışmada tablet üretiminde DLP tekniği ile çalışan 3B yazıcıların kullanılabilirliğini değerlendirmiştir. Fotoreaktif polimerler olarak PEGDA ve poli(etilen glikol) dimetakrilat (PEGDMA), etkin madde olarak ise teofilin kullanılmıştır. Baskı parametreleri (UV ışık yoğunluğu, ışığa maruziyet süresi, polimer konsantrasyonu ve tabaka kalınlığı) optimize edildikten sonra, delikli ve deliksiz olmak üzere çeşitli modellerde tabletler basılmıştır. Teofilin yükleme etkinliği, tablet ağırlığından bağımsız olarak %1 bulunmuştur. Etkin madde içeriği ve kütle tektürlülüğü incelendiğinde varyasyonun Amerikan Farmakopesi'nde yer alan sınırlar içinde olduğu görülmüştür. Salım profilleri değerlendirildiğinde, tablet üzerindeki delik sayısındaki artışla birlikte salınan etkin madde miktarının da arttığı tespit edilmiştir. Sonuç olarak, DLP'nin istenilen şekle ve istenilen salım profillerine sahip tabletler üretmede uygun bir yöntem olarak kullanılabileceği kanıtlanmıştır.

Sürekli Sıvı Arayüz Üretimi (CLIP)

Bu teknik, UV ışık kullanılarak dijital ışık projektörü ile gerçekleştirilmektedir. Fotoreaktif reçine havuzu, destek platformu, oksijen geçirgen pencere ve görüntüleme ünitesi temel bileşenleri oluşturmaktadır. Görüntüleme ünitesinden baskılanacak nesneye ait desen reçineye yansıtılmakta ve

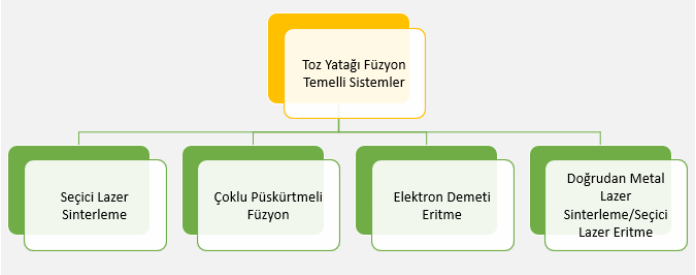
ışığın yansıdığı bölgedeki sıvı reçine hemen katılaşmaktadır. Oksijen geçirgen pencere reçine havuzunun üstünde ölü bir bölge oluşturmakta ve bu bölgedeki reçinenin katılaşması engellenmektedir. Böylece destek platformunun yukarı-aşağı hareketiyle birlikte katılaşan parça sıvı reçine havuzundan çekilmektedir. Baskılamanın katman katman yapılmaması sebebiyle diğer yöntemlere göre daha hızlıdır ve baskı kalitesi daha iyidir. Bu teknik genel olarak tablet üretimi yerine mikro iğnelere etkin madde/ler yüklemek için kullanılmaktadır (Şekil 2) [10,14,24].



Şekil 2. 3B baskılama yöntemlerinin şematik gösterimi (SLA: stereolitografi; CLIP: sürekli sıvı arayüz üretimi; BJ: bağlayıcı püskürtme; SLS: seçici lazer sinterleme; FDM: eriyik yığma modelleme) [14]

Toz Yatağı Füzyon Temelli Sistemler

Bu yöntemde toz halindeki maddelere sinterleme (kısmi yüzey eritme ve katılaşma) veya yüksek erime noktasına sahip partiküllerin düşük erime noktasına sahip bağlayıcılar ile bağlanması şeklinde bir işlem uygulanmaktadır. Sinterlemede toz madde kısmen eritilmekte, ısı ile aktive edilen kimyasal reaksiyonlar neticesinde katılaşma sağlanmaktadır. Her iki yaklaşımda da ısı uygulanması gerektiğinden bu amaç için genellikle lazer kullanılmaktadır [10,13]. SLS, çoklu püskürtmeli füzyon (MJF), doğrudan metal lazer sinterleme/seçici lazer eritme (DMLS/SLM) ve elektron demeti eritme (EBM) teknikleri bu temele dayanan sistemlerdir (Şekil 3) [9]. Günümüzde eczacılık alanında SLS ve EBM yöntemi kullanılabilmektedir.



Şekil 3. Toz yatağı füzyon temelli sistemlerin sınıflandırılması

Seçici Lazer Sinterleme (SLS)

Bu teknikte, toz halindeki parçacıklar (termoplastik polimerler) lazer kullanılarak birleştirilmektedir. Baskı işlemi sırasında toz yatağına lazerle belirli bir desen çizilmekte erime ve akabinde katılaşmayla birlikte katmanlar oluşarak nihai 3B yapı üretilmektedir (Şekil 2). SLS, eczacılık alanında yakın zamanda kullanılmaya başlanan, yüksek çözünürlüklü ve tek işlem basamaklı bir baskı teknolojisidir. Bu yöntemin özellikle ağızda dağılan tabletlerin üretimi için uygun bir yöntem olduğu görülmüştür [10,25,26].

Fina ve ark. [27] yaptığı bir çalışmada, SLS yönteminin ilaç üretimi için uygunluğu değerlendirilmiştir. İki termoplastik polimer, Kollicoat IR (%75 polivinil alkol ve %25 polietilen glikol kopolimeri) ve Eudragit L100-55 (%50 metakrilik asit ve %50 etil akrilat kopolimeri), sırasıyla hızlı ve modifiye salım yapan tabletler üretmek için seçilmiştir. Her polimer için %5, %20 ve %35 olmak üzere üç farklı etkin madde (parasetamol) oranına sahip formülasyonlar tasarlanmıştır. Toplamda altı 3B baskılı tablet baskılanmıştır. Karakterizasyon çalışmaları sonucunda bir geçimsizlik tespit edilmemiştir. Salım profilleri incelendiğinde Kollicoat içeren formülasyonlarda parasetamol salımı pH'dan bağımsız gerçekleşmiş ve formülasyondaki etkin madde oranına göre salım hızı değişmiştir. Eudragit içeren formülasyonlarda ise etkin madde miktarından bağımsız olarak pH'ya bağlı, 12 saat süresince modifiye salım profili göstermiştir

Fina ve ark. [28] yaptığı bir çalışmada, değiştirilebilir salım özelliklerine sahip silindirik ve jiroid kafes modelinde iki katmanlı yapıya sahip parasetamol içeren tabletler, polimer olarak polietilen oksit (PEO, M.A. 1 000 000), Eudragit (L100-55 ve RL) ve etil selüloz (viskozite 5.6–8 mPa·s) kullanılarak üretilmiştir. Jiroid kafes yapısının, silindirik tabletlerle karşılaştırıldığında, herbir polimer için etkin madde salımını artırdığı tespit edilmiştir. Silindirik yapıda baskılanan formülasyonların salım profiline bakıldığında, PEO içeren formülasyonda etkin maddenin %60'ı ilk 2 saatte, kalan %40'ı ise takip eden 4-5 saatte salınmıştır. Polimerin enterik özelliğinden dolayı Eudragit L içeren formülasyondan ilk bir saatte sadece %17 parasetamol ortama salınmış, pH 5.5'te etkin madde salımının daha hızlı gerçekleştiği; salımın 12 saatte tamamlandığı tespit edilmiştir. Etil selülozun kullanıldığı formülasyonda polimerin hidrofobik olması ile bağlantılı olarak ilk 8 saatte sadece %7; 24 saatte %20 parasetamol salımı gerçekleşmiştir. Eudragit L ve etil selüloz içeren formülasyonlar karşılaştırıldığında Eudragit L kullanılarak baskılanan tabletlerin porozitesinin daha fazla olduğu görülmüş, bağlantılı olarak da temas yüzeyinin daha fazla olması neticesinde etil selüloza göre 4 kat fazla parasetamol salımının olduğu tespit edilmiştir. Sonuç olarak genişleyen temas yüzeyi ve tablet gözenekliliğindeki belirgin artışın daha fazla etkin madde molekülünün difüzyonunu mümkün kıldığı tespit edilmiştir. Son olarak PEO polimeri ile jiroid kafes ve silindirik tasarımların bir kombinasyonu şeklinde yeni bir iki katmanlı konfigürasyon oluşturulmuştur. Bu formulasyondan etkin madde salım profili, klasik silindirik tablet ile jiroid kafes yapısından salım profilleri arasında gerçekleşmiştir. Sonuçta formülasyon bileşimini değiştirmek zorunda kalmadan tablet geometrisini değiştirerek etkin madde salımının modifiye etmenin mümkün olduğu bildirilmiştir.

Elektron Demeti Eritme (EBM)

Bu teknikte baskılama için gereken enerji, lazer ışınından ziyade elektron ışınından sağlanmaktadır. Elektron ışınının yüksek yoğunluğu, toz halindeki materyalleri 1000°C'ye ulaşan

sıcaklıklarda tamamen erimiş hale getirmektedir. Elektron kaynağı olarak tungstenden yapılmış bir filaman kullanılmakta ve bir elektromanyetik bobin kullanılarak dar bir ışın demeti haline getirilmektedir. Elektronlardaki enerji, ısıyı toz yatağına aktarırken toz yatağının negatif yükü artmakta, bu yükü dağıtmak için ise işlem sırasında helyum gazı salınmaktadır. Bu teknik sadece metalleri işlemek için kullanılabilmektedir. Özellikle protez yapımında ön plana çıkmaktadır. Fakat son yıllarda özellikle etkin madde içeren kemik iskelelerinin üretimi içinde araştırılmaktadır [29].

Malzeme Ekstrüzyon Temelli Sistemler

Küresel olarak ekstrüzyon, en yaygın kullanılan 3B baskı teknolojisidir ve yönteme olan ilgi, farmasötik üretimde de artmaktadır. Ekstrüzyon işleminde, malzeme robotik olarak çalıştırılan nozüllerden ekstrüde edilmektedir. Bir toz yatağı gerektiren bağlayıcı biriktirmenin aksine, ekstrüzyon yönteminde herhangi bir alt tabakaya baskı yapılabilmektedir. Bununla birlikte, bir toz yatağının olmaması nedeniyle, ekstrüde edilmiş nesneleri desteklemek için genellikle "iskele" gibi fazladan bir destek malzemesi gerekmektedir. Termoplastik polimerler, pastalar, kolloidal süspansiyonlar, silikonlar ve diğer yarı katılar dahil olmak üzere çok çeşitli malzemeler 3B baskı için ekstrüde edilebilmektedirler [13]. Bu teknoloji eriyik yığma modelleme (FDM) ve yarı katı ekstrüzyon (SSE) teknolojisi ile çalışan yazıcıları kapsamaktadır.

Yarı katı Ekstrüzyon (SSE)

Bu teknikte, yarı katı ürünü katmanlar halinde yazdırmak için bir enjektör kullanılmaktadır. Yarı katı haldeki yapı, çözücü ve polimerin bir kombinasyonu olup jel veya pasta şeklinde bulunabilmekte; ekstrüzyon 3-5 bar basınç altında gerçekleşmektedir. Bu yöntemde dikkat edilmesi gereken nokta, kuruma sırasında deformasyon veya çökme meydana gelebileceği için sistemin çökmemesi adına üst katmanların inşa edildiği alt katmanların yeterli sertliğe/dayanıklılığa sahip olup olmadığının kontrol edilmesidir [10,30].

Khaled ve ark. [31] yaptığı bir çalışmada, etkin madde olarak guaifenesin kullanılarak iki katmanlı 3B baskılanmış tabletleri SSE yöntemi ile üretilmiştir. Tablet katmanları hızlı salımın akabinde sürekli salım yapacak şekilde tasarlanmıştır. Sürekli salımı sağlayan tabaka için hidroksipropil metilselüloz (HPMC 2208) (Methocel TM K100M Premium) ve poli (akrilik asit) (PAA) (Carbopol® 974P NF) kullanılmıştır. Hızlı salım yapan diğer tabaka için bağlayıcı olarak Hypromellose® (HPMC 2910), dağıtıcı olarak mikrokristal selüloz (MCC) (Pharmacel® 102) ve sodyum nişasta glikolat (SSG) (Primojel®) kullanılmıştır. Üretilen tabletlerin ağırlık sapması, friabilite, sertlik ve kalınlık gibi fiziksel ve mekanik özellikleri değerlendirildiğinde farmakope standartlarına uygun olduğu görülmüştür. Salım çalışmaları sepet yöntemi ile yapılmış; tabletler 2 saatlik bir süre boyunca suni mide ortamına konulmuş ve ardından suni bağırsak sıvısı olarak pH 6.8 ve 0.2 M trisodyum fosfat dodekahidrat tamponuna bırakılmıştır. Salım profilleri değerlendirildiğinde ilk 30 dakika içinde hızlı salım yapan tabakadaki etkin madde ortama tamamen geçerken, 12 saatlik bir süre boyunca sürekli salım tabakasından etkin madde çıkışının sürdüğü görülmüştür. 3B baskılanmış tabletlerin salım profilleri piyasada yer alan ticari guaifenesin tabletlerinin salım profili ile karşılaştırıldığında salım özelliklerinin benzer olduğu görülmüştür.

Eriyik Yığma Modelleme (FDM)

Stratasys şirketinin kurucu ortağı Scott Crump tarafından 1989 yılında toz yataklı 3B baskılamanın sınırlamalarına bir alternatif olarak geliştirdiği bir yöntemdir. Bir dişli sistemi ile filament ısıtılmış nozula doğru gönderilmektedir. X ve Y olmak üzere iki eksende hareket edebilen nozulda filamentler ısıtılarak eritilmektedir. Termoplastik malzeme ekstrüzyon nozulundan sıkıştırılarak itilirek katmanlar oluşturulmakta, inşa platformu Z ekseninde hareket ettirilerek 3B yapı baskılanmaktadır (Şekil 2). FDM yönteminde ekstrüzyon nozulunun hareketini sağlamak için temel olarak kademeli motorlar kullanılmaktadır [32-35].

Bu yöntemde filament olarak erimiş metaller, mumlar ve termoplastik polimerler (poli(laktik asit), poli(vinil alkol) ve poli(etilen vinil asetat)) kullanılabilmektedir. FDM'nin avantajları arasında daha düşük maliyette, birden çok polimeri tek bir yapıda basabilme ve iyi bir mekanik dayanıma sahip içi boş ve gözenekli yapılar oluşturma kapasitesine sahip olması sayılabilmektedir. Bu özellikler,

FDM'nin kişiselleştirilmiş tablet üretiminde uygun bir yöntem olarak öne çıkarmaktadır. İşlemin dezavantajları arasında, uygun reolojik özelliklere sahip termoplastik polimer miktarının sınırlı olması ve uygulanan yüksek sıcaklık sebebiyle etkin maddelerin bozulma olasılığı sayılabilmektedir [10,32]. Bu dezavantajlara rağmen FDM açık ara en çok kullanılan yöntemdir [13].

Goyanes ve ark. [36] bir çalışmada, FDM yöntemi ile parasetamol ve kafein içeren 3B baskılanmış tabletler üretmiştir. Baskı işlemi için kullanılan filamentler, her etkin maddenin ayrı ayrı PVA ile karıştırılması ve sıcak eriyik ekstrüzyon yönteminin uygulanmasıyla üretilmiştir. Dört filamentin etkin madde konsantrasyonu parasetamol için %4.3 ve %8.2; kafein için %4.7 ve %9.5 olmuştur. Farklı salım profilleri elde etmek için iki farklı tasarıma sahip tabletler 3B olarak baskılanmıştır. Birinci tasarım, 1 mm'lik kafein ve parasetamol birbirini takip eden ayrı tabakalar halinde çok katlı bir kaplet şeklinde üretilirken, ikinci tasarım DuoCaplet (9.0 mm uzunluk x 3.34 mm çap) olarak adlandırılan tabletin iç kısmı kafein veya parasetamol etkin maddesini içeren bir çekirdek ve diğer etkin maddenin bulunduğu bir dış katman şeklinde üretilmiştir. Açlık durumunda gastrointestinal sistemin koşullarını simüle etmek için ilk 1 sa. 0.1 M HCl, 35 dk. modifiye edilmiş Hanks tuz çözeltisi (pH 5.6'dan pH 7.0), ve takiben modifiye edilmiş Krebs tamponu (3 sa. pH 7.0 – 7.4 ve sonra pH 6.5) içinde USP-II çözünme testi cihazı ile kapletlerden etkin maddelerin salım çalışmaları yapılmıştır. Ortam pH'sı çözünme artamlarından CO_2 ve He gazları geçirilerek ayarlanmıştır. Sonuçlar, katlı kapletlerden her iki etkin maddenin aynı anda salındığını gösterirken, çekirdek-dış tabaka kapletler yani DuoCapletler modelinde ilk olarak dış tabakaya dahil edilen etkin madde ve daha sonra iç tabakadaki etkin maddenin salımı başlamıştır. Ancak dış tabaka tam olarak çözündükten sonra iç tabakadaki etkin madde salımı gerçekleşmiştir. Dış tabakadaki etkin maddenin en az %50'si *in vitro* salımın gastrik fazında salınmıştır (kafein için ise bu oran %80'den yüksek olmuştur). İç çekirdekteki etkin madde, *in vitro* salım testinin ince bağırsak fazı boyunca 50-135 dakikalık bir gecikme süresinden sonra salınmıştır. Ekstrüde edilen filamentlerin artan etkin madde konsantrasyonu ile salım hızının arttığını, çok katmanlı ürünlerde hem parasetamol hem de kafeinin salımının eşzamanlı ve etkin maddelerin çözünürlüğünden bağımsız olduğunu belirlemişlerdir. DuoCaplet tasarımıyla, etkin maddenin salım yeri seçilerek hızlı veya gecikmeli salımın mümkün olduğu sonucuna ulaşılmış; iç bölmeden salım için gecikme süresinin dış katmanın özelliklerine bağlı olduğu gösterilmiştir.

Okwusa ve ark. [37] bir çalışmada, metakrilat polimerleri (Eudragit EPO, RL, L100-55 ve S100), hidroksipropil selüloz (HPC) ve polivinil pirolidon (PVP) bazlı teofilin içeren filamentlerin 6 ay boyunca stabilitesini araştırmıştır. HME ile üretilen filamentler, vakumlama ile/vakumlama olmadan 5°C veya 30°C / %65 bağıl nemde saklanmıştır. Daha sonra 1, 3 ve 6. aylarda filamentlerin boyutları, görünümü, termal özellikleri ve baskılanabilirliği değerlendirilmiştir. Stabilite çalışmaları sonucunda metakrilat polimerleri ile hazırlanan filamentlerin fiziksel olarak daha stabil olduğu ve FDM yöntemi ile baskılamaya daha uygun olduğu görülmüştür. HPC ve PVP bazlı filamentlerin ise higroskopik yapıları dolayısıyla camsı geçiş sıcaklıklarında bir azalma görülmüş, bu da filamentlerin esnekliğinin artmasına yol açmıştır. Neticesinde FDM ile baskılanabilirlikleri mümkün olmamıştır. Saklama sırasında teofilin miktarı ve kristal yapısı önemli ölçüde değişmemiş, fakat salım profilinde fark gözlenmiştir. Filamentlerin uzun vadeli stabilitesi, özellikle klinik uygulamalardan önce hazır filamentler ile 3B baskılama yapılabilmesi noktasında büyük önem arz etmektedir. Dolayısıyla bu ve benzeri çalışmalar büyük önem arz etmektedir.

Mürekkep Püskürtme Temelli Sistemler

Mürekkep püskürtme temelli sistemlerde bir başlıktan püskürtülen mürekkep (bağlayıcı veya malzeme) damla damla bir zeminde biriktirilip katılaştırılarak 3B nesne elde edilmektedir. Yaygın olarak kullanılan malzemeler arasında eriyik polimerler ve mumlar, UV ile kürlenebilen reçineler, çözeltiler, süspansiyonlar ve çok bileşenli sıvılar bulunmaktadır. Tüm formülasyonların püskürtmeye ve hızlı katılaşmaya uygun şekilde formüle edilmesi gerekmektedir. Ürün geometrisinin büyük ölçüde damlacık yoluna, damlacık etkisine ve yüzeyin ıslanmasına bağlı olduğu bilinmektedir. Uygulanması zor bir yöntem olup yüksek çözünürlüğü nedeniyle diğerlerinden daha avantajlı konumda olduğu bilinmektedir. Bu sistemde püskürtülen damlacıkların çapı yaklaşık 100 µm olup, yüzeyin ıslanması, çözücü buharlaşması veya büzüşme gibi sebeplerden dolayı katman kalınlığı damlacık çapından daha ince olmaktadır. Bu durumun keşfinin ardından mikropartiküller bu yöntem kullanılarak başarılı bir

şekilde baskılanabilmiştir [10,13]

Tercihe Bağlı Damlatma (Drop on Demand-DoD)

Bu yöntemde, bir kartuşa basılacak malzeme doldurulmakta, daha sonra mikroakışkan bir hazneden çıkış deliğine doğru itilmektedir. Mürekkep damlasının özellikleri, mürekkebi delikten geçirmek için kullanılan sisteme ve çıkış deliğinin özelliklerine bağlı olduğundan baskı kafası tipi termal ve piezoelektrik olarak ikiye ayrılmaktadır. Termal inkjet tekniğinde, mürekkepte bir kabarcık oluşturarak mürekkebi delikten geçmeye zorlayan bir ısıtma elemanı kullanılmaktadır. Piezoelektrik inkjette ise sıvı malzeme bir piezoelektrik kristal kullanılarak küçük bir delikten geçirilmekte ve küçük damlacıklara bölünmesi sağlanmaktadır. Sıvı malzeme ile bağlantılı olan piezoelektrik kristale bir voltaj uygulanmakta ve bu durum baskılanacak sıvı materyalde volümetrik bir değişim yaratmaktadır. Baskılamada kullanılacak sıvı ve nozül arasında oluşan basınç farkı ile damlacıklar oluşmaktadır. Bu yöntem, diğer yöntemlere göre işleme bağlamında uygun maliyetlidir; hızlıdır, düşük atık üretimi ile geniş alanlarda minimum kontaminasyon sağlamaktadır [10,38-40].

Teofilin ve metoprolol tartrat için değişebilen bir doz kontrol platformu oluşturmak üzere DoD yönteminin bir alt kategorisi olan toz üzerine damla (DoP) teknolojisine dayalı bir masaüstü 3B yazıcı kullanabilirliğinin araştırıldığı bir çalışmada, etkin madde yükleme modellerinin doz düzenlemesinin doğruluğu üzerindeki etkisi de değerlendirilmiştir. Baskılanmış tabletlerin tümü iyi mekanik özellikler ve tatmin edici yapısal bütünlük sergilemiştir. Hedef etkin madde dozlarına sahip tabletlerde doğruluk, %0.5-3.2 aralığında küçük bir varyasyon katsayısı ile %91.2-108 aralığında olmuştur. Geleneksel bölünmüş doz yöntemleriyle karşılaştırıldığında, DoP 3B baskı teknolojisi, doz düzenlemesinde daha yüksek doğruluk sergilemiş, ancak in vitro etkin madde salım davranışı üzerinde daha az etki göstermiştir. Bu çalışmadaki sonuçlar, pediyatrik hastalara yönelik kişiselleştirilmiş ilaçların üretimi için dinamik bir doz kontrol platformu oluşturmak açısından umut verici bir yöntem olarak DoP teknolojisinin uygulanabilirliğini açıkça göstermiştir [41].

Malzeme Püskürtme

Bu teknikte sıvı malzeme damlacıklar halinde bir yüzeyde biriktirilmekte ve UV ile katılaştırılarak 3B nesneler oluşturulmaktadır. Bu teknik ile nesne üretim aşamaları şu şekildedir; fotopolimer malzemeler hava içermeyen bir tanktan nozüle iletilmekte ve orada ısıtıldıktan sonra platform üzerinde çok ince bir tabaka oluşturacak şekilde damlacıklar halinde biriktirilmektedir. Son olarak, katılaşma için platform üzerindeki erimiş malzeme üzerine UV ışını tutulmakta ve ilk katman üretimi gerçekleşmektedir. Bu fotopolimerizasyon işleminde, sıvı haldeki monomerlerin/oligomerlerin katılaşması için belirli dalga boyuna sahip bir ışık kaynağı kullanılmaktadır. Pratikte 190-400 nm arasında bir dalga boyuna sahip bir ışık kaynağı tercih edilmektedir. Bir katman baskılandıktan sonra platform belirli bir katman kalınlığı seviyesine inmekte ve yeni sıvı malzeme bir önceki katman üzerine püskürtülmektedir. Birbirini takip eden her katman katılaşınca nesne üretilmektedir. Mürekkep püskürtmede sıvı veya erimiş malzeme kullanıldığından, özellikle çıkıntı bölgelerinde jel benzeri bir destek yapısına ihtiyaç duyulmakta ve bu destek yapılarının farklı yöntemler kullanılarak parçadan sonradan çıkarılması gerekmektedir [42].

Clark ve ark. [43] yaptığı bir çalışmada, çözünürlüğü düşük bir etkin madde olan karvedilolun, mürekkep püskürtme yöntemi kullanılarak 3B baskılama ile farklı geometrilere sahip tabletleri üretilmiştir. Formülasyonlar ağırlıkça %10 karvedilol, Irgacure 2959 ve fotokürlenebilir N-vinil-2-pirolidon (NVP) ve PEGDA'dan oluşmuş; çeşitli geometrilerde (halka, ağ, silindir, ince film) tabletler üretilmiş ve yüzey alanı/hacim oranları tespit edilmiştir. Karvedilol salımının, tüm geometriler için 10 saat içinde %80 oranında gerçekleştiği tespit edilmiştir. Karvedilolün salım hızı tablet geometrisine göre kıyaslandığında en hızlı salımın ince filmlerde, ardından halka ve ağ geometrilerinde ve en yavaş salımın silindirik formlarda olduğu gözlenmiştir. Yüksek hızlı salım, artan yüzey alanı/hacim oranıyla ilişkilendirilmiştir. Sonuçlara göre çeşitli salım profillerine sahip yüksek miktarda etkin madde içeren tabletlerin, tablet geometrisi değiştirilerek mürekkep püskürtme yöntemi ile üretilebileceğini gösteren ilk çalışma olmuştur.

Bağlayıcı Püskürtme (BJ)

Bağlayıcı püskürtme (BJ) tekniğinde bir toz yatağı üzerine sıvı haldeki bağlayıcı çözeltisi bir nozuldan damlacıklar şeklinde püskürtülmekte ve toz partiküllerinin bağlanmasıyla birlikte 3B nesneler elde edilmektedir. Bir rezervuarda yer alan toz, bir silindir ile bağlanan toz tabakasının üzerine örtülmekte ve nozuldan tekrar bağlayıcı püskürtülerek istenen tasarıma uygun şekilde toz partikülleri bağlanmaktadır (Şekil 2). Bu işlem 3B nesne elde edilene değin tekrarlanmaktadır. Bu teknik hem hızlı salım hem de kontrollü salım yapan dozaj formlarının tasarımı için başarılı bir şekilde uygulanabilmektedir. En büyük dezavantajları ise büyük, pahalı, temininin zor ve sadece büyük ölçekli üretime uygun olmasıdır. Amerikan Gıda ve İlaç İdaresi (FDA) tarafından onaylı bir ilaç olan Spritam® bu yönteme dayanan ZipDose teknolojisi kullanılarak üretilmiştir [44].

SONUÇ VE TARTIŞMA

3B baskılama veya eklemeli üretim teknolojisi eczacılıkta ve tıpta geçtiğimiz yıllarda kişiselleştirilmiş tedavi hedeflerinin gerçekleştirilmesi için ilaç üretiminde kullanılmaya başlanmıştır. 3B baskılama ile ilaçların bilgisayar ortamında sanal bir tasarımı yapılıp baskı kafası ve püskürtme ucuna sahip uygun bir yazıcı ile katmanlar oluşturularak baskılanması sağlanmaktadır. Keşfinden bu yana birçok yöntem geliştirilmiş olup özellikle FDM, BJ ve SLA yöntemleri ilaç formülasyonlarının araştırma ve geliştirilmesinde yaygın olarak kullanılan yöntemler olmuştur. Bu umut verici teknoloji, geleneksel teknolojik süreçlerle elde edilmesi zor olan formülasyonlar için büyük bir esneklik sunmaktadır. Çünkü konvansiyonel ilaç üretimine kıyasla tamamen yenilikçi bir şekilde, etkin madde/yardımcı madde oranının yüksek hassasiyetle ayarlanarak farklı şekillerde dozaj formlarının hazırlanmasına olanak tanımaktadır. Ancak henüz kişiselleştirilmiş ilaçlar için kalite ve emniyeti gösterecek belirlenmiş bir çerçeve bulunmamaktadır ve de bu teknolojinin sahte ilaç, yasal olmayan ilaçların yaygınlaşması ve yanlış etiketleme gibi riskleri de olabilecektir. 3B üretim için iyi imalat uygulamalarının (GMP) benimsenmesi, standart işlem prosedürlerinin oluşturulması, tüm üretim hattında ve sonrasında bitmiş üründe kalite kontrolün (örneğin tabletlerde içerik tektürlülüğü, kütle tektürlülüğü, çözünme testi) yürütülmesi gereklidir. Aynı seri içinde ve seriler arasında tekrarlanabilirlik değerlendirilmelidir. Ayrıca bu teknolojide kullanılacak yardımcı maddelerin seçimi de kritik olacaktır. Bununla birlikte Ağustos 2015'te 3B baskılanmış tablet formülasyonu olan Spritam® isimli ilacın FDA tarafından onaylanması 3B baskılamanın ticari olarak uygulanabilirliğinin kanıtı olmuştur. Diğer bir yön olarak etkin madde salım sistemlerinin ve tıbbi cihazların 3B baskılamasının, özelleştirilmiş/yenilikçi ürün üretmek için cazip bir araç olarak hizmet vereceğini de unutmamak gerekmektedir. Artan ilaç geliştirme çalışmaları, bu teknolojinin inkâr edilemez faydalarını kanıtlamaktadır, ancak tam başarı, endüstriyel ölçekte ayrıntılı yeni dozaj formlarına öncülük ettikten sonra elde edilecektir.

YAZAR KATKILARI

Kavram: S.T., N.Y.; Tasarım: S.T., Ş.G., N.Y.; Denetim: S.T., N.Y.; Kaynaklar: S.T., Ş.G., N.Y.; Malzemeler: S.T., Ş.G., N.Y.; Veri Toplama ve/veya İşleme: S.T., Ş.G.; Analiz ve/veya Yorumlama: S.T., N.Y.; Literatür Taraması: S.T., Ş.G.; Makalenin Yazılması: S.T., Ş.G., N.Y.; Kritik İnceleme: S.T., N.Y.; Diğer: -

ÇIKAR ÇATIŞMASI BEYANI

Yazarlar bu makale için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan ederler.

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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.

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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.

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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.

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- **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.

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  Example:

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  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: -

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  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.

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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/

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