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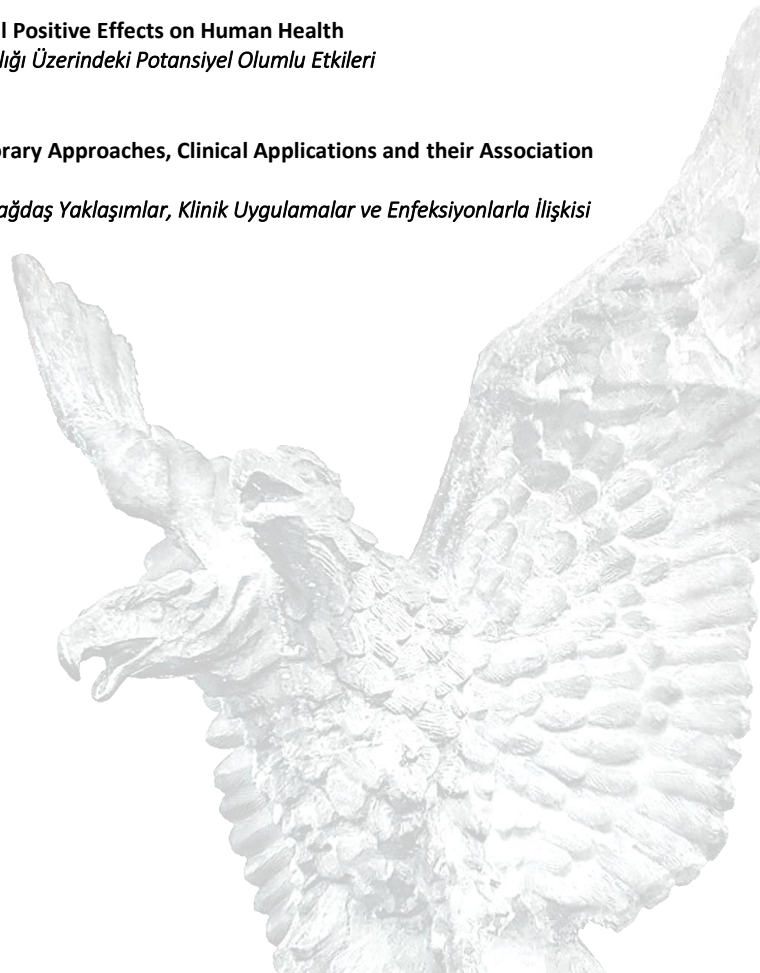

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Evaluation of Diabetic Stress and Depression in Patients with Geriatric Type 2 Diabetes

Geriatrik Diyabetli Hastalarda Diyabetik Stres ve Depresyonun Değerlendirilmesi

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ABSTRACT

Objective: This study was conducted to determine the levels of diabetes-related stress and depression in geriatric diabetic patients and to determine the relationship between them.

Methods: This descriptive, cross-sectional and correlational study was conducted between November 2020 and March 2021 with 200 individuals with type 2 diabetes who came to the endocrine and internal medicine outpatient clinic of a state hospital in Kars. Data were collected through face-to-face interviews using the Patient Information Form, Diabetic Distress Scale and Geriatric Depression Scale. Descriptive statistics, Mann-Whitney U Test and Spearman's correlation analysis were used to evaluate the data.

Results: 69% of the patients were between 65-70 years of age. The mean HbA1c value was 8.40±1.63 and the mean duration of diabetes diagnosis was 7.46±7.30 years. The mean value of Diabetes Distress Scale was 5.51±1.09 and the mean value of Geriatric Depression Scale was 16.10±6.34. There was a significant positive correlation between Geriatric Depression Scale and Diabetes Stress Scale ($p < .05$).

Conclusion: It was found that the patients had high levels of diabetes-related stress and moderate levels of depression. As geriatric depression increased, the stress experienced by the patients increased.

Keywords: Depression, distress, elderly, type 2 diabetes

ÖZ

Amaç: Bu çalışma geriatrik diyabetili hastalarda diyabete bağlı stres ve depresyon düzeylerini belirleyerek aralarındaki ilişkiyi tespit etmek amacıyla yapılmıştır.

Yöntem: Tanımlayıcı, kesitsel ve ilişki arayıcı türdeki bu çalışma, Kasım 2020 ile Mart 2021 tarihleri arasında Kars'ta bir devlet hastanesinin endokrin ve iç hastalıkları polikliniğine gelen 200 tip 2 diyabetli birey ile yürütülmüştür. Veriler Hasta Bilgi Formu, Diyabetik Distres Ölçeği ve Geriatrik Depresyon Ölçeği kullanılarak yüz yüze görüşme yoluyla toplanmıştır. Verilerin değerlendirilmesinde tanımlayıcı istatistikler, Mann-Whitney U Testi ve Spearman korelasyon analizi kullanılmıştır.

Bulgular: Hastaların %69'u 65-70 yaş arasındaydı. Ortalama HbA1c değeri 8,40±1,63 ve ortalama diyabet tanı süresi 7,46±7,30 yıldı. Diyabet Stres Ölçeği ortalama değeri 5,51±1,09 ve Geriatrik Depresyon Ölçeği ortalama değeri 16,10±6,34 idi. Geriatrik Depresyon Ölçeği ile Diyabet Stres Ölçeği arasında pozitif yönde anlamlı bir korelasyon vardı ($p < .05$).

Sonuç: Hastaların diyabetle ilişkili stres düzeylerinin yüksek ve depresyon düzeylerinin orta seviyede olduğu tespit edilmiştir. Geriatrik depresyon arttıkça hastaların yaşadığı stres de artmıştır.

Anahtar Kelimeler: Depresyon, distres, tip 2 diyabet, yaşlılık

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Introduction

This study was conducted as a descriptive, cross-sectional and correlational study. Aging is the process of irreversible differences that occur in the human body over time. As the differences occur, the prevalence of chronic diseases also increases in the elderly. Therefore, the elderly need more medical treatment and care (Rintala, 2013). With the increase in the elderly population worldwide, the incidence of chronic diseases is also increasing (Sithu et al., 2017). Furthermore, diabetes is significantly present as a chronic disease incidence worldwide. Diabetes can lead to loss of competence; development of dependency on others; changes in body appearance; causes anxiety, fear, and distress for the future; and negatively affects the physical and emotional well-being and social life of patients (Türten Kaymaz & Akdemir, 2016). Negative emotions (e.g., worry, depression, and stress) in patients can aggravate diabetes and its symptoms and increase emotional problems (Ell et al., 2015). Moreover, the physical damage caused by diabetes and its complications and the progression of the disease affects the psychological well-being more negatively, especially in the elderly. These feelings cause the progression of diabetes complications (Kasteleyn et al., 2015; Hessler et al., 2015). Since the elderly often have cognitive deficiencies, limitations in their daily activities, undiagnosed depression, and difficulties in social issues, paying attention to these issues in the elderly is necessary (Knech et al., 2011; Rasmussen et al., 2011). Furthermore, diabetes increases the tendency for depression, and depressive symptoms make it difficult to adapt to diabetes (Mazanec et al., 2011). Failure to indicate somatic symptoms (decrease or increase in appetite, insomnia, fatigue, psychomotor slowing, and so on) from the symptoms of diabetes or the side of medications make it difficult to diagnose depression as part of the process in which the disease occurs (Kocaman et al., 2007). Thus, the diabetic person should be evaluated in terms of depression if he does not participate in the treatment even though he is physically sufficient, if his complaints persist despite his medical condition with the appropriate treatment, if he does not feel well, shows less functionality than his abilities, and if he experiences a loss of interest and pleasure (Thanakwang et al., 2014; Vu et al., 2018). The risk of developing premorbid distress and depression is twice as high in diabetic patients compared with the general population (Polonsky, 2005). Consequently, distress and depression negatively affect the clinical condition and quality of life in diabetic patients (Çaklılı et al., 2020). It also causes a decrease in the patient's compliance with diabetes self-care recommendations and problems in terms of social relations (Kocaman et al., 2007). However, distress and depression are often not recognized and therefore patients cannot be properly treated in this respect (Thanakwang et al., 2014; Vu et al., 2018; Polonsky et al., 2005). When the literature is examined, the number of studies determining the levels of stress and depression due to diabetes using measurement tools specific to the elderly is limited (Çaklılı et al., 2020; Ell et al., 2015; Rintala, 2013). Thus, this study was conducted to determine the levels of distress and depression due to diabetes in elderly diabetic patients. This study sought answers to the following questions:

1) What is the level of diabetes stress in elderly people with type 2 diabetes?
2) What is the level of depression in elderly people with type 2 diabetes?
3) Is there a relationship between diabetes-related stress and depression in elderly people with type 2 diabetes?

Methods

This study was conducted as a descriptive, cross-sectional and correlational study and the ethical approval was obtained from the "Kafkas University Faculty of Health Sciences Ethics Committee" (Date: 06.10.2020, Decision no: 2020/8). All individuals included in the study were informed and signed voluntary consent forms. The population of the study consisted of patients who applied to the internal medicine and diabetes polyclinic of Kars Harakani State Hospital. The study sample consisted of 200 patients who met the inclusion criteria between November 2020 and March 2021. The inclusion criteria were the following: 65 years and older, diagnosed with type 2 diabetes for at least 1 year, taking oral antidiabetic and/or insulin therapy, having a diet program, not having diagnosed psychiatric disorder, having person, place and time orientation, and oral and written to participate in the study formed patients who gave consent. Sampling calculation was determined in G*Power 3 statistical analysis program with 95% confidence interval and 0.90 power ratio (minimum 120 persons).

Data Collection

The data were collected through patient introductory information forms prepared by the researcher Diabetes Distress Scale (DDS) and The Geriatric Depression Scale (GDS).

Patient introductory information forms

In the form created by the authors, there were questions that identify the patient's demographics and diabetes characteristics (Kocaman et al., 2007; Thanakwang et al., 2014; Vu et al., 2018; Polonsky et al., 2005).

Diabetes distress scale (DDS)

DDS was developed by Polonsky et al. (2005) to assess the psychosocial distress in diabetic patients (Polonsky et al., 2005) Çaklılı et al. (2020) conducted its Turkish validity and reliability study (Çaklılı et al., 2020). The scale consists of 17 items. Each item is evaluated between 1 and 6 points. A score of 1 means no stress, a score of 6 means serious stress. A score of ≥3 is defined as type 2 diabetes-related stress. The scale, which has 4 subscales: Emotional Burden, Doctor Stress, Regime Stress and Interpersonal Stress, evaluates the stress experienced in the last month. The original version of the DDS has been shown to be reliable alpha (α) = 0.9. In this study, alpha coefficient was 0.86.

The geriatric depression scale (GDS)

This scale was developed by Yesavage et al. (1983) and contains 30 items, each of which is rated yes or no (Yesavage et al., 1983). Items are scored as either 0 or 1 points and the total score is rated on a scoring grid. Participants with scores of 0-9 are classified as normal, those with scores of 10-19 as mildly depressed, and those with scores of 20-30 as severely depressed. Ertan and Eker (2000) conducted its Turkish validity and reliability study. In this study, alpha was 0.84 (Ertan & Eker, 2000).

Statistical Analyses

The data were analyzed using the Statistical Package for the Social Sciences IBM SPSS Corp., Armonk, NY, USA package program in a computer environment. The compliance of the data to normal distribution was examined using the Shapiro Wilk test. Kruskal Wallis and Mann Whitney U tests were used to compare data that did not conform to normal distribution. Spearman correlation analysis was used to examine the relationship between the sub-dimensions of the scales. Reliability analysis studies were done with Cronbach Alpha. $p < .05$ was considered significant.

Results

It was determined that 69.0 % of the patients were aged between 65-74 years, 54.5% were female, illiterate 46.5%, 84.0% were married. In 67.0% the income=expenditure, 83.5% were unemployed, 47.0% were living in a village, 58.5% were living with spouse, 77.5% were diagnosis duration of 11 years and above, 87.5% were regularly blood glucose, 82.5% had presence of having another disease, 82% had cardiovascular complications of diabetes, 55.0% HbA1c level; %5-10. Elderly diagnosis duration was 7.46±7.30 years and HbA1c level were 8.40±1.63 (Table 1). Regimen related distress subscale mean score; It was found to be higher and statistically significant in the elderly who were single, unemployed, living in the village, diagnosed with diabetes for more than 11 years ($p < .05$). Physician-related distress subscale mean score; high in illiterated and unemployed elderly and statistically significant ($p < .05$). Diabetes-related interpersonal distress subscale mean score; It was found to be higher and statistically significant in the elderly who are in the 75-84 age group, who are illiterate and live alone ($p < .05$). (Table 2). DDS and GDS mean scores; high school and above, diabetes-related complications and HbA1c levels more than 11% were found to be higher in the elderly who did not work, who had been diagnosed with diabetes for more than 11 years, who did not regularly measure flank glucose levels, and were statistically significant ($p < .05$). (Table 3). When the distribution of the scale mean scores was examined, the mean score of DDS subscales; emotional burden was determined to (1.31±0.35) moderate level. Regimen distress was determined to (1.62±0.42), Physician distress was determined to (1.45±0.44) and interpersonal distress was determined to (1.11±0.33) high distress. The mean score of total DDS was (5.51±1.09) high distress and the obtained mean score mean score of GDS 16.10±6.34 was high depression. It was determined that as the total mean score of DDS, emotional burden distress and regimen related distress levels increased, depression level increased. A statistically significant and a weak positive correlation was found between these scales ($p < 001$) (Table 5).

Table 1. Socio-demographic characteristics of elderly (n=200)

Variables	Number	%
Age		
65-74	138	69.0
75-84	48	24.0
85 and over	14	7.0
Gender		
Female	109	54.5
Male	91	45.5
Educational Status		
Illiterate	93	46.5
Literate	49	24.5
Primary school	42	21.0
High school and above	16	8.0
Marital Status		
Married	168	84.0
Single	32	16.0

Table 1. Socio-demographic characteristics of elderly (n=200) (Continue)

Variables	Number	%
Perceived level of income		
Income > expenditure	16	8.0
Income = expenditure	134	67.0
Income < expenditure	50	25.0
Work Status		
Working	33	16.5
Unemployed	197	83.5
Patient's residence		
Province	40	20.0
District	66	33.0
Village	94	47.0
Living arrangement		
Alone	19	9.5
Living with a spouse	117	58.5
Living with children/relatives	56	28.0
Other	8	4.0
Diagnosis duration		
1-5 years	15	7.5
6-10 years	30	15.0
>11 years	155	77.5
Regularly blood glucose checking status		
Yes	175	87.5
No	25	12.5
Presence of having another disease		
Yes	165	82.5
No	35	17.5
***Diabetes-related complications**		
Serebro-vascular diseases	152	76.0
Cardiovascular diseases	164	82.0
Peripheral diseases	112	56.0
Diagnosis duration		
1-5 years	15	7.5
6-10 years	45	22.5
>11 years	130	70.0
Total HbA1c level		
<5	20	10.0
5-10	110	55.0
>10	70	35.0

Diabetes characteristics of elderly	Min	Max	Mean	SD
Diagnosis duration (year)	2	50	7.46	7.30
HbA1c	5	15.60	8.40	1.63

Table 2. Socio-demographic characteristics by DDS and GDS scores

Diabetes Distress Scale	Emotional burden Distress mean±SD	Regimen related Distress mean±SD	Physician-related Distress mean±SD	Diabetes-related interpersonal Distress mean±SD	DDS Mean±SD	GDS Mean±SD
Age						
65-74	13.45±3.64	16.26±4.28	14.66±4.48	10.38±3.24	55.77±11.60	15.55±6.05
75-84	12.66±2.91	15.75±4.13	14.37±4.13	12.16±3.49	52.95±7.21	17.08±7.32
85 and over	12.42±3.20	17.28±4.45	14.42±5.43	10.85±3.89	56.00±14.82	18.14±4.84
Test and p	KW:2.067, *p > .05*	KW: 3.251, *p > .05*	KW: 0.706, *p > .05*	KW:9.244, *p < .05*	KW: 3.975, *p > .05*	KW: 3.877, *p > .05*
Gender						
Female	13.01±3.66	15.81±4.17	14.53±4.37	11.09±3.55	57.45±11.70	17.46±6.34
Male	13.40±3.43	16.69±4.34	14.63±4.56	11.16±3.18	54.90±10.08	14.46±5.97
Test and p	t:0.767, *p > .05*	t:0.451, *p > .05*	t:0.166, *p > .05*	t:0.152, *p > .05*	t:0.924, *p > .05*	t:3.428, *p < .001*
Educational Status						
Illiterate	13.08±3.71	16.34±4.67	15.00±4.65	12.06±3.51	54.49 ±11.83	17.52±6.54
Literate	13.06±2.60	16.10±3.84	13.73±.76	10.57±2.76	54.46±7.93	15.02±5.81
Primary school	14.28±3.54	16.09±3.49	13.19±3.93	11.42±3.59	54.00±9.61	14.85±5.95
High school and above	11.37±4.52	16.12±5.12	11.87±6.11	10.37±3.64	57.75±16.63	14.37±6.46
Test and p	KW:6.952, ***p < .05***	KW:0.846, *p > .05*	KW:4.100, *p < .05*	KW:5.628, *p < .05*	KW:2.310, *p < .05*	KW:11.659, *p < .001*
Marital Status						
Married	13.14±3.36	15.43±4.14	14.69±4.33	10.75±3.34	55.39±10.82	15.66±6.10
Single	13.43±4.51	16.36±4.34	14.00±5.05	11.16±3.18	53.62±11.91	18.37±7.12
	t:0.420, *p > .05*	t:1.127, ***p < .05***	t:0.803, *p > .05*	t:0.683, *p > .05*	t:0.836, *p > .05*	t:2.237, *p < .001*
Perceived level of income						
Income > expenditure	11.08±3.71	16.34±4.67	14.00±4.65	11.06±3.51	54.49±11.83	14.52±6.54
Income = expenditure	11.06±2.60	16.10±3.84	14.73±4.76	10.57±2.76	54.46±7.93	15.02±5.81
Income < expenditure	14.28±3.54	16.09±4.49	15.19±5.43	11.42±3.59	57.00±9.61	17.85±5.95
	F:2.837, ***p < .001***	F: 0.053, *p > .05*	F: 1.265, *p > .05*	F:1.265, *p > .05*	F: 0.581, *p > .05*	F: 3.069, *p < .001*
Work Status						
Working	13.16±3.82	15.00±3.52	13.16±3.67	10.58±3.64	51.90±9.53	13.67±8.87
Unemployed	17.13±3.52	16.39±4.36	14.85±4.56	11.21±3.35	55.62±11.21	16.76±6.17
	t:1.499, ***p < .05***	t:1.680, ***p < .05***	t:1.943, ***p < .05***	t:0.955, *p > .05*	t:1.736, ***p < .001***	t:2.537, ***p < .001***
Elderly's residence						
Province	13.35±3.83	17.30±4.68	15.30±5.12	11.60±3.47	57.55±12.27	14.90±6.17
District	12.95±3.07	15.46±3.30	14.69±4.38	11.13±3.25	54.25±10.39	15.66±6.33
Village	13.29±3.77	16.27±4.43	14.19±4.19	10.91±3.55	54.68±10.80	16.91±6.61
	F:0.226, *p > .05*	F: 2.346, ***p < .001***	F: 0.902, *p > .05*	F:0.574, *p > .05*	F: 1.258, *p > .05*	F: 1.685, *p > .05*

Table 2. Socio-demographic characteristics by DDS and GDS scores (Continue)

Diabetes Distress Scale	Emotional burden Distress mean±SD	Regimen related Distress mean±SD	Physician-related Distress mean±SD	Diabetes-related interpersonal Distress mean±SD	DDS Mean±SD	GDS Mean±SD
Living arrangement						
Alone	12.78±1.98	14.94±2.69	15.15±3.56	12.15±2.63	55.05±6.05	17.26±6.27
Living with spouse	13.23±3.39	16.17±4.28	14.52±3.97	10.68±3.33	54.62±10.23	15.43±6.07
Living with children/relatives	13.53±3.78	18.50±8.36	14.57±5.16	11.89±3.56	56.39±11.62	17.17±6.72
Other	11.25±6.34	16.39±3.78	14.00±7.63	9.75±3.15	53.50±22.90	15.50±7.34
	KW:1.050, *p > .05*	KW: 1.369, *p > .05*	KW: 0.155, *p > .05*	KW:2.727, ***p < .05***	KW: 0.384, *p > .05*	KW: 1.209, *p > .05*

t: Independent Sample t test, KW: Kruskal-Wallis H, F: ANOVA, *p < .05*, DDS: Diabetic Distress Scale, GDS: Geriatric Depression Scale, SD: Standart Deviation

Table 3. Diabetes characteristics of elderly by DDS and GDS scores

Diabetes Distress Scale	Emotional burden Distress Mean±SD	Regimen related Distress Mean±SD	Physician-related Distress Mean±SD	Diabetes-related interpersonal Distress Mean±SD	DDS Mean±SD	GDS Mean±SD
Diagnosis duration						
1-5 years	11.25±3.63	11.14±3.20	12.02±3.20	10.20±3.12	52.30±9.45	15.12±6.35
6-10 years	12.47±2.14	12.46±3.66	13.32±3.46	10.09±3.22	53.17±7.32	15.42±6.56
>11 years	13.66±2.20	17.85±4.17	13.23±3.31	11.42±3.07	57.85±9.78	18.58±6.48
	KW:1.478	KW: 4.452	KW: 0.752	KW:0.364	KW: 5.420	KW: 4.765
	p > .05	***p < .05***	*p > .05*	*p > .05*	***p < .05***	***p < .05***
Regularly blood glucose checking status	13.12±3.41	15.42±4.65	13.36±3.24	11.45±3.28	51.12±8.45	17.54±5.94
Yes	13.20±3.08	15.24±4.28	13.47±3.46	11.36±3.07	57.69±9.05	13.18±6.12
No	MWU:0.652, *p > .05*	MWU:0.563, *p > .05*	MWU:0.166, *p > .05*	MWU:0.245, *p > .05*	MWU:4.948, ***p < .001***	MWU:3.428 ***p < .001***
Diabetes-related complications	12.46±3.13	15.42±4.52	12.24±4.32	10.57±3.87	57.88±9.43	18.12±6.14
Yes	12.65±3.43	15.74±4.07	12.88±4.10	11.50±3.6	51.06±6.41	14.23±5.24
No	t:0.654, *p > .05*	t:0.346, *p > .05*	t:0.240, *p > .05*	t:0.134, *p*>0.05	t:2.474, ***p < .001***	t:2.289, ***p < .001***
Total HbA1c level						
<5	12.17±2.35	14.25±3.32	14.89±5.23	11.78±3.72	51.11±6.09	13.15±5.22
5-10	13.63±3.14	15.63±4.65	14.23±5.18	12.35±2.65	53.25±6.35	13.43±6.18
>10	13.78±3.25	15.74±4.12	14.64±5.64	12.41±3.88	57.88±10.74	17.89±7.72
	KW:0.150	KW: 0.367	KW: 0.089	KW:0.328	KW: 11.841	KW: 10.189
	p > .05	*p > .05*	*p > .05*	*p > .05*	***p < .001***	***p < .001***

t: Independent Sample t test, KW: Kruskal-Wallis H, F: ANOVA, *p* < .05, DDS: Diabetic Distress Scale, GDS: Geriatric Depression Scale, SD: Standart Deviation

Table 4. Elderly's DDS and GDS score averages

DDS	Mean±SD	Min-Max
DDS- Emotional Burden	1.31±0.35	0.6-2.2
DDS - Regimen Distress	1.62±0.42	0.6-2.9
DDS – Physician Distress	1.45±0.44	0.5-2.4
DDS - Interpersonal Distress	1.11±0.33	0.4-1.8
DDS -Total	5.51±1.09	2.7-5.8
GDS	16.10±6.34	2.00-30.00

DDS: Diabetic Distress Scale, GDS: Geriatric Depression Scale, SD: Standart Deviation, Min: Minimum, Max: Maximum

Table 5. Correlation between elderly's DDS and GDS score averages

		GDS
DDS	r	0.274
	p	***p < .001***

* r: Sperman Correlation Coefficient

Discussion

This study was conducted to examine the relationship between diabetes-related stress and depression in the elderly with type 2 DM. It was determined that patients had high diabetes-related stress levels and moderate depression levels. As geriatric depression increased, the stress experienced by the patients also increased.

The domain deficit score (DDS) emotional burden distress subscale mean score of elderly diabetes patients included in this study was found to be higher in illiterate, low income, and unemployed elderly. Similarly, a study that the psychosocial adjustment of diabetic patients with low education, low income, and unemployment was low (Çaklılı et al., 2020). Moreover, Naskar et al. (2017) determined that individuals with diabetes who have low income and educational level have problems psychologically adapting to the disease (Naskat et al.,2017). The literature stated that having a regular and sufficient income is an important factor in developing healthy living behaviors and psychosocial adaptation in patients (Polonsky et al., 2005; Çaklılı et al., 2020; Yesavage et al., 1983; Ertan & Eker; 2000; Naskar et al., 2017). The necessity of the existence and competence of material and spiritual resources about the disease so that the sick individual can adapt to the disease (Tahanakwang et al., 2014). Previous studies showed that low-income individuals lack adequate support and resources to effectively manage diabetes (Tahanacwang et al., 2014; Vu et al., 2018; Polonsky et al., 2005; Çaklılı et al., 2020). Moreover, the emotional burden is more vulnerable in the psychological health of diabetic patients. Living with illness requires rigorous management including taking medicines, obligations with regards to diet, physical activity, and heedful monitoring to control glucose/insulin level in the body. Thus, patients get frustrated and feel overwhelmed by emotional burden (Kocaman et al., 2007; Tahanacwang et al., 2014; Vu et al., 2018; Polonsky et al., 2005; Çaklılı et al., 2020).

The regimen-related distress subscale mean score was found to be higher and statistically significant in the single, unemployed, village-dwelling elderly people diagnosed with diabetes >10 years. Sidhu et al. (2017) and Ell et al. (2015) found that the level of regimen-related distress is high in elderly people who have received diabetes treatment for a long time, depending on the stemming of concerns about diet, physical activity, and medication (Sidhu et al., 2017; Ell et al., 2015). Also, when the literature is examined, Kasteleyn et al. (2015) stated that the problems related to diabetes increase as the duration of diabetes increases (Kasteleyn et al., 2015; Hessler et al., 2015). Furthermore, a study found that as the duration of diabetes increases, the adherence to treatment of chronic diseases due to diabetes decreases (Rasmussen et al., 2011). In this study, the social support needs of the elderly also increased due to the presence of other different chronic diseases other than diabetes. Therefore, the average scores of the distress related to the treatment regimen and compliance of the single and unemployed elderly are considered high.

The physician-related distress subscale mean score was found to be higher in illiterate and nonworking elderly patients and was statistically significant. Similarly, Sidhu et al. (2017) and Hessler et al. (2015) found that diabetic patients do not attend regular medical visits and are not conscious enough of their disease (Sidhu et al., 2017; Hessler et al., 2015). Tudies found that patients' follow-up compliance was insufficient in their studies with diabetic patients (Knech et al., 2011; Naskar et al., 2017). Moreover, another study diabetes individuals were inadequately compliant with follow-up, diet, and treatment (Siddiqui, 2014). The fact that the patients are elderly and have low education levels can be considered as the reason for this situation.

The diabetes-related interpersonal distress subscale mean score was found to be higher and statistically significant in the elderly in the 75–84 age group, with high school education and above, and living alone. In chronic diseases, support from the family and the environment is an important resource in coping and adapting to the disease (Rasmussen, 2011). The literature stated that the individual's mental state and social life are also negatively affected depending on the physiopathological changes that occur due to diabetes (Sidhu et al., 2017; Türten Kaymaz & Akdemir, 2016; Ell et al., 2015; Kastaleyn et al., 2015; Hessler et al., 2015). The study conducted by Kocaman et al. (2007) with individuals with chronic diseases determined that the most affected areas were professional life and social environment (Kocaman et al., 2007). Many studies, the area where the best adaptation to diabetes was observed was associated with the extended family (Ell et al., 2015; Kastaleyn et al., 2015; Tahanacwang et al., 2014). Elderly who have family communication and support positively affect adaptation to the disease and social life. Lack of family support, an important element of social support in the elderly living alone, causes the elderly to be unable to cope with illnesses, increase illness-specific problems, and reduce the quality of life by causing adjustment disorders and psychosocial problems (Siddiqui et al.,

2014). Furthermore, a study found higher mean depression scores and lower physical and mental subdimensions of quality of life in elderly people living alone (Chin et al., 2017). Following the literature, the high average score for distress due to interpersonal relationships is thought to be that the 75–84 age group experiences complications due to diabetes, their social support is insufficient because they live alone, and their adaptation to lifestyle changes and illness is low (Adakan et al., 2017; Aljohani et al., 2021).

DDS and GDS mean scores of high school education and above were found to be higher in elderly people who did not work, who had been diagnosed with diabetes for >11 years, who did not regularly measure blood glucose levels, and who have diabetes-related complications and HbA1c levels >11. This study determined that they experienced at least one complication due to diabetes and 82%, at most, had cardiovascular complications of diabetes.

A study that individuals with high educational status and those who do not work have a low adaptation to diabetes (Chin et al., 2017). Moreover, studies have reported that depression levels increase in the elderly who develop complications due to diabetes (Rasmussen et al., 2011; Mazanec et al., 2011; Kocaman et al., 2007; Tahanacwang et al., 2014; Vu et al., 2014; Polonsky et al., 2005).

A study found that individuals with complications due to diabetes have low quality of life and high levels of depression and distress (Adakan et al., 2017). Furthermore, studies found that depression and disability significantly increased as the rate of complications due to diabetes increased (Kocaman et al., 2007; Tahanacwang et al., 2014; Vu et al., 2014; Polonsky et al., 2005, Çaklılı et al., 2020; Yesavege et al., 1983).

Similarly, a study stated that as the duration of diabetes increases, complications associated with diabetes and accordingly stress levels increase (Kastaleyn et al., 2015). Vu et al. (2014) found that elderly people with a high level of education, who have been diagnosed with diabetes for a long time, have an irregular blood glucose level, and have high levels of diabetes-related complications and HbA1c also have a high level of depression (Vu et al., 2014). Chin et al. (2017) found that the depressive symptoms of elderly diabetic patients who were single and diagnosed with diabetes for >10 years were identified to be associated with diabetes control and had significantly higher plasma glucose and HbA1c concentration (Chin et al., 2017).

GDS average score; female, singles were found to be higher in the elderly with low income and statistically significant ($p < .05$)

Studies observed that female patients had difficulties psychologically adjusting to diabetes and women with diabetes have low psychological adjustment levels (Naskar et al., 2017; Chin et al., 2017; Adakan et al., 2017). A study found that female, single, and low-income diabetic patients have high levels of depression (Adakan et al., 2017). Furthermore, Vu et al. found that the levels of depression are high in elderly diabetes patients who are women and single (Vu et al., 2014).

When the distribution of the scale mean scores was examined, the mean score of the DDS emotional burden distress subscale was 13.19 ± 3.55 (moderate level). Moreover, regimen-related, physician-related, and diabetes-related interpersonal distress were determined as 16.21 ± 4.26, 14.58 ± 4.45, and 11.12 ± 3.38 (high distress). The mean score of total DDS was 55.11 ± 10.99 (high distress) and the obtained mean GDS score was 16.10 ± 6.34 (high depression).

The total mean score of DDS, emotional burden distress, regimen-related distress, and depression levels increased. Moreover, a statistically significant and positive correlation was found between these scales. A positive and statistically significant relationship was found between DDS and depression scale. Similar to our study, depression and distress increased statistically significantly in Chin et al. (2017) study. Elderly diabetic patients who have received insulin and diet therapy for a long time have high levels of stress and depression (Chin et al., 2017; Adakan et al., 2017). Studies also emphasized that insulin-treated patients may more frequently experience some negative emotions (e.g., hopelessness, dissatisfaction, and feeling punished) compared with patients who received oral drug regimens (Chin et al., 2017; Adakan et al., 2017; Katon et al., 2004; Bai et al., 2018). Furthermore, determined that depressive symptom and diabetes-related stress levels are high in elderly diabetic patients (Katon et al., 2004). Sidhu et al., (2017) diabetes distress, and depression were positively associated. Conversely, emotional burden and regimen-related distress subscales are the strongest correlates (Sidhu et al., 2017). In addition, Hessler et al. (2015) also identified a bidirectional association between regimen-related distress and depression (Hessler et al., 2015). This significant relationship between diabetes stress and depression experienced by patients may be caused by the patients' low level of education and their inability to control the disease due to non-compliance with the treatment regimen

Conclusion

In conclusion, the prevalence of diabetes-related distress and depression among patients with type 2 diabetes mellitus was high in this study. The elderly with high school and above have been found to have high levels of depression and distress in elderly people who do not work, have been diagnosed with diabetes for more than 11 years, do not measure their blood glucose level regularly, and have Diabetes-related complications and HbA1c levels >11. When the distribution of the scale mean scores was examined, the mean score of DDS subscales; Emotional Burden Distress was determined to moderate level. Regimen-Related Distress, Physician-Related Distress and Diabetes-Related Interpersonal Distress were determined to high level. The mean score of Total DDS was high distress and the obtained mean score mean score of GDS was high depression. It was determined that as the total mean score of DDS, Emotional burden Distress and Regimen related Distress levels increased, depression level increased. A statistically significant and positive correlation was found between these scales.

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Familial Hypercholesterolemia: Evaluating CRP/Albumin Ratio and Blood Count Parameters

Ailesel Hiperkolesterolemi: Crp/Albümin Oranı ve Kan Sayımı Parametrelerinin Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to evaluate the CRP-to-albumin ratio (CAR) and complete blood count parameters in patients with familial hypercholesterolemia (FH).

Methods: A retrospective study included 101 patients (61 female) and 35 healthy controls (18 female) who visited our hospital from January 2015 to June 2018. Serum levels of total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), albumin, and CRP were measured using the Hitachi 917 biochemistry analyzer. Complete blood count was performed with the Abbott CELL-DYN Ruby® hematology analyzer. Statistical analysis was conducted with SPSS 27.

Results: The FH patients had significantly higher median values of TC (328 mg/dL), TG (218 mg/dL), LDL-C (269 mg/dL), CRP (5.6 mg/dL), WBC (8.3×10^3/uL), NEU (4.9×10^3/uL), PLT (286×10^3/uL), and CAR (0.128), compared to the healthy controls. Significant differences were found between the two groups in TC, TG, LDL-C, albumin, CRP, CAR, WBC, NEU, LYM, and PLT ($p<.05$). The area under the curve (AUC) for CAR was 0.715, indicating its potential to distinguish FH patients from healthy controls (95% Confidence Interval: 0.626-0.824).

Conclusion: CAR is a valuable inflammatory marker for diagnosing and monitoring familial hypercholesterolemia, showing significant differences between FH patients and healthy controls.

Keywords: CRP-to-albumin ratio, familial hypercholesterolemia, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio

ÖZ

Amaç: Bu çalışmanın amacı, ailesel hiperkolesterolemili (FH) hastalarda CRP/albümin oranı (CAR) ve tam kan sayımı parametrelerini değerlendirmektir.

Yöntem: Ocak 2015 ile Haziran 2018 arasında hastanemize başvuran 101 hasta (61 kadın) ve 35 sağlıklı kontrol (18 kadın) üzerinde retrospektif bir çalışma yapıldı. Hastaların serumundaki toplam kolesterol (TC), trigliseritler (TG), LDL-kolesterol (LDL-C), HDL-kolesterol (HDL-C), albümin ve CRP düzeyleri Hitachi 917 biyokimya analizörü kullanılarak ölçüldü. Tam kan sayımı Abbott CELL-DYN Ruby® hematoloji analizörü ile gerçekleştirildi. İstatistiksel analiz SPSS 27 programı kullanılarak yapıldı.

Bulgular: FH hastalarında, sağlıklı kontrollere kıyasla TC (328 mg/dL), TG (218 mg/dL), LDL-C (269 mg/dL), CRP (5.6 mg/dL), WBC (8.3×10^3/uL), NEU (4.9×10^3/uL), PLT (286×10^3/uL) ve CAR (0.128) medyan değerleri anlamlı derecede yüksekti. TC, TG, LDL-C, albümin, CRP, CAR, WBC, NEU, LYM ve PLT değerleri açısından iki grup arasında anlamlı farklar bulundu ($p<.05$). CAR' ın eğri altındaki alan (AUC) değeri 0.715 olarak hesaplandı, bu da CAR' ın FH hastalarını sağlıklı kontrollerden ayırt etme potansiyelini gösterdi (Güven Aralığı: %95, 0.626-0.824).

Sonuç: CAR, ailesel hiperkolesterolemi tanı ve takibinde değerli bir inflamatuar belirteç olup, FH hastaları ile sağlıklı kontroller arasında önemli farklılıklar göstermektedir.

Anahtar Kelimeler: Ailesel hiperkolesterolemi, CRP/albümin oranı, nötrofil/lenfosit oranı, trombosit/lenfosit oranı

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Introduction

Familial hypercholesterolemia (FH) represents an autosomal dominant hereditary anomaly related to lipid metabolism. Increased plasma concentrations of low-density lipoprotein cholesterol (LDL-C) disrupt vascularization and subsequently promote the untimely onset of coronary artery disease (CAD). High LDL-C levels also increase the susceptibility to heart disease by 2.5 to 10 times. The factor most likely to cause increased mortality associated with FH is cardiovascular disease (CVD). FH often occurs together with metabolic disorders such as hypercholesterolemia, insulin resistance, diabetes mellitus, and obesity which serve as possible predisposing factors for inflammatory pathologies that underlie atherosclerotic lesions. Atherosclerosis is a chronic immune inflammatory disease, characterized by a protracted and inflammatory process, that impairs vascular physiology and is an important precursor to various CVDs such as myocardial infarction and strokes (Falk, 2006; Hansson, 2005). It is also an important common disease cause of global mortality. Multifaceted interactions of genetic and environmental determinants are recognized to be involved in its etiology. Major risk factors leading to atherosclerosis are hypercholesterolemia, hypertension, diabetes mellitus, smoking, and adiposity [high body mass index (BMI)—measured as weight (in kg) divided by squared height (in m2)] according to the 2021 European Society of Cardiology Guidelines on cardiovascular disease prevention in clinical practice (Ross, 1999). These factors influence the formation of atherosclerosis and pathogenic blood flow through complex interactions. The onset of inflammation is initiated by the infiltration of cholesterol-laden lipoproteins into the vascular intima and the subsequent triggering of the secretion of proinflammatory cytokines by the sustained activation of macrophages. In addition, these cytokines spark the hepatic synthesis of inflammatory proteins such as C-reactive protein (CRP). Consequently, the persistence of chronic vascular inflammation is deemed paramount in this context (Ikonomidis et al., 1999, Hirschfield, 2003).

CRP, one of the proteins called acute phase reactants produced by the liver and fat cells, is an indicator of inflammatory processes, enabling a direct quantification of the magnitude of the inflammatory response. Recently, the high-sensitivity (hs)-CRP test has been used as an inflammation biomarker and gained recognition as a significant cardiovascular risk determinant (Visseren et al., 2021). Albumin, another hepatocyte-synthesized protein, assumes a pivotal role in upholding blood colloidal osmotic pressure, facilitating diverse molecular transport, and exhibiting antioxidant properties. Low albumin concentrations may indicate malnutrition or malabsorption, liver failure or diseases, renal impairment, and some inflammatory and infectious diseases. Many studies have demonstrated a robust inverse correlation between serum albumin levels and CRP concentrations (Sheinenzon et al., 2021, Eckart et al., 2020). The CRP-to-albumin ratio (CAR) is derived by dividing the CRP concentration (mg/L) by the albumin concentration (g/L). High CARs, signify an underlying disequilibrium between inflammatory processes (reflected by CRP levels) and nutritional status (reflected by albumin levels), prominently manifesting in cases characterized by severe or prolonged inflammation. While the magnitude of this ratio may exhibit inter-individual variability, it has been identified as a promising prognostic indicator across a spectrum of serious medical pathologies, encompassing septic conditions (Günes et al., 2021), cardiovascular diseases, neoplastic disorders (Arakawa et al., 2021, Ishizuka et al., 2016, Kinoshita et al., 2015) and acute kidney injury (Yu et al., 2020). For example, according to a retrospective study, significant associations were found between the elevated CAR and mortality rates at presentation to the emergency department, especially in individuals over 65 years of age (Park et al., 2018).

The amount of circulating LDL-C plays a role in the regulation of T and B lymphocytes, which have important roles in the innate immune response. In particular, an increase in the amount of oxidized LDL-C decreases the number of regulatory T lymphocytes, which are key atheroprotective cells. As a result, the numbers of T helper 1 and T helper 2 increase, resulting in increased secretion of proinflammatory cytokines and atherosclerotic inflammation (Ait-Oufella et al., 2006). Under hypercholesterolemic conditions, an increase in the number of circulating neutrophils and constitutively active neutrophils may be observed (Farah et al., 2010). In addition, serum levels of neutrophil chemoattractants are increased by hypercholesterolemia, thus increasing neutrophil mobilization. Neutrophilia in FH is associated with increased degranulation and the release of large amounts of cytotoxic and destructive factors that can cause vascular tissue destruction. For example, neutrophils produce large amounts of reactive oxygen radicals via myeloperoxidase and NADPH oxidase, which are involved in the formation of oxidized LDL-C, impairing the performance of endothelial cells and leading to endothelial dysfunction (Malle et al., 2006).

Neutrophil to lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) are considered to be predictive parameters of systemic inflammation and are also some of the routine tests used to predict cardiovascular and peripheral vascular diseases (Condado et al., 2016). PLR is predicted as a new predictor of in-hospital mortality in patients in the cardiac intensive care unit. Patients with higher PLR levels have been observed to be associated with cardiac diseases such as congestive heart failure, arrhythmias, atrial fibrillation, and valvular disease (Zhai et al., 2021). When people with angiographically confirmed coronary occlusion were compared with healthy young individuals, a significant association between decreased HDL-cholesterol (HDL-C) levels

and increased PLR levels was reported (Tok et al., 2014). The principal objective of this investigation was to evaluate the CAR and complete blood count parameters in patients diagnosed with FH.

Methods

This retrospective study included a total of 136 participants, 101 patients with FH aged ≥18 years (61 female) and 35 healthy controls with LDL-C<160 mg/dL (18 female) using an electronic database of individuals admitted to Erzurum Regional Training and Research Hospital between January 2015 and July 2018. All procedures involving participants and data were in accordance with the revised Helsinki Declaration of 2000 and the study was approved by Erzurum Regional Training and Research Hospital, Medical Ethics Committee (approval date: 08.11.2018 and number: 2018/17-160). The patients were diagnosed with FH according to recommendations of the Dutch Lipid Clinic Network after a comprehensive physical examination and an extensive evaluation of their family medical history and laboratory results. Then, the patients with FH were divided into three subgroups according to LDL-C levels: mildly increased LDL-C (G3, [N=30]: LDL-C 190–249 mg/dL); moderately increased LDL-C (G2, [N=37]: LDL-C 250–329 mg/dL); and severely increased LDL-C (G1, [N=34]: LDL-C >330 mg/dL) and, each group compared with healthy ([N=35]: LDL-C 48-129 mg/dL). The patients with secondary hyperlipidemia due to diabetes, hypothyroidism, hyperthyroidism, acute or chronic renal failure, adrenal disorders, chronic liver disorders, malignancies, inflammatory diseases, and acute or chronic infection diseases were excluded from the study.

Venous blood samples were taken from patients in the morning, after 8-12 hours of fasting, into gel vacuum tubes (Vacusera, Turkey) for biochemistry and hematology testing. Total cholesterol (TC), triglyceride (TG), LDL-C, HDL-C, and albumin levels in the serum of the patients were measured using the enzymatic photometric method and hs CRP was measured using the immunonephelometric method on the Hitachi 917 biochemistry analyzer (Boehringer Mannheim, USA). Complete blood count was measured by the CELL-DYN Ruby® hematology analyzer (Abbott Diagnostics, USA). Then, the CAR, NLR, and PLR were calculated based on these count measurements. NLR is calculated by dividing the absolute neutrophil count by the absolute lymphocyte count and PLR is calculated by dividing the absolute platelet count by the absolute lymphocyte count. The two-level (normal and pathologic) internal quality control materials provided by kit manufacturers (Bio-Rad, Hercules, CA, USA) were routinely analyzed once a day, and the one-level external quality control program materials (Bio-Rad, Hercules, CA, USA) were analyzed monthly. All the results were acceptable during the study.

Statistical Analysis

All data were analyzed using the IBM SPSS© Statistics (version 27.0) program (SPSS, Chicago, USA). The normal distribution of continuous variables was assessed through the Kolmogorov-Smirnov test. For comparing control and patients' independent groups, the independent samples t-test was employed under conditions of normal distribution, while the Mann–Whitney U test was applied when normal distribution assumptions were not met. Where the parametric test assumptions were met, the one-way ANOVA test was used; otherwise, the Kruskal–Wallis test was used to compare differences in clinical-biochemical parameters between the subgroups. Comparative analyses concerning categorical variables were performed using the chi-square test and, where applicable, Fisher's exact test. Continuous variables were expressed as mean ± standard deviation (SD) or medians and the 1st and 3rd quartiles (Q1–Q3), and categorical variables as frequencies and percentages. The correlations between two continuous variables were assessed using the Pearson correlation test when normal distribution criteria were satisfied, and the Spearman correlation test was utilized when such conditions were not met. Linear regression analysis was employed to investigate the association between numerical variables. The Receiver Operating Characteristic (ROC) curve analysis was implemented to ascertain the diagnostic utility of continuous variables. The Area Under the Curve (AUC) value shows the overall discriminatory ability of the CAR. The AUC value of 1 indicates perfect discrimination, while the AUC value of 0.5 suggests no discrimination (equivalent to random guessing). The results (except age) are shown as mean ± standard deviation for parameters with normal distribution, and as median and 1st–3rd quartiles for parameters not showing normal distribution. Ages are shown as median and minimum–maximum. The differences between groups were considered significant if the p-value was less than .05 (two-tailed).

Results

A total of 136 individuals over 18 years old participated in our study, the control group consisted of 35 [39±14 (18–68) years] individuals, and the patient group consisted of 101 [40±14 (18–77) years] individuals diagnosed with FH. Of these, 50% of the participants were female (n= 18) in the control group, whereas 61% of FH patients were female (n= 61). There were no statistically significant differences between both groups in gender and age (p>.05). The median and Q1–Q3 or mean ± standard deviation values of TC (mg/dL), TG (mg/dL), LDL-C (mg/dL), HDL-C (mg/dL), Albumin (mg/dL), CRP (mg/dL), WBC, neutrophil (NEU), lymphocyte (LYM), platelet (PLT), CAR, NLR, PLR were 328 (274–398), 218 (171–368), 269 (229–340), 48 (41–58), 43 (38–45), 5.6 (1.6–10), 8.3 ± 1.94.9 ± 1.7, 2.57 ± 0.9, 286 (236–331), 0.128 (0.043–0.244), 1.87 (1.21–2.62), 113 (87–148) for FH patients and161±23, 84±37, 95±20, 47 (42–58), 45±2.6, 2 (1–3), 6.5 ± 1.2, 3.7±1, 2.21±0.49, 223±50, 0.044 (0.042–0.051), 1.58 (1.21–2.12), 104±28for healthy controls, respectively. There were significant differences between two groups in TC, TG, LDL-C, AL, CRP, CAR, WBC, NEU, LYM, and PLT (p<.05). The characteristics, biochemical and hematological results for the patients with FH and controls are seen in Table 1, and for subgroups in Table 2. CAR was the only parameter among all the investigated that showed significant differences in pair wise comparisons between all groups (p<.05). The medians of the CAR in the control and patients subgroups (G1, G2, or G3) are seen in Figure 1.

Table 1. The characteristics, biochemical, and hematological results of the patients with familial hypercholesterolemia and healthy controls

	Control (N=35)	FH (N=101)	P-value
Age (years)	39±14 (18–68)	40±14(18–77)	NS
Gender (Female: N, % /Male: N, %)	18.51 / 17.49	61.60 / 40.40	NS
TC (mg/dL)	161±23	328 (274–398)	.001*
TG (mg/dL)	84±37	218 (171–368)	.001*
LDL-C(mg/dL)	95±20	269 (229–340)	.001*
HDL-C(mg/dL)	47 (42–58)	48 (41–58)	NS
ALB (g/dL)	45±2.6	43 (38–45)	.001*
CRP (mg/L)	2 (1–3)	5.6 (1.6–10)	.002*
WBC (K/µL)	6.5 ± 1.2	8.3 ± 1.9	.001*
NEU (K/µL)	3.7±1	4.9 ± 1.7	.001*
LYM (K/µL)	2.21±0.49	2.57 ± 0.9	.03*
PLT (K/µL)	223±50	286 (236–331)	.001*
CAR	0.044 (0.042–0.051)	0.128 (0.043–0.244)	.001*
NLR	1.58 (1.21–2.12)	1.87 (1.21–2.62)	NS
PLR	104±28	113 (87–148)	NS

Note: FH, familial hypercholesterolemia; CRP, c-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; WBC, white blood cells; NEU, neutrophil; LYM, lymphocyte; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CAR, CRP-to-albumin ratio; NS, non-significant. The results (except age) are shown as mean ± standard deviation for parameters with normal distribution or as median and 1st–3rd quartiles for parameters not showing normal distribution. Ages are shown as median and minimum–maximum.*The differences between groups were considered significant if the p-value was less than .05 (two-tailed).

Table 2. The characteristics, biochemical, and hematological results of the subgroups of the patients with familial hypercholesterolemia and healthy controls

Parameters		Control (N=35)	G1 (N=34)	G2 (N=37)	G3 (N=30)	P value
Age (years)		39±14 (18–68)	47±15 (18–77)	52±10 (24–59)[ab]	25±4 (20–44)[abc]	.001[a]
Gender	Male (n,%)	17 (% 49)	14 (% 42)	11 (% 30)	15 (% 50)	NS
	Female (n,%)	18 (% 51)	20 (% 58)	26 (% 70)	15 (% 50)	
TC (mg/dL)		161±23	417 (392–481)[a]	325 (307–346)[b]	258 (254–275)[ab]	.001[a]
TG (mg/dL)		84±37	468 (260–683)[a]	206 (160–284)[ab]	218±81[ab]	.001[a]
LDL-C (mg/dL)		95±20	362 (339–390)[a]	268 (258–278)[b]	212±15[b]	.001[a]
HDL-C (mg/dL)		47 (42–58)	56±23[a]	47±10[b]	46 (42–58)	NS
ALB (g/dL)		45±2.6	40 (33–44)[a]	44 (41–46)[ab]	43±5[ab]	.001[a]
CRP (mg/L)		2 (1–3)	7.2 (1.8–12.6)[a]	5.9 (2.5–9.1)[a]	3.1 (1.3–7.2)[ac]	.003[a]
WBC (K/µL)		6.5 ± 1.2	8.7 ± 2.3[a]	7.8 ± 1.6	8.48 ± 1.1.7	.001[a]
NEU (K/µL)		3.7±1	5.4±2.1[a]	4.4±1.3	5±1.6[a]	.001[a]
LYM (K/µL)		2.2±0.5	2.4±0.9[a]	2.6±0.9[a]	2.7±0.8[a]	.03[a]
PLT (K/µL)		223±50	278 (233–353)[a]	289±81[ab]	288±57[c]	.001[a]
CAR		0.044 (0.042–0.051)	0.189 (0.054–0.339)[a]	0.134 (0.059–0.220)[ab]	0.068 (0.031–0.172)[ac]	.001[a]
NLR		1.5 (1.2–2.1)	2 (1.3–3.6)[a]	1.7 (1.2–2.3)	1.5 (1.1–2.6)[a]	NS
PLR		104±28	127 (96–183)[a]	110 (83–146)[ab]	105 (90–126)	NS

Note: FH, familial hypercholesterolemia; CRP, c-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; WBC, white blood cells; NEU, neutrophil; LYM, lymphocyte; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CAR, CRP-to-albumin ratio; NS, non-significant. The results (except age) are shown as mean ± standard deviation for parameters with normal distribution or as median and 1st–3rd quartiles for parameters not showing normal distribution. Ages are shown as median and minimum–maximum. p<.050 was considered statistically significant. [a]p<.050, vs. the control group. [b]p<.050, vs. the G1 group. [c]p<.050, vs. the G2 group

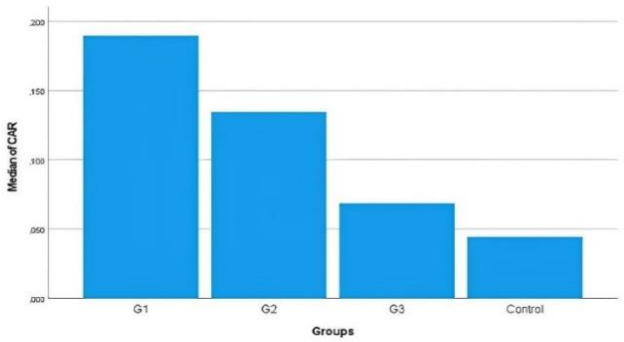

Figure 1. The median values of CRP-to-albumin ratio for the subgroups of patients with familial hypercholesterolemia (G1, G2, G3) and the controls.

The ROC curve analysis of the CAR for distinguishing FH from the controls is presented in Figure 2, showing the relationship between sensitivity and 1-specificity for different threshold values of the CAR. The AUC value was 0.715 (95% confidence interval [CI]: 0.626–0.824, P=0.0001) for CAR. The point of 0.075 on the curve for the CAR exhibited a sensitivity of 62% and a specificity of 94% for distinguishing FH from the controls.

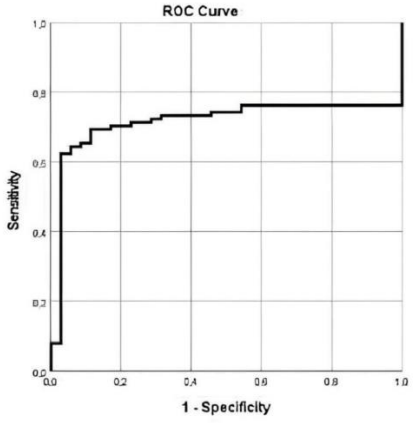

Figure 2. The ROC curve analysis for CRP-to-albumin ratio for distinguishing familial hypercholesterolemia from the controls

Discussion

FH is an inherited disorder of lipid metabolism, and the risk of CAD is high in patients with FM (Falk et al., 2006, Hansson, 2005). Therefore, timely detection of FH before the complications of it begin is very important. Unfortunately, the widespread lack of awareness regarding FH tends to hinder accurate diagnosis of it. Implementation of systematic screening programs for high-risk populations, such as those with a family history of FH or heart disease at an early age, will be useful in early diagnosis of the disease. FH can be diagnosed according to clinical criteria such as the Simon Broome or Dutch Lipid Clinic Criteria. These criteria include high LDL-C levels, family history, and tendon xanthomas (cholesterol deposits under the skin). Nevertheless, the diagnosis and management of FH also require collaboration between primary care physicians, cardiologists, laboratory specialists, and genetic counselors (WHO, 1999). We performed this study considering that the albumin and CRP may provide information about the disease in addition to classical lipid tests in FH. To our knowledge, this study is the first to evaluate the CARin patients with FH.

CAR, which has recently been used in the diagnosis and follow-up of many diseases, is an important parameter that deserves more comprehensive research, considering the severity of FH and the potential to prevent its complications through early diagnosis (Visseren et al., 2021, Eckart et al., 2020). The AUC value of 0.715 calculated for CAR shows good diagnostic ability in familial hypercholesterolemia in the study. Moreover, the p-value of 0.0001 confirms the statistical significance of this discriminatory capacity and increases the robustness of the diagnostic efficiency of this ratio. Current literature predominantly suggests that CAR can be used as a predictor of various medical conditions, including malignancies, sepsis, and septic shock. For example, Kinoshita et al. (2015) investigated the association between survival of patients with hepatocellular carcinoma using a cut-off value of 0.037 for CAR. The findings conclusively demonstrate a significant association between the elevated CAR (The optimal cutoff level ≥0.037) and both tumor progression and decline in liver functional reserve. In addition, the study highlights the plausible role of CAR as a potential prognostic indicator in patients with hepatocellular carcinoma. A comprehensive cohort study of 205 patients was conducted by Yamagata et al. (2021) between 2015 and 2018 to elucidate the prognostic utility of inflammatory markers in oral squamous cell carcinoma (OSCC). The rigorous review revealed CAR exhibiting 59.3% sensitivity and 75.3% specificity when set to a cutoff threshold of 0.032. As a result, CAR has been considered an important prognostic marker for OSCC. This study also validates the potential integration of CAR into routine medical evaluations, coupled with its convenient and rapid acquisition, thus establishing its reputation as a valuable tool in predicting outcomes for patients with OSCC.

The point of 0.075 on the curve for the CAR exhibited a sensitivity of 62% and a specificity of 94% for distinguishing FH from the controls, and it provided significant diagnostic ability. So, a sensitivity rate of 62% showed that the test was suitable for accurately identifying true positives, and a specificity rate of 94% showed that it was quite good at identifying true negatives. The specificity obtained with our findings is higher than that of CARs in studies involving various cancer patients, which is very promising for new studies. This high specificity rate serves as a positive confirmation of the diagnostic robustness and discriminatory potential of our approach and contributes to the overall significance of the results of our study.

CRP testing is widely used in patients with acute pancreatitis both at hospital admission and during treatment follow-up (Staubli et al., 2015). For instance, Piñerúa-Gonsálvezet al. (2023) investigated the correlation between CAR and the severity of acute pancreatitis. In particular, the results showed that an AUC value of 0.68for CAR exceeded the discriminatory capacity of the

Ranson criteria, which had an AUC value of 0.62. Regarding the prognosis of severe acute pancreatitis, a reasonably chosen cut-off value of 7.51 was determined to be optimal, providing a sensitivity of 63.4% and a specificity of 65.6%. These findings demonstrate the potential of CAR to predict the severity of acute pancreatitis. In addition, a study by Wang et al. (2010) showed that decreased albumin levels and elevated CRP levels were predictive of a poor prognosis in patients with acute pancreatitis. These findings also support the concept that the measurement of CRP and albumin levels can be used as predictive markers to assess the risk of death in individuals with acute pancreatitis.

The study performed by Aksu et al. (2019) investigated the association between CAR and stent restenosis with demographics of patients suffering from ST-segment elevation myocardial infarction (STEMI). As a result of the study, a high CAR emerged as a predictive factor for SR in STEMI cases, with a defined cutoff value of 1.25. Its predictive capacity has been highlighted with 84% sensitivity and 70% specificity. Similar to any other research, the cohort study involving 344 patients suffering from acute coronary syndrome revealed a significant result. It has been confirmed that high CAR shows remarkable efficacy in predicting the prognosis of moderate to high SYNTAX scores, thus exceeding the predictive capacity of CRP and albumin alone (Çağdaş et al., 2019). Considering that acute coronary diseases represent the primary cause of death among individuals suffering from FH, and in light of the rather high sensitivity values obtained in our study, our thesis proposes the usage of CAR as a powerful tool to measure the diagnosis and severity of FH.

In our study, as a result of the comparison between the group with the highest LDL-C levels and the control group, WBC, NEU and LYM counts were also significantly higher, which may lead to a judgment in line with studies (Taghizadeh et al., 2020) on the presence of endothelial dysfunction and atherosclerotic inflammation with increased oxidized LDL-C levels. In addition, the significant difference in PLR level between the control group, group 1 and group 2 is consistent with the fact that it is a routine test used in the prediction of cardiovascular diseases (Condado et al., 2016). The significant difference in the NLR ratio between the control group and group 1 in our study, which has a strong correlation with atherosclerotic cardiovascular disease and is considered as an inflammatory biomarker, confirms the possibility of cardiovascular disease with a higher rate in FH patients (Hu et al., 2020).

Conclusion

The findings of our study emphasize the importance of CAR as a diagnostic indicator for FH. Its significance is further amplified by its suitability for assessment through routine clinical tests, its rapid and simple result generation, and its exceptional sensitivity in comparison to numerous alternative diagnostic markers. Thanks to its potential as a valuable prognostic marker, our study will contribute to the literature on the use of CAR in the diagnosis and follow-up of many diseases such as FM. We would like to add that additional prospective studies involving larger and more diverse populations, conducted in multiple research centers, are mandatory to rigorously assess the utility of the CAR as a reliable diagnostic for FH and predictor of mortality. The limitations of our study were as follows: First, there isn't any reliable gold standard method for selecting patients with FH. Moreover, there were more females than males in each group. The sample sizes of each subgroup were too small and. Additionally, our patient groups may differ in the usage of some medications, and we did not have detailed information about whether these drugs affected any test results.

Ethics Committee Approval: Ethical approval was obtained from the "Erzurum Regional Training and Research Hospital, Medical Ethics Committee" (Approval date 08.11.2018 and number: 2018/17-160).
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Kaju (*Anacardium occidentale* L.) Bitki Ekstrelerinin Anksiyolitik Etkisinin Biyoinformatik Temelli Geliştirilen Lipidom Analiz ile Beyin Dokularında Araştırılması: Zebra Balığı Modellemesi

Investigation of Anxiolytic Effect of Cashew (*Anacardium occidentale* L.) Plant Extracts in Brain Tissues by Bioinformatics Based Lipidome Analysis: Zebrafish Modeling (Chemometric Analysis)

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ÖZ

Amaç: Bu çalışmada, predatör stresi ile zebra balıklarında oluşturulan anksiyete modelinde, kaju (*Anacardium occidentale*) meyvesinin metanol ekstresinin anksiyolitik etkisi ve bu etkinin moleküler mekanizması incelenmiştir. Çalışmanın temel amacı, kaju metanol ekstresinin zebra balığı modelinde anksiyolitik etkisini belirlemek ve bu etkilerin altında yatan biyokimyasal mekanizmaları lipidomiks analizleri aracılığıyla moleküler düzeyde açıklamaktır.
Yöntem: Deneyde, predatör stresine maruz bırakılan 18 zebra balığı rastgele üç gruba ayrılmıştır. Üç hafta süresince, her gruba farklı bir uygulama yapılmıştır: Birinci gruba farmasötik referans ilaç olarak diazepam, ikinci gruba 100 gram toz haline getirilmiş kaju meyvesinin metanolde maserasyon yöntemiyle hazırlanmış ekstresi, üçüncü gruba ise kontrol grubu olarak yalnızca standart yem verilmiştir. Bu süreç boyunca balıkların davranışsal ve biyokimyasal değişimleri izlenerek anksiyolitik etkinin değerlendirilmesi amaçlanmıştır.
Bulgular: Deneyler sonucunda, kaju ekstresi ile tedavi edilen deney grubu ve diazepam ile tedavi edilen kontrol grubu başarıyla ayrıştırılmıştır. Gerçekleştirilen ayrım analizlerinin doğruluğu, hassasiyeti ve özgüllüğü ek deneylerle doğrulanmıştır. Anksiyete durumundaki zebra balıklarının beyin dokusunda metabolit profili önemli ölçüde değişim göstermiş olup, çalışmada anlamlı farklılık gösteren toplam 64 metabolit tespit edilmiştir.
Sonuç: Zebra balığı modelinde, deney grubu kaju ekstresi ile, kontrol grubu ise diazepam ile tedavi edilmiştir. Yapılan analizler sonucunda, kaju ekstresinin içerdiği anakardik asit ve triptofan bileşikleri sayesinde anksiyolitik etkisinin belirgin olduğu ve bu etkinin bilimsel olarak doğrulandığı gösterilmiştir.

Anahtar Kelimeler: *Anacardium occidentale*, anksiyete, anakardik asit, lipidomiks, tof ms/ms, zebra balığı

ABSTRACT
Objective: In this study, the anxiolytic effect of methanol extract of cashew (*Anacardium occidentale*) fruit and the molecular mechanism of this effect were investigated in an anxiety model induced in zebrafish by predator stress. The main objective of the study was to determine the anxiolytic effect of cashew methanol extract in a zebrafish model and to explain the biochemical mechanisms underlying these effects at the molecular level through lipidomics analyses.
Methods: In the experiment, 18 zebrafish exposed to predator stress were randomly divided into three groups. Each group received a different treatment for three weeks: the first group received diazepam as a pharmaceutical reference drug, the second group received 100 grams of powdered cashew fruit extract prepared by maceration in methanol, and the third group received only standard feed as a control group. During this process, the aim was to evaluate the anxiolytic effect by monitoring the behavioral and biochemical changes of the fish.37.8% of the participants were male.
Results: As a result of the experiments, the experimental group treated with cashew extract and the control group treated with diazepam were successfully differentiated. The accuracy, sensitivity and specificity of the discrimination analyses performed were confirmed by additional experiments. The metabolite profile in the brain tissue of zebrafish in the anxiety state showed significant changes, and a total of 64 metabolites were detected that showed significant differences in the study.
Conclusion: In the zebrafish model, the experimental group was treated with cashew extract, and the control group was treated with diazepam. As a result of the analyses, it was shown that the anxiolytic effect of cashew extract was evident thanks to the anacardic acid and tryptophan compounds it contained, and this effect was scientifically confirmed.

Keywords: Anacardic acid, *Anacardium occidentale*, anxiety, lipidomix, tof ms/ms, zebra fish

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Giriş

Bitkisel kaynaklı bileşiklerin farmakolojik potansiyelinin değerlendirilmesi, geleneksel tıptan modern tıbba uzanan köklü bir geçmişe sahiptir. Özellikle anksiyolitik özelliklere sahip bitkisel tedaviler, anksiyete bozukluklarına yönelik mevcut tedavi seçeneklerine alternatif veya tamamlayıcı yaklaşımlar olarak giderek daha fazla ilgi görmektedir. Bu bağlamda, kaju (*Anacardium occidentale*) bitkisinin anksiyolitik özelliklerine odaklanmak, doğal kaynaklı potansiyel tedavi seçeneklerinin keşfinde önemli bir adım olabilir (Dendena & Corsi, 2014).

A. occidentale L., Anacardiaceae familyasına ait tropik bir ağaç olup, özellikle kaju fıstığı ve meyvesiyle tanınmaktadır. Bu bitkinin başlıca biyoaktif bileşenleri arasında anakardik asitler, tanenler, flavonoidler ve diğer fenolik bileşikler yer almaktadır. Söz konusu bileşiklerin anti-inflamatuar ve antioksidan özelliklere sahip olduğu bilinmektedir (Akinpelu, 2001). Ayrıca, yapılan bazı ön çalışmalar, kaju ekstrelerinin anksiyete üzerinde olumlu etkiler gösterebileceğini ortaya koymuştur. Ancak, bu bulguların daha geniş ölçekli ve kontrollü çalışmalarla doğrulanması gerekmektedir (Konan & Bacchi, 2007; Razali et al., 2008). Araştırmalar, kaju ağacı ekstrelerinin hem fıstık hem de elma dahil olmak üzere potansiyel sağlık faydaları sunduğunu göstermiştir. Bu faydalar arasında antidiyabetik, kansere karşı koruyucu ve gastroprotektif etkiler bulunmaktadır. Aynı zamanda antiseptik, antioksidan, antiviral ve antifungal gibi farmakolojik etkileri için kullanılmaktadır. Antidepresan etkileri üzerindeki araştırmalar ise hala devam etmektedir. Ağacın kabuğu ve yaprakları, ishal ve diş ağrıları gibi çeşitli rahatsızlıkları tedavi etmek için geleneksel ilaçlarda kullanılmaktadır (Lim, 2010).

Kaju, vitaminler, mineraller, yağ asitleri, proteinler ve anakardik asit, tanenler, saponinler gibi biyolojik açıdan aktif fitokimyasallar içermektedir (Togola et al., 2020). Bu bileşenler, anti-inflamatuar ve nöroprotektif özellikler göstererek sinir sistemi üzerinde olumlu etkilere sahip olabilir. Özellikle anakardik asit ve polifenoller, anksiyete gibi psikolojik rahatsızlıkların hafifletilmesinde potansiyel faydalar sağlayabilir. Anksiyete, zaman zaman normal kabul edilse de aşırı ve kontrol edilemez hale geldiğinde yaşam kalitesini düşüren bir sağlık problemine dönüşebilir. Klinik olarak anksiyete bozukluğu tanısı için, kişinin en az altı ay boyunca belirgin veya belirsiz kaygı yaşaması ve bu durumun günlük işlevselliğini etkilemesi gerekmektedir. Genetik yatkınlık, çevresel faktörler ve beyin kimyasındaki dengesizlikler gibi çeşitli etmenler anksiyetenin gelişiminde rol oynar. Anksiyete bozuklukları; panik bozukluk, sosyal anksiyete ve genelleşmiş anksiyete bozukluğu gibi alt türlere ayrılır (Bandelow et al., 2017).

Anksiyete tedavisinde bireysel farklılıklar göz önünde bulundurularak psikoterapi, özellikle bilişsel davranışçı terapi (BDT), yaygın bir yaklaşım olarak öne çıkmaktadır. Ayrıca, farmakolojik tedavi ve bitkisel bileşiklerin anksiyolitik potansiyeli üzerine yapılan araştırmalar, alternatif yaklaşımların gelişmesini desteklemektedir. Kaju ekstresi gibi doğal bileşiklerin anksiyete üzerindeki etkilerinin incelenmesi, nörolojik sağlık üzerindeki potansiyellerini anlamamıza katkı sağlayabilir (Bandelow et al., 2017).

İlaç tedavisi, anksiyete yönetiminde önemli bir rol oynar. SSRI'lar ve SNRI'lar gibi antidepresanlar, anksiyete semptomlarını kontrol altına almak için sıklıkla kullanılır. Ayrıca, bazı durumlarda benzodiazepinler kısa süreli tedavi için tercih edilebilir, ancak bağımlılık riski taşır. Yaşam tarzı değişiklikleri, düzenli egzersiz, yeterli uyku, sağlıklı beslenme ve meditasyon, anksiyeteyi hafifletebilir. Mindfulness ve yoga gibi teknikler de stres yönetiminde faydalıdır. Anksiyete tedavisi, bireysel ihtiyaçlara göre özelleştirilmeli ve doğru tedavi kombinasyonu, etkili bir yönetim sağlar. Anksiyete bozuklukları, yaşam kalitesini olumsuz etkileyen yaygın bir psikiyatrik rahatsızlıktır (Remes et al., 2016).

Geleneksel anksiyete tedavileri çoğunlukla farmasötik ilaçlar içerir, ancak bu ilaçlar yan etkilere yol açabilir ve her hastada aynı derecede etkili olmayabilir. Bu nedenle, daha az yan etkiye sahip doğal alternatiflerin araştırılması önemlidir. Anksiyolitik ilaçlar, genel anksiyete bozukluğu, sosyal anksiyete bozukluğu, panik bozukluğu ve bazı durumlarda obsesif kompulsif bozukluk gibi farklı anksiyete türlerinin tedavisinde kullanılır. Ayrıca, cerrahi işlemler öncesi veya diğer tıbbi prosedürlerde de tercih edilebilir. Benzodiazepinler, hızlı etki gösterse de uzun süreli kullanımlarda bağımlılık riski taşır, bu nedenle dikkatli kullanılmalıdır (Longo & Johnson, 2000).

Buspiron gibi ilaçlar, bağımlılık riski düşük ve daha yavaş etkili olup uzun tedavi süreçleri gerektirir. Anksiyete tedavisinde ilaçlar ve psikoterapi, özellikle bilişsel davranışçı terapi, daha etkili sonuçlar sağlar. Anksiyolitik ilaçlar benzodiazepinler, SSRI'lar, SNRI'lar ve diğerleri olarak dört gruba ayrılır; her biri farklı etki mekanizmalarına ve yan etki profillerine sahiptir. Benzodiazepinler bağımlılık riski taşırken, SSRI ve SNRI'lar alkolle alınmamalıdır. Yan etkiler genellikle hafif olup sedatif etkiler dikkat gerektiren durumlarda göz önünde bulundurulmalıdır. Bu çalışmada, zebra balığı modeli kullanılarak kaju bitkisinin anksiyolitik etkileri, biyoinformatik temelli lipidom analizleriyle incelenmektedir. Zebra balığı, insan benzeri sinir sistemi yapısı ile anksiyolitik etkilerin lipidomik yollar üzerindeki etkilerini aydınlatmak için ideal bir modeldir (Karasakal, 2021).

Bu çalışmanın amacı, lipidom verilerini istatistiksel analizlerle birleştirerek kaju ekstresinin anksiyolitik etkilerini moleküler düzeyde incelemektir. Elde edilen veriler, kaju bitkisinin beyin dokularındaki lipidomik yolları nasıl etkilediğini ve hangi metabolitlerin düzeylerinde değişiklik meydana geldiğini ortaya koyacaktır. Bu analizlerin ışığında, kaju bitkisinin anksiyolitik etkilerinin mekanizmalarını açıklayacak bir model geliştirilecektir. Çalışma, bitkisel ilaçların anksiyolitik potansiyelini ve tedavi hedeflerini daha iyi anlamanın yanı sıra, kaju bitkisinin beyin metabolizması üzerindeki etkilerini değerlendirerek anksiyete bozukluklarının tedavisinde bitkisel tedavi yöntemlerinin potansiyelini açığa çıkarmayı amaçlamaktadır.

Yöntem

Bu çalışmada, araştırma için AB hattı zebra balığı larvaları (*Danio rerio*) kullanılmıştır. Balıklar, kapalı devre zebra balığı ünitesinde yetiştirilmekte olup, fotoperiyodları 10 saat karanlık ve

14 saat aydınlık olacak şekilde düzenlenmiştir. Döllenmeden itibaren 10. güne kadar paramecium ile beslenen balıklar, 10. günden sonra günlük olarak canlı Artemia salina ve pul yemle beslenmeye devam edilmiştir. Çalışmada, 72 saatlik larva balıklar kullanılmıştır. Ayrıca çalışmada kullanılan cihaz ve materyallerin kullanım amaçları da Tablo 1' de belirtilmiştir.

Tablo 1. Deneyde kullanılan cihaz ve materyaller

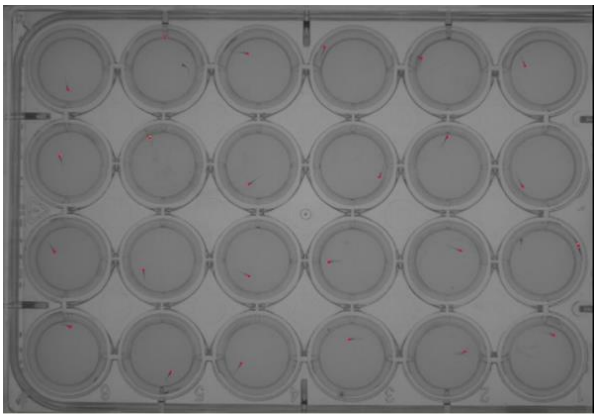
Kuruluşta Bulunan Altyapı/Ekipman Türü, Modeli (Laboratuvar, Araç, Makine-Teçhizat, vb.)	**Çalışmada Kullanım Amacı**
Kapalı devre zebra balığı üretim sistemi	Anaç zebra balıklarının beslenmesi, üremesi ve zebra balığı yumurtası elde etmek için kullanılmıştır.
Soğutmalı santrifüj	Metabolom analizleri için örnek hazırlama aşamasında kullanılmıştır.
Otoklav	Sterilizasyon için kullanılmıştır.
-20 derin dondurucu	Numune hazırlamada inkübasyonda kullanılmıştır.
+4 buzdolabı	Isıya duyarlı sarf malzemelerin muhafazasında kullanılmıştır.
Ultra sonik su banyosu	Doku homojenizasyonu aşamalarında kullanılmıştır.
-80 derin dondurucu	Örneklerinin muhafazası için kullanılmıştır.
TOF-MS	Metabolom analizlerinde kullanılmıştır.
İnkübatör	Alınan yeni nesil balıkların 120. saatlerine kadar sabit sıcaklıkta tutulması için kullanılmıştır.

Çalışma Kurgusu ve Düzeni

Çalışmada, toplam üç grup oluşturulmuştur (Tablo 2). Deneyde kullanılan predatör strese maruz kalmış 18 zebra balığı üç gruba ayrılmıştır. Pozitif kontrol grubunda, altın standart olarak bilinen diazepam kullanılarak anksiyete modeli oluşturulmuş zebra balıklarına verilmiştir. Deney grubuna, 3 hafta boyunca metanol ekstratı (100 ppm) içeren yem verilmiş, kontrol grubuna ise aynı süre boyunca sadece normal yem verilmiştir. Üç hafta sonunda, tüm gruplar analizlere tabi tutulmuştur. Anksiyete testi, zebra balığındaki anksiyete benzeri davranışları test etmek için literatürde yaygın olarak kullanılan yeni dalma tankı yöntemiyle (Şekil 1) yapılmıştır. Bu testler, kaju ekstresinin anksiyolitik etkilerini değerlendirmek için kullanılmıştır.

Tablo 2. Deney grupları

Deney Grupları	**Ekstraksiyon Yöntemi**	**Doz**	**Beslenme Süresi**	**Predatör Stresi**	**Anksiyete Testi**
Pozitif Kontrol Grubu (PK)	Farmasötik Formülasyon (Diazepam)	10 mg/L	3 Hafta	Var	Var
Deney Grubu (Kaju Metanol Ekstresi)	Metanol	100 ppm	3 Hafta	Var	Var
Kontrol Grubu	-	-	3 Hafta	Var	Var



Şekil 1. Yeni dalma tankı yöntemi

Deneyde Kullanılan İlaç Uygulaması

Deneyde Pozitif Kontrol Grubu'ndaki (PK) zebra balığı larvalarına her 24 saatte bir 10 mg/L Diazepam uygulanmıştır. Uygulama toplam 3 hafta sürdü ve Diazepam larvalara suya eritilmiş formda eklenerek verilmiştir.

Ekstraksiyon

Kuru kaju meyvesinin 100 gr'ı homojen bir partikül boyutu elde edilene kadar öğütülerek ince toz haline getirilmiştir. Elde edilen toz, her biri 300 mL saf metanol içeren kapalı kaplarda, sürekli hareketli ortamda 3 gün boyunca maserasyona bırakılmıştır. Maserasyon işlemi, her gün 8 saatlik periyotlar halinde gerçekleştirilmiş ve her periyot sonunda çözeltiler süzülerek ayrılmıştır. Elde edilen süzüntüler, çözücünün uzaklaştırılması amacıyla rotary evaporatörde düşük basınç altında buharlaştırılmış ve yoğunlaştırılmış metanol ekstreleri elde edilmiştir. Günlük olarak elde edilen konsantre ekstreler birleştirilmiş, hassas terazide tartılmış ve nihai verimi belirlenmiştir. Ekstraksiyon sürecinin tekrarlanabilirliğini ve ekstraktın kimyasal içeriğinin tutarlılığını değerlendirmek amacıyla, elde edilen ekstraktın toplam fenolik bileşik miktarı, toplam flavonoid içeriği ve belirleyici fitokimyasal bileşenleri spektrofotometrik ve kromatografik yöntemlerle analiz edilmiştir. Bu sonuçlar, literatürde bildirilen benzer çalışmalardaki verilerle karşılaştırılarak metodun geçerliliği değerlendirilmiştir. Son olarak, elde edilen metanol ekstresi, anksiyolitik ajan olarak model organizmada kullanılmak üzere -80°C'de muhafaza edilmiştir. Diyette kullanılacak miktarın belirlenmesine bağlı olarak ekstraksiyon sürecinde elde edilen ekstre miktarı optimize edilmiştir.

Lipidom Profili

Zebra balıklarından alınan beyin doku örnekleri, toz haline getirilip homojenize edilmiştir. 1 mL metanol/kloroform karışımı kullanılarak örneklerdeki proteinler çöktürülmüş ve yağ asitleri ekstrakte edilmiştir. Örnekler, analiz süresine kadar -80°C'de saklanmıştır. Supernatant kısmı, vakum altında uçurularak izopropanol, su ve metanol içeren mobil fazda çözündürülmüştür. Bu aşamada, her örnekten 10 µL alınarak kalite kontrol (QC) analizi için bir havuz oluşturulmuştur. Lipidomik analiz, ters faz C18 kolon kullanılarak gerçekleştirilmiştir. Gradiyent elüsyonla yapılan analizde enjeksiyon hacmi 4 µL, kolon sıcaklığı ise 50°C olarak ayarlanmış ve QTOF–MS cihazı pozitif modda ESI iyonizasyonu ile çalıştırılmıştır. Cihaz parametreleri, daha önceki çalışmalarımıza dayalı olarak 70-1500 m/z aralığı, 5 Hz tarama frekansı, 200°C kuru gaz sıcaklığı ve nebulizör basıncı 37 psi olarak belirlenmiştir. Bu parametreler, pilot çalışmaya dayalı olarak optimize edilmiştir. Analiz sırasında her 8 örnekte bir kalite kontrol numunesi cihaza verilmiştir. Örnekler, kalite kontrol örnekleriyle normalize edilmiş ve rastgele sıralanarak cihaza verilmiştir. Lipid anotasyonu, iterated DDA yöntemiyle 70-200, 200-350, 350-500, 500-750, 750-1000, 1000-1250 ve 1250-1500 m/z aralığında tandem MS/MS analizleri yapılarak gerçekleştirilmiştir. Prekürsör ve ürün iyonları tespit edilerek her bir lipidin tanımlanması sağlanmıştır. MS/MS spektrumları, Lipidblast, HMDB ve METLIN gibi referans kütüphanelerindeki spektrumlarla karşılaştırılarak pikler tanımlanmıştır. Elde edilen veriler, MATLAB 2021a yazılımında yer alan PLS Toolbox 8.5 ile karşılaştırmalı biyoinformatik analizlere tabi tutulmuştur. XCMS pik dedeksiyonu, m/z değerlerinin listelenmesi ve MS/MS spektrum eşleşmeleri sonrası veriler MATLAB programına aktarılmıştır. MATLAB içerisinde, grubumuzca hazırlanan kodlar kullanılarak, düşük yoğunluklu (≤5000), tekrarlanabilirliği olmayan (%BSS ≥ 30) ve QC örneklerinde yer almayan (false peak) m/z değerleri filtre edilmiştir. Bu işlemlerden sonra, PLS Toolbox yazılımında yer alan OPLS-DA modellemesi ile karar/destek modeli oluşturulmuş ve istatistiksel olarak anlamlı lipidler için VIP skorları ve fold değişimleri hesaplanmıştır. Yolak analizleri için PIUMET ve Metaboanalist 6.0 programları kullanılarak hedeflenmiş ve hedeflenmemiş lipidom analizleri yapılmış, böylece zebra balıklarında anksiyete modeli oluşturulmuş ve lipidomik strateji geliştirilmiştir.

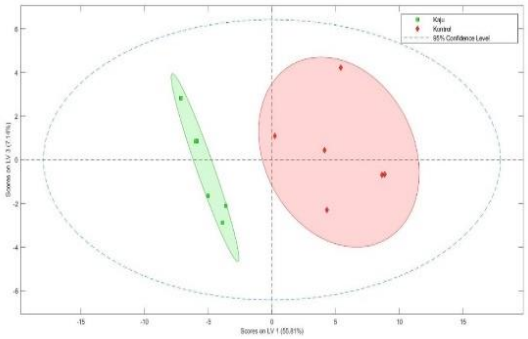
Bulgular

Model organizmada istatistiksel olarak anlamlı farklılık göstermiş annotasyonu yapılan lipid molekülleri Tablo 3'de verilmiştir.

Kemometrik analizler

Kısmi en küçük kareler diskriminant analizi (PLS-DA)

Bu analiz, çok değişkenli verilerde gruplar arasındaki farkları ve sınıflandırmayı değerlendirmeye olanak tanır. Grupların birbirinden nasıl ayrıldığını daha net bir şekilde görmek için ise skor grafiği kullanılabilir (Şekil 2).

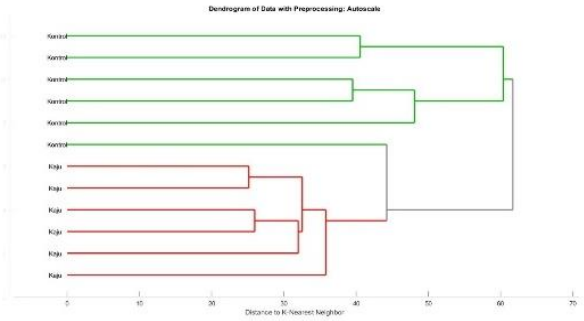


Şekil 2. PLS-DA skor grafiği: kaju ekstresi ve diazepam gruplarinin lipit profil ayirimi

Yeşil kareler kaju ekstresi ile tedavi edilen zebra balıklarını (Deney Grubu), kırmızı elmaslar ise diazepam ile tedavi edilen zebra balıklarını (Pozitif Kontrol Grubu) temsil etmektedir. Bu dağılım, iki grubun birbirinden ne kadar ayrıldığını veya örtüştüğünü görsel olarak ortaya koyar. PLS-DA model parametreleri ise Tablo 4'de sunulmuştur.

Hiyerarşik küme analizi

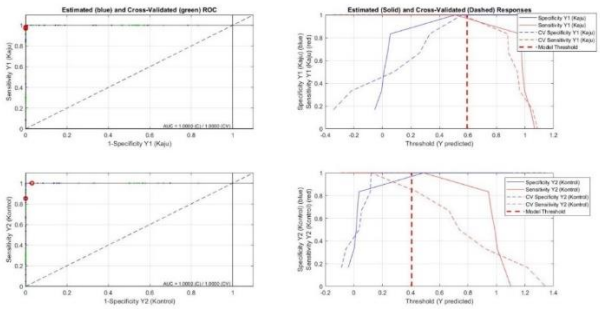
Bu grafik, kontrol ve deney gruplarının belirgin şekilde ayrıldığını ortaya koymaktadır. Yeşil çizgiler diazepam ile tedavi edilen zebra balıklarını (Pozitif Kontrol Grubu), kırmızı çizgiler ise kaju ekstresi ile tedavi edilen zebra balıklarını (Deney Grubu) temsil etmektedir. Şekil 3' deki dendogramda rastgele oluşturulan grupların birbirinden açıkça ayrıldığı gözlemlenmiştir. Bu çizgilerin birbirine karışmaması ayrımın başarılı şekilde yapıldığının kanıtıdır.



Şekil 3. Hiyerarşik küme analizi dendogrami: kaju ekstresi ve diazepam gruplarinin lipit profil kümelenmesi

Alıcı çalışma karakteristiği (ROC) analizi

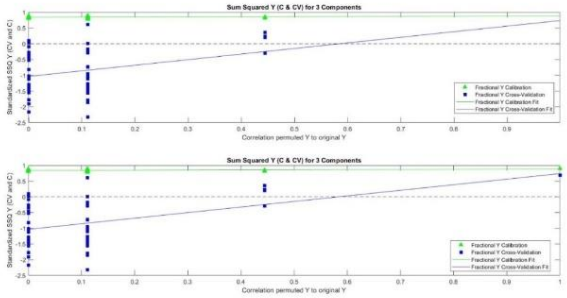
Bu grafikler, modelin doğruluğunu ve performansını değerlendirmek için kullanılan ROC eğrilerini ve duyarlılık (sensitivity) ile özgüllük (specificity) analizlerini göstermektedir. ROC eğrilerindeki AUC değerleri hem kalibrasyon hem de çapraz validasyon veri seti için 1.0000'dir (Şekil 4).



Şekil 4. PLS-DA modeli ROC eğrisi

Permütasyon analizi

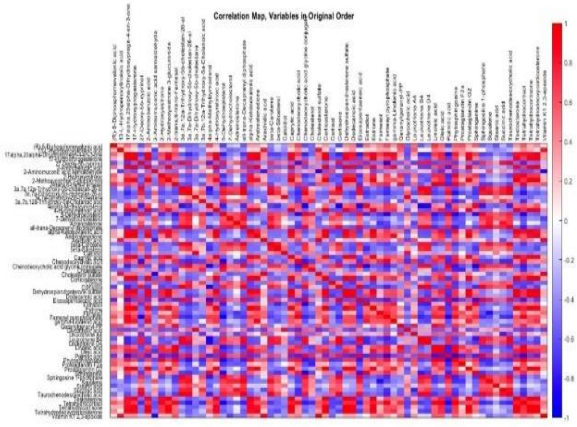
Bu analiz, tedaviyle elde edilen başarının rastgele olup olmadığının anlaşılmasına yardımcı olur. Modelin doğruluğu hakkında bilgi verir. 50 kombinasyon yapılmış ve bunların performansları değerlendirilmiştir (Şekil 5).



Şekil 5. Permütasyon analizi grafiği

Korelasyon Matrisi

Korelasyon haritası, çalışmada incelenen çeşitli lipidlerin ve metabolitlerin birbirleriyle olan ilişkilerini gösterir. Bu tür haritalar, biyolojik sistemlerdeki moleküler etkileşimlerin anlaşılmasına yardımcı olur ve belirli biyokimyasal yolakların potansiyel işlevleri hakkında bilgi sağlar (Şekil 6).



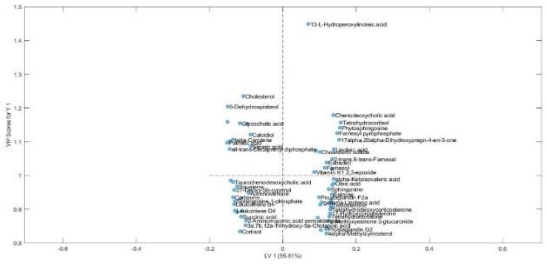
Şekil 6. Korelasyon matrisi

Şekil 6'daki kırmızı kareler, iki değişken arasında yüksek pozitif korelasyonu (1'e yakın) gösterir; bu, her iki değişkenin birlikte arttığını veya azaldığını ifade eder. Mavi kareler ise negatif korelasyonu (−1'e yakın) simgeler; bu da bir değişken artarken diğerinin azaldığını gösterir. Beyaz kareler, korelasyonun olmadığı (0) veya çok düşük olduğu durumu yansıtır.

Her bir karedeki renk yoğunluğu, korelasyon katsayısının büyüklüğünü belirtir ve katsayının büyüklüğü, iki değişken arasındaki ilişkinin gücünü gösterir. Eğer belirli bir metabolit grubu arasında güçlü bir pozitif korelasyon bulunuyorsa, bu metabolitlerin aynı biyokimyasal yolakta yer alabileceğini veya benzer biyolojik işlevler üstlenebileceğini düşündürebilir.

Projeksiyonda Değişken Önem (VIP)

Bu analiz sayesinde PLS-DA modelindeki değişkenlerin modelin açıklayıcı gücüne katkıları değerlendirilir. Şekil 7'de çalışmamız için oluşturduğumuz modele katkıları olan lipidler ve bu katkıların dereceleri gösterilmiştir.

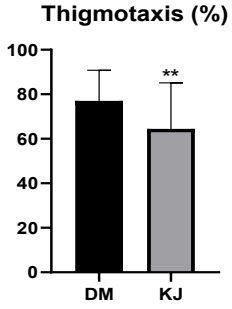


Şekil 7. VIP skorları

Hareket Eksenine Göre Anksiyete Ölçümü

Thigmotaxis, normal eksende hareket etmeme olarak tanımlanabilir ve hayvanların anksiyete ve stres düzeylerini değerlendirmede önemli bir göstergedir. Bu yöntem, anksiyolitik ilaçların etkinliğini değerlendirmek için kullanılır. Hayvanlar, anksiyete seviyeleri yüksek olduğunda çevreyi aktif olarak keşfetmek yerine test alanının duvarlarına yakın kalma eğilimindedir (Kankaynar et al., 2023). Zebra balıkları da anksiyete durumunda havuzun kenarlarına yakın yüzme eğilimi göstermiştir (Şekil 8).

Model organizmalar, deney boyunca kamerayla izlenerek hareket eksenlerine göre anksiyete tedavi süreçleri değerlendirilmiştir.



Şekil 8. Diazepam ile tedavi edilen pozitif kontrol grubu (siyah sütun) ve kaju ekstresi verilmiş deney grubunun (gri sütun) Thigmotaxis grafiği

Tablo 3. Model organizmada istatistiksel olarak anlamlı farklılık göstermiş lipid molekülleri

	m/z	Kat Oranı	p.value	Düzenlenme
(R)-5-Diphosphomevalonic acid	331,0027	0,92781	,03	Azalmış düzenleme
13-L-Hydroperoxylinoleic acid	295,2271	0,073297	,02	Azalmış düzenleme
17alpha,20alpha-Dihydroxypregn-4-en-3-one	261,2213	0,076841	,01	Azalmış düzenleme
17-Hydroxyprogesterone	330,2272	0,02293	,04	Azalmış düzenleme
27-Deoxy-5b-cyprinol	219,1743	4,178679	,01	Artmış düzenleme
2-Aminobenzoic acid	138,0538	0,06368	,01	Azalmış düzenleme
2-Aminomuconic acid semialdehyde	164,0296	1,84265	,04	Artmış düzenleme
2-Hydroxyestrone	309,1559	0,092307	,03	Azalmış düzenleme
2-Methoxyestrone 3-glucuronide	459,206	0,034747	,05	Azalmış düzenleme
4alpha-Methylzymosterol	327,337	0,735435	,04	Azalmış düzenleme
4-Hydroxyretinoic acid	300,1806	0,031115	,04	Azalmış düzenleme
5-Dehydroepisterol	369,3541	2,941342	,01	Artmış düzenleme
7-Dehydrocholesterol	385,3428	9,999824	,01	Artmış düzenleme
Adrenosterone	301,1648	2,332575	,03	Artmış düzenleme
all-trans-Decaprenyl diphosphate	859,5817	5,227941	,01	Artmış düzenleme
alpha-Ketoisovaleric acid	116,0494	0,057547	,03	Azalmış düzenleme
Androstenedione	287,2029	0,06182	,05	Azalmış düzenleme
Arachidic acid	335,2792	0,432094	,02	Azalmış düzenleme
Beta-Carotene	555,4452	7,195638	,01	Artmış düzenleme
Beta-Sitosterol	397,3785	2,324958	,02	Artmış düzenleme
Calcidiol	400,3424	3,175361	,03	Artmış düzenleme
Caprylic acid	167,1066	0,057443	,05	Azalmış düzenleme
Cholesterol	369,3524	2,14952	,01	Artmış düzenleme
Cholesterol sulfate	423,332	0,282695	,01	Azalmış düzenleme
Corticosterone	311,2044	0,079365	,04	Azalmış düzenleme
Cortisol	345,2096	1,322263	,04	Artmış düzenleme
Cortisone	361,1924	3,587769	,02	Artmış düzenleme
Dodecanoic acid	223,1692	0,082277	,01	Azalmış düzenleme
Eicosapentaenoic acid	325,2201	2,599403	,01	Artmış düzenleme
Estradiol	201,1599	0,208228	,01	Azalmış düzenleme
Estrone	293,1389	0,035491	,05	Azalmış düzenleme
Gamma-Linolenic acid	233,2263	0,106889	,04	Azalmış düzenleme
Glycocholic acid	448,2907	1,313115	,01	Artmış düzenleme
Leukotriene B4	269,2476	2,250562	,02	Artmış düzenleme
Leukotriene D4	479,269	1,917981	,03	Artmış düzenleme
Linoleic acid	245,2262	0,213346	,01	Azalmış düzenleme
Oleic acid	153,1275	0,297716	,01	Azalmış düzenleme
Palmitic acid	279,2304	1,921963	,01	Artmış düzenleme
Phytosphingosine	282,2778	0,313419	,01	Azalmış düzenleme
Prostaglandin F2a	119,0854	0,607344	,03	Azalmış düzenleme
Sphinganine	284,2944	0,237686	,01	Azalmış düzenleme
Sphingosine 1-phosphate	402,221	2,87104	,03	Artmış düzenleme
Squalene	411,398	1,306625	,01	Artmış düzenleme

Tablo 3. Model organizmada istatistiksel olarak anlamlı farklılık göstermiş lipid molekülleri (Devamı)

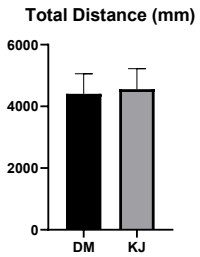
	m/z	Kat Oranı	p.value	Düzenlenme
Stearic acid	267,268	2,144304	,02	Artmış düzenleme
Succinic acid	119,0351	1,77832	,04	Artmış düzenleme
Testosterone	271,2053	0,110067	,05	Azalmış düzenleme
Tetrahydrocortisol	283,2817	0,21907	,01	Azalmış düzenleme
Tetrahydrocortisone	347,226	0,170456	,03	Azalmış düzenleme
Tetrahydrodeoxycorticosterone	357,25	0,2614	,04	Azalmış düzenleme
Vitamin K1 2,3-epoxide	467,3581	0,353988	,03	Azalmış düzenleme

Tablo 4. PLS-DA model performans metriği değerleri

Özellik		Sınıf 1	Sınıf 2
Duyarlılık (Cal)	:	1.000	1.000
Özgüllük (Cal)	:	1.000	1.000
Duyarlılık (CV)	:	1.000	1.000
Özgüllük (CV)	:	1.000	1.000
Sınıflandırma Hatası (Cal)	:	0	0 0
Sınıflandırma Hatası (CV)	:	0	0 0
Kalibrasyonun Karekök Ortalama Hatası	:	0.108122	0.108122
Çapraz Doğrulamanın Karekök Ortalama Hatası	:	0.222994	0.222994
Bias	:	0	0
Çapraz Doğrulama Yanlılığı	:	0.0130765	-0.0130765
R^2 (Cal)	:	0.953238	0.953238
R^2 (CV)	:	0.826215	0.826215

Toplam Kat Edilen Mesafeye Göre Anksiyete Ölçümü

Zebra balığı modellerinde, anksiyete ve stres terapilerinin değerlendirilmesi, balığın kat ettiği toplam mesafenin ölçülmesiyle izlenebilir (Şekil 9). Kaygılı veya stresli zebra balıkları sıklıkla daha az hareket ederler veya bir köşede sabit kalma eğilimindedir. Zebra balığındaki kaygı, donma ve düzensiz hareket atakları olarak kendini gösterir. Donma, solungaçlar ve gözler hariç, 2 saniye veya daha uzun süren hareketin tamamen durmasıyla karakterize edilir. Sonuç olarak, toplam kat edilen mesafe dahil olmak üzere lokomotor aktiviteyi değerlendirmek, zebra balığı modellerinde kaygıyla ilişkili davranışları ve olası terapilerin etkinliğini analiz etmek için etkili bir yaklaşımdır (Hamilton et al., 2021; Ranjith et al., 2022).



Şekil 9. Diazepam ile tedavi edilen pozitif kontrol grubu (siyah sütun) ve kaju ekstresi verilmiş deney grubunun (gri sütun) kat ettikleri mesafe grafiği

Tartışma

Anksiyete, yaygın olarak görülen bir ruhsal bozukluktur ve kontrol edilemeyen kaygı ile huzursuzluk durumu olarak tanımlanır. Psikoterapi ve davranışsal tedavinin yanı sıra, anksiyete tedavisinde en yaygın yöntem ilaç kullanımına dayanmaktadır. Ancak ilaçların yüksek yan etkileri ve bırakıldığında yoksunluk sendromuna yol açabilmesi, tedavide beklenen başarıya ulaşılamaması, alternatif tedavi yöntemlerine olan talebi artırmıştır. Bu bağlamda, tıbbi bitkiler, görece güvenilirlikleri, düşük yan etkileri ve maliyetleri ile etkili bir tedavi alternatifi olarak öne çıkmaktadır (Bandelow et al., 2017; Bayir et al., 2023; Karaarslan et al., 2024).

Kaju kabuğu (*A. occidentale*), ekonomik olmasının yanı sıra, zengin kimyasal içeriği nedeniyle ilaç endüstrisinde sıkça tercih edilmektedir. İçeriğindeki anakardik asit ve triptofan, antitümör ve antimikrobiyal aktiviteleriyle daha önce çeşitli çalışmalara konu olmuştur. Ancak, kaju kabuğunun anksiyolitik etkileri üzerine yapılmış sınırlı sayıda çalışma bulunmaktadır ve bunlar çoğunlukla kemirgenler üzerinde gerçekleştirilmiştir (Baptista et al., 2018; Baptista et al., 2020; Siracusa et al., 2020). Zebra balıkları ile ilgili ise herhangi bir veri bulunmamaktadır. Bu çalışmamız, kaju kabuğundan elde edilen sekonder metabolitlerin farmakolojik etkilerini araştırarak, anksiyolitik etkileri konusunda önemli veriler sunmayı hedeflemiştir. Elde edilen bulgular, bu metabolitlerin anksiyolitik etki potansiyelini ortaya koymaktadır.

Tablo 3'te, model organizmada tespit edilen 64 farklı lipitin anlamlı farklılıklar gösterdiği belirtilmiştir. Bu veriler, kaju

ekstraktının lipid profili üzerindeki etkilerini net bir şekilde ortaya koymaktadır. Şekil 4'deki Kısmi Bileşen Analizi (PLS-DA) skor grafiği, kaju ve kontrol gruplarının belirgin bir şekilde ayrıldığını göstermektedir. Bu, kaju grubunun kontrol grubuna kıyasla farklı bir bileşen profiline sahip olduğunu ortaya koymaktadır. Şekil 3'teki hiyerarşik küme analizi dendogramında, rastgele oluşturulan grupların birbirinden açıkça ayrıldığı gözlemlenmiştir. Bu ayrımın netliği, analizin başarıyla gerçekleştirildiğinin bir göstergesidir. Şekil 4'teki PLS-DA Modeli ROC eğrilerindeki AUC değerlerinin 1.0000 olması, modelin iki grup arasında mükemmel bir ayrım yapabildiğini ve doğruluğunun çok yüksek olduğunu gösterir. Bu, kaju ekstrası alan ve almayan balıkların yüzde yüz doğrulukla ayırt edilebileceğini kanıtlamaktadır. Bu grafik, kaju verilen deney grubunun diazepam verilen kontrol grubuna göre çok daha farklı bir lipid profiline sahip olduğunu da göstermektedir.

Permütasyon analizi grafiğinin sağında kalan değerler kendi diazepam ve kaju değerlerimiz. Solunda kalanlar ise iterasyonu yapılan değerleri gösterir. İterasyonu yapılan değerlerin hepsi ana modelden daha kötü performans göstermiş. Bu da elde edilen başarının rastgele olmadığını bize kanıtlar (Şekil 5). Şekil 6' daki korelasyon matrisinde, "17-hydroxyprogesterone" ve "17alpha, 20alpha-Dihydroxy-4-pregnene-3-one" bileşenleri arasında yüksek pozitif korelasyon gözlemlenmiştir. Bu, bu bileşenlerin aynı biyokimyasal yolda işlev gördüğünü veya birlikte düzenlendiğini gösterebilir. "Cortisol" ve "17 hydroxyprogesterone" arasındaki negatif korelasyon, bu bileşenlerin farklı biyolojik süreçlerde yer aldığını veya birbirini dengeleyici işlevlere sahip olabileceğini düşündürmektedir. Şekil 7'de kaju ekstresi almış zebra balığı modelinde en fazla artış gösteren lipit olan 13-L-hidroperoksilineleik asit dikkat çekmektedir. Linoleik asit, vücutta araşidonik asite dönüşerek prostaglandinler ve lökotrienler gibi biyolojik olarak önemli bileşiklerin oluşumuna katkı sağlar. Linoleik asit, aynı zamanda anti-enflamatuvar ve anti-seboreik etki gösterdiği bilinen bir bileşiktir (Innes & Calder, 2018; Lin et al., 2017).

Thigmotaksis, zebra balığı larvalarında anksiyete indeksinin belirleyicisi olarak kabul edilir ve genel olarak canlının çevresel uyaranlara duyarlılığının bir göstergesidir (Pir et al., 2024). Şekil 8'deki thigmotaxis oranı, kaju ekstresi verilmiş deney grubunun daha düşük bir orana sahip olduğunu göstermektedir. Bu, kaju ile beslenen model organizmada anksiyete durumunun azaldığını, yani tedaviye yanıt gösterildiğini düşündürmektedir. Şekil 9'da, zebra balıklarının kat ettiği toplam mesafeler kıyaslanarak iyileşme düzeyleri analiz edilmiştir. Diazepam verilen grubun deney grubuna kıyasla daha az hareket ettiği gözlemlenmiştir, bu da kaju ekstresinin diazepam benzeri anksiyolitik etki göstermiştir.

Sonuç

Elde edilen bulgular, kaju ekstresinin anksiyete tedavisinde potansiyel bir tedavi alternatifi olabileceğini göstermektedir. Yapılan metabolit analizleri ve kemometrik değerlendirmeler, kaju ekstrasının vücutta önemli değişikliklere yol açtığını ve anksiyete tedavisinde faydalı olabilecek metabolitlerin bulunduğunu ortaya koymuştur. Gelecekteki çalışmalar, kaju ekstresinin insanlarda uygulanabilirliğini ve anksiyete tedavisindeki etkinliğini daha ayrıntılı bir şekilde inceleyerek, bu bitkinin tedaviye katkısını daha iyi anlamamıza yardımcı olacaktır.

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Antioxidant Potential and Phenolic Content of *Plantago major* L.

Plantago major'un Antioksidan Potansiyeli ve Fenolik İçeriği

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ABSTRACT

Objective: This study aimed to evaluate the antioxidant capacity and bioactive compound content of the aerial parts of *Plantago major*, a medicinal plant with potential therapeutic applications.

Methods: The aerial parts were extracted using 70% methanol, and the solvent was evaporated. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified. Antioxidant capacity was assessed using DPPH, ABTS, and FRAP assays.

Results: The DPPH radical scavenging assay showed an IC_{50} value of 127,33 ± 1,07 µg/mL, while the ABTS assay revealed an IC_{50} value of 46,74 ± 2,53 µg/mL. The FRAP assay demonstrated a reducing power equivalent to 44,62 ± 0,61 mg TE/g extract.

Conclusion: The high phenolic and flavonoid content of *Plantago major* extract contributes significantly to its strong antioxidant activity. These findings suggest its potential application as a natural antioxidant source in the pharmaceutical and nutraceutical industries.

Keywords: ABTS, Antioxidant, DPPH, FRAP, *Plantago major* L.

ÖZ

Amaç: Bu çalışma, terapötik potansiyeli sahip, tıbbi bir bitki olan *Plantago major*'un toprak üstü kısımlarının antioksidan kapasitesini ve biyoaktif bileşik miktarını değerlendirmeyi amaçlamıştır.

Yöntemler: Toprak üstü kısımlar %70 metanol kullanılarak ekstre edilmiş ve çözücü buharlaştırılmıştır. Toplam fenolik içerik (TPC) ve toplam flavonoit içerik (TFC) miktarları belirlenmiştir. Antioksidan kapasitesi DPPH, ABTS ve FRAP analizleri ile değerlendirilmiştir.

Bulgular: DPPH serbest radikal süpürme testi IC_{50} değerini 127,33 ± 1,07 µg/mL olarak gösterirken, ABTS testi IC_{50} değerini 46,74 ± 2,53 µg/mL olarak ortaya koymuştur. FRAP analizi ise indirgeme gücünün 44,62 ± 0,61 mg TE/g ekstrakt eşdeğerinde olduğunu göstermiştir.

Sonuç: *Plantago major* ekstraktının yüksek fenolik ve flavonoid içeriği, güçlü antioksidan aktivitesine önemli ölçüde katkıda bulunmaktadır. Bu bulgular, bitkinin doğal bir antioksidan kaynağı olarak farmasötik ve nutrasötik endüstrilerinde potansiyel kullanımını desteklemektedir.

Anahtar Kelimeler: ABTS, Antioksidan, DPPH, FRAP, *Plantago major* L.

Introduction

The genus *Plantago* L. (Plantaginaceae) is represented worldwide by more than 200 species and in Turkey by 27 species. *Plantago major* L., a perennial herbaceous plant, is one of the most widespread species within the genus. It is characterized by its rosette-forming leaves, which are broadly ovate with prominent parallel venation, and small, inconspicuous flowers arranged in dense spikes. The plant thrives in a wide range of habitats, including meadows, roadsides, and disturbed areas, and is well-adapted to diverse environmental conditions (Haddadian et al., 2014; Kolak et al., 2011).

Pollen research has demonstrated that *P. major* was introduced into the Nordic nations concomitant to the establishment of the first crude cultivated fields in the stone age around 4000 years ago (Samuelsen, 2000). P. major was spread all over the world by humans starting in Europe. The Indians called it 'White man's footprint' since it was found everywhere Europeans had gone. The genus name *Plantago*, which comes from the Latin planta, which means sole of the foot, has been derived from this (Samuelsen, 2000).

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P. major has a long history of being used traditionally to treat wounds. In the first century, Dioscorides wrote about it in "De materia medica." The leaves were used as a remedy for dog bites (Roca-Garcia, 1970). It is known that in Turkey, it is used externally in folk medicine for furuncle, mastitis, wounds, anti-inflammatory, vaginitis, conjunctivitis (Gözcü, Korkmaz, et al., 2024; Yiğit & Gözcü, 2024).

Traditional medicine has long utilized *P. major* for its anti-inflammatory and antimicrobial properties, yet the specific compounds responsible for these effects remain poorly understood. A detailed phytochemical and bioactivity analysis is therefore necessary to scientifically validate these traditional claims and identify potential therapeutic applications. In previous phytochemical analyses conducted in this direction, the presence of alkaloids (Samuelsen, 2000), caffeic acid derivatives (Skari et al., 1999), flavonoids (Skari et al., 1999), and iridoids (Taskova et al., 1999) in the herba of the plant was reported. Again, in studies conducted on the aerial parts of the plant, it has been reported that it has antiulcerogenic (Ragheb et al., 2021), anticancer (Daştan et al., 2016), antibacterial (Sousa et al., 2025), antiviral (McCutcheon et al., 1995), anti-inflammatory (Núñez Guillén et al., 1997), analgesic (Núñez Guillén et al., 1997), antioxidant and free radical scavenger (Stanisavljević et al., 2008) activities.

The aerial parts of *P. major* were subjected to methanolic extraction to evaluate their antioxidant and phytochemical properties. The study comprehensively assessed the *P. major* extract's which is traditionally used in folk medicine in Erzincan radical scavenging capacity using ABTS and DPPH assays, alongside its reducing power potential. Furthermore, the total phenolic and total flavonoid contents were quantified, providing valuable insights into the plant's bioactive compound profile. This study emphasizes *Plantago major*'s importance as a possible natural antioxidant source and its use in the functional food, pharmaceutical, and nutraceutical industries.

Methods

Plant Material and Extraction

The plant material was collected in May 2024 from Yanızbağ, Erzincan, Türkiye (39° 48' 23.94"N, 39° 22' 54.31"E), located at an altitude of 1324 m. Fresh *Plantago major* was used for extraction. The herbarium specimens were prepared by drying the collected plant material under shaded conditions with adequate ventilation to ensure preservation and prevent degradation of morphological and chemical characteristics. The authentication of voucher specimens was conducted by Prof. Dr. Ali Kandemir and deposited at the Herbarium of Erzincan Binali Yıldırım University in Erzincan, Turkiye (Akşit 13885)

In a shaded area, the plant material (Herba) was allowed to dry at room temperature. A laboratory mill was used to crush the dried aerial parts into a fine powder, and 12 g of the powdered material was macerated in 70% methanol (3 × 500 mL) at room temperature (It was used to extract both lipophilic and hydrophilic secondary metabolites). This process was performed using a mechanical mixer for 8 hours per day over 3 consecutive days to maximize extraction efficiency. After that, the methanolic extract was filtered and It was vacuum concentrated at 40 °C in a rotating evaporator. Following dry concentration, 2.4 g of methanolic extract was obtained. The obtained methanolic extract was stored at +4 °C away from light and moisture for further analysis.

DPPH·Free Radical Scavenging Ability

The free radical scavenging activity was evaluated using a modified version of the Akman et al. method (Akman et al., 2023). The experiment involved preparing a 0.26 mM DPPH· solution and stock solutions of extracts at 1 mg/mL. The stock solutions were taken in varying volumes (20-400 µL) and adjusted to a final volume of 3 mL with MeOH. After adding 1 mL of the DPPH· solution, each solution was vortexed and incubated for 30 minutes at room temperature. At the end of the incubation time, each reaction mixture's absorbance was measured at 517 nm. The method was repeated six times, and the mean and standard deviation of the findings were determined. The absorbance results were converted to a percentage of DPPH· scavenging activity, and the IC_{50} (µg/mL) for each extract was calculated.

ABTS·+ Radical Scavenging Ability

The extract's ABTS·+ radical scavenging activity was established by refining the Re et al. Method (Re et al., 1999). In short, 2.45 mM $K_2S_2O_8$ (potassium persulfate) solution and 2 mM ABTS (2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) solution were produced in 0.1 mM pH: 7.4 phosphate buffer. The ABTS·+ radical was produced by mixing these two solutions at a ratio of 1:2. It was subsequently kept at room temperature for 12 hours in the dark. Following that, stock solutions containing 1 mg/mL of the test samples were prepared. After adding 3 mL of phosphate buffer to extract solution (20–400 µL), 1 mL of the ABTS·+ solution was added to each solution. After 60 minutes of incubation at room temperature in the dark, the absorbance was measured at 734 nm. The method was repeated six times. The extract's IC_{50} (µg/mL) is calculated by converting the read absorbance measurements to % activity. The findings were compared to BHT, BHA, and trolox, which are common antioxidants.

Ferric Reducing Power

Akyüz's method was adjusted for the sample's reducing power. 250 µL of extract was diluted with 1.25 mL of $K_3Fe(CN)_6$ solution after preparing up to 1.25 mL with 0.2 M phosphate buffer (pH 6.6). Following a 20-minute incubation period at 50°C in a water bath, the mixture was allowed to cool to room temperature. The cooled liquid was combined with 1.25 mL of TCA (10 %) and 0.25 mL of $FeCl_3$ (0.1 %), and the absorbance of the mixture at 700 nm was then measured. The calibration curve was used to convert the absorbance of the samples to mmol TE activity/g. Trolox concentrations ranging from 10 to 100 µmol/L made up the calibration curve. Absorbances were measured

three times in order to calculate mean values and standard deviations (Akyüz, 2019).

Total Phenolic Content

The extract's total phenol content (TPC) was determined, and the findings were expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract), according to Gözcü et al. stock solutions containing 1 mg/mL of extract and gallic acid were made. 4.5 milliliters of distilled water were used to dilute 0.1 milliliters of the extract stock solution. It was prepared up to 5 mL using 0.1 mL of Folin-Ciocalteu reagent and 0.3 mL of Na_2CO_3 (2%) in total. It was vortexed and incubated for 120 minutes in the dark after 10 minutes at room temperature. At 760 nm, the solution's absorbance was measured in triplicate (Gözcü, Akşit, et al., 2024).

Total Flavonoid Content

The total flavonoid content (TFC) in the extract was measured using spectrophotometric technique with aluminum chloride. The standard utilized was quercetin. Methanol was used to create stock solutions of the extract and quercetin (1 mg/mL). A cuvette was filled with 0.1 mL of extract stock solution, 4.7 mL of methanol, 0.1 mL of 10% $AlCl_3$, and 0.1 mL of 1 M ammonium acetate solution. The cuvette was then vortexed. At room temperature, the mixture was incubated for 45 min. A spectrophotometer was used to measure the absorbance at 415 nm following incubation. Different concentrations of quercetin (1-800 µg/mL) were used to create a calibration curve. The extract's total flavonoid content (TFC) was determined using a calibration curve and represented as mg of quercetin equivalent (QE) per gram. The mean and standard deviation of the TFC were calculated (Chang et al., 2002).

Statistical analysis

The Kruskal–Wallis test was utilized to assess statistical signifcance. Data analysis was carried out using SPSS software (IBM SPSS Statistics 20, IBM Corporation, Armonk, NY, USA), with a signifcance level established at p=0.05. The IC_{50} values for the extracts are reported as mean±standard deviation

Results

The antioxidant capacity and phenolic composition of the methanolic extract of *Plantago major* were evaluated using various spectrophotometric methods. The findings, presented in Table 1, revealed significant antioxidant activity and bioactive compound content, highlighting the potential of *P. major* as a natural antioxidant source. The free radical scavenging activities of the extract were assessed using DPPH and ABTS assays. The DPPH assay showed an IC_{50} value of 127.33 ± 1.07 µg/mL, indicating moderate radical scavenging capacity compared to standard antioxidants such as BHA (8.04 ± 0.69 µg/mL), BHT (10.70 ± 0.0.73 µg/mL), and trolox (8.50 ± 0.77 µg/mL). Similarly, the ABTS assay demonstrated stronger activity, with an IC50 value of 46.74 ± 2.53 µg/mL, suggesting that *P. major* contains compounds capable of neutralizing ABTS radicals effectively. However, its activity was lower compared to standard antioxidants such as BHA (6.33 ± 0.19 µg/mL), BHT (9.42 ± 0.63 µg/mL), and trolox (7.07 ± 0.15 µg/mL), indicating that *P. major* exhibits promising antioxidant potential.

Table 1. Total phenolic (TPC), flavonoid (TFC), DPPH, ABTS, and FRAP values of methanolic extract of *P. major*

Sample and standards	DPPH IC_{50} (µg mL^{-1})	ABTS IC_{50} (µg mL^{-1})	Total phenolics mg GAE g^{-1} Extract	Total flavonoids mg QE g^{-1} Extract	Reducing power mg TE g^{-1} Extract
Plantago major	127.33±1.07	46.74±2.53	46.02±0.48	12.86±1.27	44.62±0.61
Trolox	8.50±0.77	7.07±0.15	-	-	-
BHA	8.04±0.69	6.33± 0.19	-	-	338.57± 0.31
BHT	10.70±0.73	9.42±0.63	-	-	257.80± 1.24
Ascorbic acid	9.91±0.87	8.25±0.41	-	-	394.17±0.98

The reducing power of the extract, evaluated using the FRAP assay, was 44.62 ± 0.61 mg TE/g-1 extract. While the reduced capacity was lower than that of synthetic antioxidants such as ascorbic acid (394.17 ± 0.98 mg TE/g-1), BHA (338.57 ± 0.31 mg TE/g-1), and BHT (257.80 ± 1.24 mg TE/g-1), it still indicates a notable potential for electron donation, contributing to the overall antioxidant activity of the extract.

The total phenolic content (TPC) of the extract was measured as 46.02 ± 0.48 mg GAE/g -1 extract, and the total flavonoid content (TFC) was 12.86 ± 1.27 mg QE/g -1 extract. These findings confirm the rich phenolic and flavonoid profile of *P. major*, which aligns with previous studies reporting the plant's high content of bioactive compounds with antioxidant properties.

Discussion

Antioxidants are molecules able to inhibit the oxidation of other molecules by eliminating free radicals or decreasing their formation. In biological systems the effectiveness of an antioxidant system is fundamental because of the constant generation of free radicals inside the organism at several sites, which potentially may cause oxidative damage and consequent loss of function of proteins, lipids and nucleic acids. The effectiveness of antioxidants against oxidative damage in biological environments is directly related to their chemical structure. The ability to chelate transition metals or to interact

with membranes is affected by several factors, such as type, reactivity, and stability of the free radical formed (Dorta et al., 2008; Halliwell et al., 1995; Rice-Evans et al., 1997).

The antioxidant and phenolic composition of *Plantago major* observed in the present study is supported by previous phytochemical investigations of this plant. Numerous bioactive compounds have been isolated and identified from the leaves and aerial parts of *P. major*, demonstrating a wide array of biological activities. Among the compounds isolated and identified in earlier studies are acteoside (verbascoside) (Eldesoky et al., 2018), plantamajoside (Mazzutti et al., 2017), aucubin (Mazzutti et al., 2017), catalpol (Rahamouz-Haghighi et al., 2023), luteolin (Beara et al., 2009), luteolin-7-O-glucoside (Beara et al., 2009), apigenin (Rahamouz-Haghighi et al., 2023), and ferulic acid (Bourne et al., 1999). These compounds have been linked to significant antioxidant, anti-inflammatory, and antimicrobial properties.

Acteoside (verbascoside) and plantamajoside, two phenylethanoid glycosides abundantly found in *P. major*, have been extensively reported to possess potent antioxidant activity due to their capacity to scavenge free radicals and chelate metal ions. For instance, Lin et al. demonstrated the strong radical scavenging and lipid peroxidation inhibition potential of acteoside (Li et al., 2018). Similarly, plantamajoside was reported to exhibit both antioxidant and UV-protective properties (Samuelsen, 2000).

The iridoid glycosides aucubin and catalpol are also prominent phytoconstituents of *P. major*. Aucubin has shown hepatoprotective and anti-inflammatory effects, as highlighted by Huang et al. (Huang et al., 2022), while catalpol has been recognized for its neuroprotective and antioxidant activities (Jiang et al., 2015). These compounds likely contribute to the moderate DPPH and ABTS radical scavenging activities observed in the current study.

Luteolin and its derivative, luteolin-7-*O*-glucoside, are flavonoids commonly detected in *P. major*. Both have been demonstrated to have strong antioxidant and anti-inflammatory properties (Ahmadi et al., 2020; De Stefano et al., 2021). demonstrated that luteolin effectively inhibits oxidative stress and inflammatory mediators, aligning with the high phenolic content reported in this study. Additionally, apigenin, a less abundant flavonoid in *P. major*, has been documented for its antioxidant and anticancer properties (Imran et al., 2020; Tian et al., 2021).

Ferulic acid, a hydroxycinnamic acid found in the aerial parts of *P. major*, is another compound with significant antioxidant potential. Kikuzaki et al. (Kikuzaki et al., 2002) reported its ability to protect against oxidative stress by scavenging free radicals and inhibiting lipid peroxidation.

These data suggest that the antioxidant activities seen in the methanolic extract of *P. major* are due to the synergistic actions of its phenolic components, glycosides, and flavonoids. Although the extract performed less well than standard synthetic antioxidants such as BHA, BHT, and trolox in some tests, the presence of naturally occurring bioactive compounds with multifunctional properties makes *P. major* a promising source of natural antioxidants for pharmaceutical and nutraceutical applications.

Conclusion

This study shows that *Plantago major*'s methanolic extract has a high antioxidant capacity and phenolic content. In the DPPH and ABTS evaluations, the extract demonstrated moderate free radical scavenging activity as well as detectable reducing power, which was supported by its high phenolic and flavonoid content. These effects are most likely attributed to bioactive substances such as acteoside, plantamajoside, aucubin, catalpol, luteolin, and ferulic acid, which contribute to the plant's antioxidant, antiinflammatory, and antibacterial capabilities. The extract's natural composition and multifunctionality emphasizes its potential for pharmaceutical and nutraceutical uses, even if its activity was lower than that of synthetic antioxidants such as BHA, BHT, and trolox. According to these results, *P. major* is a potential natural antioxidant source that deserves more research into its bioactive components and potential medical applications.

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COVID-19 Pandemisi, Kronik Hastalıklar ve Geleneksel, Tamamlayıcı ve Fonksiyonel Tıp Uygulamaları

COVID-19 Pandemic, Chronic Diseases, Traditional, Complementary and Functional Medicine Practices

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ÖZ

Yeni Koronavirüs Hastalığı COVID-19, Çin'in Wuhan kentinde 2019 yılının sonlarına doğru ortaya çıkan ateş, dispne ve öksürük gibi semptomlar gösteren, 13 Ocak 2020'de tanımlanan bir virüstür. 2023 yılında hala etkisini sürdüren virüs birçok kez mutasyona uğramış ve yeni varyantlar ortaya çıkmıştır. COVID-19 son derece hızlı çoğalarak vücudun tüm sistemlerini ele geçiren oldukça bulaşıcı bir hastalıktır. Hastalık diyabet, hipertansiyon, astım, böbrek yetmezliği ve immün yetmezliği gibi komorbiditeye sahip bireylerde daha ağır seyretmektedir.

COVID-19 için henüz etkililiği ve güvenilirliği kesin olarak kanıtlanmış bir tedavi bulunmamaktadır ve bu durum hastaları Geleneksel, Tamamlayıcı ve Fonksiyonel Tıp Uygulamalarını (GETAT) kullanmaya sevk etmektedir. Bu yazıda COVID-19 pandemisinde kronik hastalıklar ve GETAT kullanımı ele alınmıştır.

Anahtar Kelimeler: COVID-19, Kronik hastalıklar, GETAT

ABSTRACT

New Coronavirus Disease COVID-19 is a virus that was identified on January 13, 2020, and showed symptoms such as fever, dyspnea and cough, which appeared in Wuhan, China, towards the end of 2019. The virus, which is still effective in 2023, has mutated many times and new variants have emerged. COVID-19 is a highly contagious disease that multiplies extremely quickly and takes over all systems of the body. The disease is more severe in individuals with comorbidities such as diabetes, hypertension, asthma, kidney failure, and immune deficiency.

There is no treatment yet with proven efficacy and safety for COVID-19, and this prompts patients to use Traditional, Complementary, and Functional Medicine Practices (T&CM). This article discusses chronic diseases and T&CM use in the COVID-19 pandemic.

Keywords: COVID-19, Chronic diseases, T&CM

Giriş

2019 yılının bitmesine günler kala Çin'de ortaya çıkıp haftalar içinde bütün dünyayı etkileyen emsali görülmemiş bir salgın başlamıştır. Korona virüslerin sebep olduğu bu hastalık Corona Virüs Hastalığı (COVID-19) olarak adlandırılmıştır (Dünya Sağlık Örgütü, 2023).

Çok kısa sürede virüs ile enfekte olan kişi sayısı milyonlara ulaşmıştır (Dünya Sağlık Örgütü, 2023). Hastalığın hızlı bir şekilde geniş sahalara çok kısa sürede yayılmasının sebebi kişiden kişiye kolay bir şekilde bulaşmasıdır (Wang ve ark., 2020a). COVID-19'un Diabetes Mellitus (DM), Hipertansiyon (HT), kalp hastalığı gibi komorbiditelerle birlikte olması, semptomların şiddetini arttırmakta, hastalığın seyrini olumsuz yönde etkilemekte ve mortalite oranlarını arttırmaktadır (Guan ve ark., 2020a).

Pandemi döneminde COVID-19'un kesin bir tedavisi bulunmaması sebebiyle daha önce farklı alanlarda kullanılan ilaçların tedavi edici özelliğinden yararlanılmıştır (DSÖ, 2023). COVID-19 spesifik tedavisindeki bu belirsizlikler Geleneksel, Tamamlayıcı ve Fonksiyonel Tıp Uygulamalarının (GETAT) gündeme gelmesine neden olmuştur. Toplumun büyük bir kesimi gerek hastalığın bulaşmasından

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korunmak gerekse hastalık döneminde ortaya çıkan semptomları hafifletmek amacıyla GETAT yöntemlerine başvurmuştur (Ang ve ark., 2020; Hijazi ve ark., 2023; Jeon ve ark., 2022; Parvizi ve ark., 2022).

COVID-19

COVID-19, ateş, dispne ve öksürük gibi semptomlar gösteren, 13 Ocak 2020'de tanımlanan bir virüstür (Sağlık Bakanlığı, 2023b). Ülkemizde COVID-19 vakası 11 Mart 2020'de görülmüştür. Aynı tarihte Dünya Sağlık Örgütü (DSÖ) tarafından COVID-19 pandemi olarak ilan edilmiştir ve başta Wuhan kenti olmak üzere birçok ülkede sıkı önlemler ve karantinalar başlamıştır (Türkiye Bilimler Akademisi, 2020).

COVID-19 virüsü damlacık yolu ve hasta bireylerin sekresyonları ile temastan sonra ellerin burun, göz ve ağız teması sonrası bulaşır. Hasta bireylerin öksürme hapşırma sonrası havada asılı kalan mikro partiküllere temas sonucu bulaş gerçekleşmesi hastalığın yayılmasını kolaylaştırıp hızlandırmıştır (Türken & Köse, 2020).

COVID-19 her hastada farklı şiddetlerde semptomlar göstererek ortaya çıkar. Bazı hastalarda hafif üst solunum yolu enfeksiyonu (ÜSYE) belirtileri görülürken bazı hastalarda bu durum kendini ağır pnömöni tablosu olarak ortaya çıkarır (Tan ve ark., 2022). Hastalarda en çok görülen semptomlar dispne, yüksek ateş, geçmeyen öksürüktür. Bunun yanında şiddetli baş ağrısı, eklem ve kas ağrısı, tat ve koku kayıpları ile bulantı ve kusma da görülebilmektedir (Huang ve ark., 2020a).

COVID-19 salgınının kontrol altına alınabilmesi için erken dönemde tanılanması ve tedaviye başlanması son derece önemlidir (Pascarella ve ark., 2020). Tanı için yapılan toraks tomografisinde tutulumlar görülmektedir (Huang ve ark., 2020b). COVID-19 tanısı koymak için moleküler ve serolojik testler kullanılır. Moleküler testlerden olan rRT-PCR (gerçek-zamanlı reverse transkripsiyon polimeraz zincir reaksiyonu) hastalardan alınan nazofarengeal ve orofarengeal sürüntüler ile pnömönisi olan hastalardan alınan bronkoalveolar lavaj sıvısı ve balgam örnekleri ile yapılır. Serolojik testlerde konakçının virüse karşı geliştirdiği antikorları belirlemek için hızlı ve güvenli testlerdir (Dülger & Ekici, 2020). Görüntüleme testlerinde ki bulgular COVID-19'a özgü olmasa da temas öyküsü olan ve klinik tablosu uyumlu olan hastalarda tanı koymak için yardımcı olur (Goudouris, 2021).

COVID-19 için henüz etkililiği ve güvenilirliği kesin olarak kanıtlanmış antiviral bir tedavi bulunmamaktadır. COVID-19 virüsünün hızlı bir şekilde yayılması ve hastalığın ölümcül olması sebebiyle ülkemizde ve başta Avrupa ve ABD olmak üzere diğer ülkelerde de daha önce başka hastalıkların tedavisinde kullanılan güvenli olduğu gösterilen, ruhsatlandırılmış antiviral tedavinin COVID-19 karşı etkili olduğu belirlenmiş ve kullanılmaya başlanmıştır. Kullanılmaya başlanan bu ilaçların güvenilirlik ve etkililik ile ilgili çalışmaları hala devam etmektedir (Sağlık Bakanlığı, 2023a).

COVID-19 ve Kronik Hastalıklar

COVID-19 yaşlı bireylerde ve komorbiditeye sahip yetişkinlerde daha ağır seyretmektedir. Literatürde COVID-19'un kardiyovasküler hastalık, obezite, DM, HT, KOAH, böbrek yetmezliği ve immün yetmezliği olan hastalar için potansiyel risk faktörü olduğu belirtilmiştir (Sandalcı ve ark., 2020).

Wang ve ark., 2020b yılında bir çalışma yapmış ve bu çalışmada 138 COVID-19 vakası incelenmiş ve hastaların %46.4'ünde komorbidite varlığı saptanmıştır. Çin'de yapılan bir diğer çalışmada 1590 COVID-19 hastası ele alınmış ve hastaların %25'inin en az bir komorbiditeye sahip olduğu saptanmıştır. Bu araştırmaya göre en yaygın görülen komorbiditeler %16.9 ile HT, %8.2 ile DM'dir (Guan ve ark., 2020b; W. Wang ve ark., 2020b). Çin'de yapılan başka bir çalışmada 726.314 COVID-19 vakası incelenmiş genel vaka ölüm oranı %2.3 iken, komorbiditesi olan vakaların ölüm oranının daha yüksek olduğu belirlenmiştir (Wu & McGoogan, 2020).

Pandemi süresince aktivite azlığı, beslenme alışkanlıklarında değişim ve hastaneye başvuruda zorluklar yaşanması gibi nedenlerle DM başta olmak üzere ve kronik hastalıklara sahip bireylerde COVID-19 enfeksiyonuna yatkınlık geliştiği gözlemlenmiştir. COVID-19 pandemisinden korunmada özellikle komorbidesi olan hastalarda aşılama daha da önem kazanmıştır (Ho ve ark., 2021).

COVID-19'dan Korunma

DSÖ, COVID-19 virüsünden korunmak ve hastalığın yayılmasını önlemek için el hijyeni, temizlik, sosyal mesafe izolasyonu, maske kullanımı ve aşı yaptırmayı önermektedir. COVID-19 ile mücadelede en çok tercih edilen korunma yöntemlerinden biri el hijyeni uygulamalarıdır. Kolay uygulanabilir olması, etki oranının yüksek olması, maliyetinin az ve ulaşılabilir basit bir yöntem olması tüm dünyada yaygın bir şekilde uygulanmasını sağlamıştır (Erkal ve ark., 2020).

COVID-19 virüsünün yüzeylerde belirli bir süre canlılığını koruması temizliğin bu dönemde çok önemli olduğunun göstergesidir. Hasta çıkartıları ile kontamine olan yüzeylerin uygun temizlik maddeleri ile temizlenmesi hastalığın yayılmasını önlemektedir (Hollis ve ark., 2021). Sosyal mesafe kurallarına uyulması da COVID-19 virüsünden korunmak için en çok kullanılan politikalardan biri olmuştur (Feldman ve ark., 2022).

Hastalık solunum yoluyla bulaştığı için maske kullanımı korunmada ilk sıralarda yer alan yöntemlerden olmuştur. Maske kullanımı diğer korunma yöntemleriyle birlikte kullanıldığında daha etkili olmaktadır (Özgür ve ark., 2022). Ülkemizde de ortak kullanılan alanlarda ve kalabalık ortamlarda maske kullanım zorunluluğu getirilmiştir. Maskenin doğru kullanımı ile ilgili afişler asılıp broşürler dağıtılıp halkın bilinçlenmesi sağlanmıştır (Candevir ve ark., 2021).

Geleneksel, Tamamlayıcı ve Fonksiyonel Tıp Uygulamaları (GETAT)

İnsanlık var oluşundan beri hastalıkla mücadele de şifa bulmak için doğadan yararlanma yolunu izlemiştir. Bu yönelim GETAT yöntemlerinin ortaya çıkmasına olanak sağlamıştır. Dünya Sağlık Örgütü (DSÖ) GETAT'ı; "Fiziksel ve ruhsal hastalıklardan korunma, bunlara tanı koyma, iyileştirme veya tedavi etmenin yanında sağlığın iyi sürdürülmesinde de kullanılan, farklı kültürlere özgü teori, inanç ve deneyimlere dayalı, izahı yapılabilen veya yapılamayan bilgi, beceri ve uygulamaların bütünüdür" şeklinde tanımlamaktadır (DSÖ, 2019).

Tüm dünya ülkelerinde olduğu gibi ülkemizde de GETAT uygulamalarına yer verilmiştir. GETAT ile ilgili ilk düzenleme 1991 yılında yapılmıştır. Türkiye'de Geleneksel ve Tamamlayıcı Tıp Anabilim Dalı, Sağlık Hizmetleri Genel Müdürlüğü bünyesinde 2012 yılında kurulmuştur. GETAT yönetmeliği ise 27 Ekim 2014 tarihinde ve 29 158 sayılı Resmi Gazete 'de yayınlanmıştır. İlgili yönetmelikte 15 GETAT uygulaması tanımlanmıştır. Bu uygulamalar; akupunktur, apiterapi, fitoterapi, hipnoz, sülük uygulamaları, homeopati, kayropraktik, kupa uygulaması, larva uygulaması, mezoterapi, osteopati, proloterapi, ozon uygulaması, refleksoloji ve müzik terapisidir (Resmi-Gazete, 2014). GETAT Uygulamaları 5 sınıfta incelenmektedir. Bunlar:

Biyolojik Temelli Uygulamalar: Yaşam tarzı değişiklikleri, vitamin ve mineral desteği, bitkiler (fitoterapi, aromaterapi) vb.

Manipülatif Beden Temelli Uygulamalar: Bu yöntemler arasında, acupressure, masaj, terapi, refleksoloji, akupunktur yer almaktadır. Zihin Beden Temelli Uygulamalar: Sanat terapi, biofeedback, dans terapi, hipnoz meditasyon, müzik terapi, dua, psikoterapi, relaksasyon, yoga vb.

Enerji Temelli Uygulamalar: Terapötik dokunma, reiki, biyoelektromanyetik vb.

Tıp Pratiği Uygulamaları: Ayurveda, homeopati vb.

COVID-19 Pandemisinde GETAT Yöntemlerinin Kullanımı

COVID-19'un kesin bir tedavisinin olmayışı tüm dünya çapında insanlarda endişeye yol açmış ve farklı tedavi ve önleme yöntemleri aramaya yönlendirmiştir. Tüm toplumlarda enfekte olma olasılığını azaltmak, bağışıklığı artırmak ve bulaş durumunda enfeksiyonun ilerlemesini hafifletmek için alternatif tedavi seçeneklerini deneme eğilimi başlamıştır (Alyami ve ark., 2020). Özellikle medyada COVID-19 enfeksiyonunun tedavisi ve önlenmesine katkı sağladığı düşünülen takviye gıda ve vitamin reklamlarının giderek artması toplumun bu ürünleri tüketmeye özendirmiştir (Hamulka ve ark., 2020). Çin'de yapılan araştırmalarda toplumun COVID-19 enfeksiyonunun önlenmesi, tedavisi ve rehabilitasyonunda GETAT uygulamalarından etkin olarak faydalanıldığı saptanmıştır (Huang ve ark., 2020a; Shankar ve ark., 2020). Parvizi ve ark.'larının İran'da yaptığı bir çalışmada ise COVID-19 tanılı hastaların %69'unun GETAT yöntemlerini kullandığı ve en çok tercih edilen bitkisel ürünlerin zencefil, kekik ve çörek otu olduğunu saptamıştır (Parvizi ve ark., 2022).

Ülkemizde Karataş ve ark.'larının yaptığı pandemi döneminde toplumun %39,3'ünün GETAT yöntemlerini kullandığını, katılımcıların %30,8 inin bitkisel ilaçları, %23,8'inin ise vitamin ve besin takviyelerini kullanmayı tercih ettiği saptamıştır (Karataş ve ark., 2021).

Literatürde COVID-19 ile enfekte olmuş hastalara yüksek miktarda C vitamini verilmesinin enfeksiyonu önemli ölçüde azaltabileceği hatta durdurulabileceği ve yüksek D vitamini dozlarının faydalı olabileceği savunulmakla birlikte bu vitaminlerin, COVID-19 enfeksiyonunu önlemede ve tedavisinde etkili olduğunu destekleyecek kesin bir kanıt bulunmamaktadır (Nilashi ve ark., 2020a; Nilashi ve ark., 2020b).

İtalya'da COVID-19 hastalarıyla yürütülen randomize kontrollü bir çalışmada müzik terapi müdahalesinin ardından hastalarda görülen yorgunluk, üzüntü, korku ve endişe yoğunluğunda önemli bir düşüş olduğu tespit edilmiştir (Giordano ve ark., 2020).

Kim ve ark.,'larının bibliyometrik analiz yöntemi kullanarak yaptıkları çalışmada, COVID-19 pandemisi döneminde GETAT yöntemlerinin kullanımı ile ilgili 16 yayın değerlendirilmiş ve bu araştırmalarda genellikle fitoterapi, akupunktur ve tai chi gibi Çin tıbbı uygulamalarının kullanıldığı saptanmıştır. Kim ve ark.'larının çalışmalarının sonucunda GETAT yönteminin COVID-19 tedavisinde uzun süre kullanılmaya devam edeceğinin tahmin edildiği ve kanıt düzeyi yüksek çalışmalara ihtiyaç olduğunu belirtmiştir (Kim ve ark., 2022).

Sonuç

Etkileri hala devam eden ve yeni varyasyonlarından bahsedilen COVID-19 virüsü ile mücadelede kronik hastalığa sahip olan bireylerin korunması önemini halen sürdürmektedir. Pandemi döneminde dünya genelinde kullanım oranı artan GETAT yöntemlerinin doğru ve etkili bir şekilde kullanılabilmesi, hastaların olası zararlı etkilerden korunabilmesi için bu yöntemler hakkında uzmanlar tarafından toplumun bilgilendirilmesi ve takip edilmesi gerekmektedir. GETAT yöntemlerinin COVID-19 virüsü üzerine etkilerinden bahsetmek için yeni bilimsel çalışmalara ihtiyaç vardır.

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Edible Insects as An Alternative Food Source and Their Potential Positive Effects on Human Health

Alternatif Bir Besin Kaynağı Olarak Yenilebilir Böcekler ve İnsan Sağlığı Üzerindeki Potansiyel Olumlu Etkileri

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ABSTRACT

The difficulty of safe/quality transportation, the rapidly increasing population and the production of protein products obtained from animals have led us to think that they can be used as an "alternative protein" source. It is reported in the literature that insects are a good source of macro (especially protein, fat and chitin) and many bioactive nutrients. The digestibility rate of edible diseases by human physiology is high, along with the physiology of the progress of the insect species. In the literature, protein content and bioavailability are compared with other plant/animal protein sources, where a good “alternative” food source can be found. In addition to its anti-carcinogenic, anti-bacterial, anti-microbial, anti-inflammatory and immuno-modulatory activities; Many clinical treatments are claimed in different areas of blood glucose levels and blood lipid profile. Authorities and current studies expressing their opinions in this field point out that edible parts of the edible parts may become a part of the more widespread human nutrition in the future as an "alternative" food source to meet the daily needs of the human body. However, comprehensive, safe and hygienic standards should be developed and implemented in order to ensure the consumption of edible insects, which have the potential of alternative food sources for the nutrition of the human population in the future.

Keywords: Edible insects, entomophagy, nutritional value, protein content

ÖZ

Güvenli/kaliteli besine ulaşımın zorlaşması, hızla artan nüfus ve nüfus artışı ile ilişkili olarak hayvansal kaynaklı protein gereksiniminin artması ile “alternatif bir protein” kaynağı olarak böceklerin kullanılabileceğini düşündürmüştür. Böceklerin iyi bir makro (*özellikle protein, yağ ve kitin)* ve birçok biyoaktif besin bileşeni kaynağı olduğu literatürde bildirilmektedir. Yenilebilir böceklerin insan fizyolojisi tarafından sindirilebilirlik oranı böceğin türü ile ilişkili olmakla birlikte, yüksektir. Literatürde protein içeriği ve biyoyararlılığı diğer bitkisel/hayvansal kaynaklı protein kaynakları ile karşılaştırıldığında iyi “alternatif” bir besin kaynağı olabilecekleri düşünülmektedir. Anti-kanserojenik, anti-bakteriyel, anti-mikrobiyal, anti-inflamatuvar, immüno-modülatör etkinliklerinin yanı sıra; kan glukoz düzeyleri ve kan lipit profilinin iyileştirilmesinde kullanılabilecekleri birçok klinik çalışmada iddia edilmektedir. Bu alanında görüş bildiren otoriteler ve güncel çalışmalar, yenilebilir böceklerin insan vücudunun günlük gereksinimlerini karşılamak için “alternatif” bir besin kaynağı olarak gelecekte daha yaygın olmakla birlikte insan beslenmesinin bir parçası olabileceğine dikkat çekmektedir. Ancak gelecekte insan nüfusunun beslenmesi için alternatif besin kaynağı potansiyeli olan yenilebilir böceklerin tüketiminin sağlanması için kapsamlı, güvenli ve hijyenik standartlar geliştirilmeli ve uygulanmalıdır.

Anahtar Kelimeler: Besin değeri, entomofaji, protein içeriği, yenilebilir böcekler

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Introduction

In recent years, with the increasing population of the world, there have been difficulties in accessing adequate, healthy and safe food. While people's dietary habits have changed in recent years, the consumption of protein-rich foods of animal origin and the amount of daily requirements of people have increased in parallel with the spread of Western-type nutrition models, the available food sources (both animal and vegetable) have decreased. As a result of this situation, it is thought that food sources will be insufficient for the world population in the coming years; for this reason, alternative "consumable other sources" alternative to "consumed foods" are also being investigated today (Kuder & Demir, 2023). According to the report published by the Food and Agriculture Organization of the United Nations (FAO) in 2017, it is estimated that by 2050, the consumption of protein from animal sources may be about 2 times higher than at present. The same report also noted the increase in daily protein intake in high-income countries over the past 50 *years (1961: 39 g/person; 2011: 52 g/person- an increase of 33%).* It is estimated that in high-income countries, daily protein intake will reach 54 grams and 57 grams per capita in 2030-2050, respectively; in low-income countries, it will reach 22 grams and 25 grams (FAO, 2017). With the simultaneous increase in daily protein consumption per capita and population, it is one of the notable titles that traditional protein sources cannot meet the amount demanded and alternative protein sources are needed in order to solve this global problem (Aksoy & El, 2021). In response to this demand, the FAO has indicated "edible insects" as an alternative protein source due to their high-quality protein (amino acid profile) and micronutrient source and lower greenhouse gas effect compared to animal-derived proteins (Kaldırım & Keser, 2023). From the past to the present, insects are widely consumed on many continents of the world, especially in Asia, Africa and Latin America. It is estimated that insects are widely consumed by at least 2 billion people worldwide. Attitudes towards the acceptance of insect consumption are associated with sociocultural and psychological reasons. Even though edible insects are not widely consumed in Western societies and there is a prejudice against insect consumption; current studies report that the food labeling studies and the way of use that can be done for these alternative food sources will contribute to an increase in consumer acceptance (Raheem et al., 2019; Muslu, 2020). "Entomophagy" is a concept that refers to the use of insects as food. The trend towards entomophagy, an old concept, has increased in order to develop an additional strategy for animal October to solve the growing need for sustainability, food insecurity in the face of climate change. Edible insects have been characterized as a new source of high-quality proteins as an alternative to traditional animal-derived foods (Erdoğan et al., 2021; Imathiu, 2020). In addition to being considered a rich source of macro/micronutrients, edible insects are also known to have many benefits from an environmental point of view (sustainability). In the studies conducted, edible insect production is associated with less water requirements and lower greenhouse gas effect compared to pig, cattle, chicken farming. In one study, the water requirement of cricket production per gram of protein was 0.7-0.8 grams, while; it has been stated that the requirement of bovine protein per gram is about 16.8 grams. In the same study, methane and carbon dioxide emissions per kilogram of cricket were found to be 0.7 and 7.6 grams, respectively; for cattle, they were found to be 114 grams and 285 grams. There are also advantageous aspects of edible insects such as the need for less growing space, high feed conversion efficiency and high reproduction rate (Imathiu, 2020; Nowakowski et al., 2021).

Due to the fact that insects have not been used in human nutrition in Europe in the past, there are no legal regulations regarding their use, but appropriate regulations should be made on this subject, assuming that insects will be more involved in human nutrition in the future. In the *FAO 2013 Edible Insects Future Prospects for Food and Feed Security Report*, it has been reported that these alternative food sources may be a sustainable food source in the future and a comprehensive analysis has been presented. In the same report, insect consumption was encouraged due to its high-quality nutrient content (Kaldırım & Keser, 2023; Erdoğan et al., 2021). In its *2021 Report*, *the European Food Safety Authority (EFSA)* argued that insects can be considered as an alternative to basic animal sources for human nutrition, such as chicken, pork, beef and fish, and there may be various legal requirements affecting their use. EFSA has listed the insects that have a high potential to be used as food and feedstuff in agriculture as follows; black fly, black soldier, yellow flour worm, moth, silkworm, cricket and grasshopper (Kaldırım & Keser, 2023; EFSA, 2015). It has been determined that there are >1900 edible insect species worldwide. The most used ones in human nutrition are "caterpillars, wasps, ants, bees, grasshoppers, crickets, Augustan beetles, termites, dragonflies and flies" (Liceaga et al., 2022). It is thought that protein, lipids, fiber (pulp) and some micronutrient elements found in insects may have an important place in human nutrition; these nutritional components may have potential positive effects on human health. In the current literature, edible insects contain antioxidant/anti-inflammatory nutritional elements; they may have anti-bacterial, anti-obesogenic activity, immunomodulatory effects; serum glucose levels may be important for regulating the blood lipid profile (reduced risk of cardiovascular disease); as well

as having positive gastrointestinal (GI) and microbiota effects, as well as various potential health effects are claimed (Liceaga et al., 2022; Zhou et al., 2022) (Figure 1). Edible insects are among the headlines that attract attention every day Decently due to their alleged potential health benefits. In this review, the current literature has been reviewed and the total protein, total lipid, chitin content of edible insects, as well as other nutritional components that stand out in terms of human nutrition; their potential positive effects on human health have been discussed. It is aimed to present an alternative perspective on the consumption of edible insects, which are thought to find more places in human nutrition in the future, in the light of current literature.

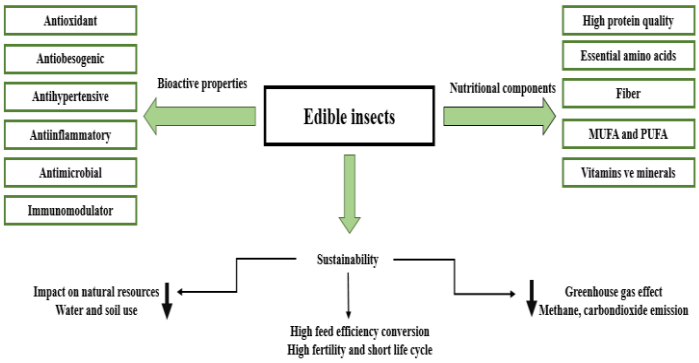

Figure 1. The reasons for using edible insects as a food source and their positive effects on health. †MUFA: Monounsaturated fatty acids; †PUFA, Polyunsaturated fatty acids

Components of Macro/Micronutrient Elements that Come to the Fore in Edible Insects

For many years >2,000 insect species have been used as a basic food source in many regions of the world. In the studies conducted, it is reported that edible insects contain high amounts of important nutritional components in terms of human nutrition. Although it varies depending on the species, it has been shown that about 77% to 98% of the total weight of edible insects is digestible in terms of human physiology, but the analyses conducted in the field of digestibility and bioavailability of edible insects are not yet sufficient in the literature and more research is needed on this issue. As a result of the studies, insect species, processing method, antinutritional factors were identified as factors affecting digestibility and bioavailability. Alternative these food sources can be consumed raw or processed, roasted, fried, boiled. The nutritional composition may vary depending on the genus, stage of development, mode of nutrition and processing method of the insect. Insects are seen as a rich source of energy, protein, fats and minerals and are associated with positive effects on human health (Imathiu, 2020; Nowakowski et al., 2021). Nutritional element compositions prepared by considering the amount of dry matter of edible insect species that are widely consumed are given in Table 1 (Castro et al., 2018).

Table 1. Macro and some micronutrient components prepared by considering the amount of dry matter of edible insects that are widely consumed

Insect species (100g per)	*Protein (g)*	*Fat (g)*	*Carbohydrate (g)*	*Mineral (g)*	*Energy (kcal)*
Coleoptera	3.7-54	3.7-52	12-34	1-3	126-574
Flies	17.5-67	4.2-31	8.38-23	1.24-8	199-460
Insects	33-65	7-54	7-19	1-19	329-622
Bees, Wasps, Ants	1-81	1.3-62	5-94	0-6	234-593
Butterflies and moths	13.2-69.6	7-77	3-41	2-8	126-762
Grasshoppers and crickets	13-77	2.4-25.14	16-30	2-27	117-436

Total Protein Content and Amino Acid Profile

When the dry weight of insects is considered, proteins constitute the largest component of the total mass. It has been reported that the total protein content of edible insects is high (7% to 91% by dry weight), the amino acid profile is balanced (Zhou et al., 2022; Huis, 2016). In a study, the nutritional composition of 6 different insects, including cricket, giant flour worm, yellow flour worm, silkworm, Java grasshopper and paddy grasshopper, were studied by drying at 60-70 degrees Celsius (°C) in the range of 12-24 hours. As a result of the protein analysis performed by the Kjeldahl method, it has been reported that the protein compositions of insects differ significantly from each other (they contain protein that varies by 32.59-76.69% for every 100 grams of dry weight). Among these 6 species, cricket is the species with the lowest protein content with a ratio of 32.59% Dec. Edible insect alternatives Compared to soybeans, which are a plant-based protein source (they contain 35.8% plant-based protein), it was thought that insects might also be a good protein alternative (Kuntadi Adalina & Maharani, 2018). Insects, which are rich in protein, have a wide range of amino acids and a large amount of essential amino acid content (Zhou et al., 2022). In another study in which the protein and amino acid composition of 4 insects, namely Bombay grasshopper, scarab beetle, grasshopper and mulberry silkworm, was investigated; As a result of the analysis performed according to the Kjeldahl method, 100 grams of insects had protein contents that differed in the range of 27-54 grams. The insect samples in the same study contained 18 of the 20 amino acids; the essential amino acids contained all of them (the highest proportion of essential amino acids was 44%

mulberry silkworm; the lowest rate is 33% in the Bombay locust), and it has been reported that the amino acid “leucine” has the highest concentration among these insect species (Köhler et al., 2019). On the other hand, it has been shown that the average amino acid score of edible insects is between 46-96% (in human nutrition Decrees that the basic amino acid score should be at least 40%). Edible insects, in relation to the species, are 67-98% digestible; therefore, they can be described as a source of high-quality protein. Compared to animal-derived and vegetable-derived protein sources such as beef, chicken, eggs, edible insects can be characterized as “nutritious” in the human diet and are thought to have potential positive effects on health (Imathiu, 2020; Nowakowski et al., 2021). In a study conducted, the amino acid profile and quality of moth caterpillar, termite, cricket and grasshopper insect species common in Nigeria were examined. As a result of the examination conducted using the Technican sequential multi-sample amino acid analysis, it was found that edible insects are good sources of various essential amino acids such as lysine, threonine, leucine, isoleucine, valine, phenylalanine and tyrosine. In the analysis of protein quality studied by the corrected protein digestibility method, digestibility was found to be in the 76-98% range (Inje et al., 2018).

Total Fat Content and Fatty Acid Profile

The second highest amount of edible insects’ nutritional component in the analysis of the composition of the nutrients in fats. The total fat content, although the amount varies in different stages of the life of the insects, 80% of the triacylglycerols (TG) and 20% trans fatty acids. While the total fat content of insects is the highest at the “larval” stage, which is the beginning of life, the amount in dry weight can vary by about 10% to 50% (Castro et al., 2018; Lucas et al., 2019). The total lipid content of insects may vary depending on many factors such as “species, diet, extraction method and environmental differences”. Considering the amount of dry matter, the fat content of grasshoppers varies between 17-28%, while the larvae of the black soldier fly have a fat content of 6.6-39%, and the yellow flour wolf has a fat content of 21-31% range of grasshoppers have a fat content of 6.6-39%. It has been reported that flour wolf contains a higher amount of oil when comparing the oil content with soybeans, which contain about 20% oil by dry matter weight (Oonincs et al., 2019; Paul et al., 2017). In the analyses carried out, it was shown that oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acids constitute the fatty acid components of edible insects. These alternative food sources are rich in monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids; they contain a high amount of C:18 fatty acids. In the “pupal” stage, saturated fatty acids are more dominant compared to unsaturated fatty acids; in the ”adult" period, this is the opposite (Castro et al., 2018; Meyer-Rochow et al., 2021). When the nutritional values of meat and edible insect species in the human diet are compared, it is concluded that both meat and insects are rich in protein, fat, minerals and vitamins, including essential amino acids and PUFA, which are considered essential for human physiology. In the same study, it was also reported that the types of fatty acids found in the highest amounts in meats and insects are “oleic acid” and “linoleic acid”. In addition to meat, it can be concluded that insects are also nutritious sources (Orkusz, 2021).

Contents of The Chitin

One of the important components in the recognition of insects as a valuable alternative food source is the content of chitin. Chitin is a natural carbohydrate polymer that is most found in nature after cellulose. Its basic molecular structure is given in Figure 2 (Orkusz, 2021). It is described as the main component found in the shells of crustaceans, the cell wall of fungi and the exoskeleton of insects (Rehman et al., 2023; Abram et al., 2019). Insects may contain different amounts of chitin in relation to the stage of development. The older the insect, the greater the content of the kit. In the studies conducted, it has been reported that edible insects contain an average of 15-25% chitin (yellow flour worms have a chitin content of 16-17%, house cricket has a chitin content of 4-7% and silkworm has a deciency of 3-20%. Insects can also be described as a good source of fiber (pulp) due to the amount of chitin they contain (23, 25).

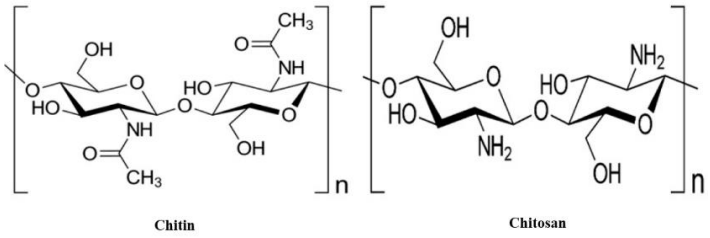

Figure 2. Chitin and chitosan structures

Potential Positive Effects of Insects on Human Health

Current literature draws attention to the fact that edible insects can be part of the daily diet to meet the daily requirement of the human body. Bioactive nutritional components found in insects may have various functional properties that make a potential contribution to human health. Thanks to the macro- and micronutrient elements contained in edible insects; it can provide anti-cancer, anti-bacterial, anti-microbial, anti-inflammatory, immunomodulatory effects through different mechanisms; it is noted that they may influence improving blood sugar and blood lipid profile (Zhou et al., 2022; Roos & Huis, 2017). Some biological activities and potential mechanisms of action associated with bioactive nutrient components found in the

structure of these alternative food sources were summarized in Figure 3 (Zhou et al., 2022).

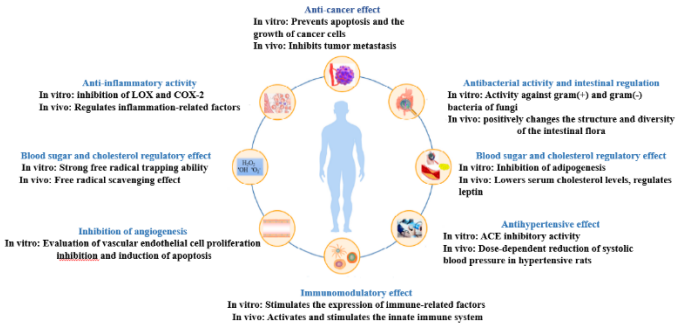

Figure 3. Some biological effects and potential mechanisms of action associated with some bioactive nutritional components found in edible insects

Edible insects naturally bioactive food components that are included in several of antioxidants (Aox) is known to have effect. Hydrolysis products obtained from insects by different methods (some substances such as peptides and chitosan) have different degrees of Aox activity. These components have anti-inflammatory effect, many of the ones that have protein-based while; LOX peptide substances (lipoksijenaz), COX-2 (Cyclooxygenase-2) and nuclear factor kappa-B (NF-kB) signaling by inhibiting anti-inflammatory effects, there are studies that support that it can have. “Glycosaminoglycans”, a type of polysaccharide found in crickets, have been shown to have an anti-inflammatory effect by inhibiting C-reactive protein (CRP) and Rheumatoid Factor (RF) (Zhou et al., 2022; Nowakowski et al., 2021). On the other hand, the antibacterial activities of insects have been associated with inhibiting Gram (+) and Gram (-) bacteria. The inhibition of these bacteria supports the effects of improving intestinal health. In recent years, the positive effects of edible insects on the intestinal microbiota have been associated with “chitin”. In animal studies, it is noted that chitin optimizes intestinal health by increasing short-chain fatty acids (SCFA) in the intestines and inhibits pathogenic bacteria (Zhou et al., 2022; Kipkoech, 2023). In one study, the effect of applying black soldier fly-larval flour on the microbiota and the production of SCFA’s was studied; as a result, it was shown that there was an increase in SCFA’s (a good prebiotic nutrient component in terms of microbiota) in the microbiota (Borrelli et al., 2017). Chitin can also bind cholesterol, reducing its absorption and helping to ensure the elimination of excess cholesterol; it activates natural/adaptive immune cells, induces cytokine production, and may exert an immuno-modulatory effect by activating macrophages (Kipkoech et al., 2023; Roos & Huis, 2017). The fatty acid profile contained in yellow flour worms, crickets and housefly maggots may have a positive effect on the risk of developing CVD. At the same time, thanks to the glucose-aminoglycan content found in crickets, a decrease in serum blood sugar and LDL cholesterol levels can also be achieved (Nowakowski et al., 2021). Studies conducted in mice have drawn attention to the fact that insects contain bioactive components that can be effective in controlling body weight. A study conducted in obese mice showed that consumption of yellow flour worm larva powders can lead to a decrease in body weight by reducing fat accumulation in adipocytes and serum TG levels. The anti-hypertensive effect of edible insects is explained by the fact that they contain powerful ACE inhibitor components. In one study, it was pointed out that silkworm, yellow flourworm, wasp and wax moths contain peptides that can show hypotensive activity (Roos & Huis, 2017). Its anti-carcinogenic activity is one of the most important potential effects of insects. As a result of another study, it was found that silkworm pupal protein hydrolysate prevents proliferation by inducing apoptosis (Zhou et al., 2022).

Consumer Acceptance

2100 insect species in about 80 countries around the world are widely consumed by edible insect ethnic groups. Although edible insects have been part of the daily diet in Asia for many years, the acceptance of insects in Western countries is still quite low (Tekiner et al., 2022; Gkinali et al., 2024). Although it is considered a new practice, insect consumption in Europe dates to Ancient Roman times. In Ancient Rome, insects were consumed as a luxury food, but they were also consumed as a response to food shortages (Platta et al., 2024). Even though their consumption dates to a long history, they are met with significant skepticism, security concerns and alienation in Western societies. Research shows that the acceptability of insect-based foods is influenced by various factors such as sociodemographic characteristics, psychological factors, environmental awareness, knowledge and awareness, cultural norms, social influences and product characteristics (Rehman & Ogrinc, 2024). In a study focusing on the accessibility of edible insects to consumer acceptance, frequency of consumption and motivation, it was concluded that religion and traditions are the main factors affecting entomophagy (Anagonou et al., 2023). In a study conducted on domestic tourists in Turkey, it was found that the participants' perceptions of insect consumption showed a significant difference according to sociodemographic characteristics such as gender, educational level and personality traits (Karaman & Bozok, 2023). Studies have highlighted the role of emotional variables in the development of negative attitudes. The most important of this disgust, and the perception of risk has been identified as neofobi food (Vanutelli et al., 2024).

Potential Health Risks

It is also very important to evaluate the potential health benefits of edible insects given above in terms of food safety (Lisboa et al., 2024; Aguilar-Toala et al., 2022). Before these insects are recommended as a “safe food”, it will be important to evaluate the associated risk factors as well. Edible insects have the potential hazards based on (a) chemical risks, (b) microbial

risks, and (c) are to be considered as a potential allergen (Hassan et al., 2024). The “chemical threats” carried by edible insects can be listed primarily as containing mycotoxins, heavy.

Conclusion

It is thought that insects can be used as an “alternative protein” source due to the increasing difficulty of access to reliable and high-quality food in recent years, the rapidly increasing population and the increase in the need for animal-derived protein in connection with population growth. It is reported in the literature that insects are a good source of macro and micronutrients. The digestibility rate of edible insects by human physiology is high, although it is related to the type of insect. It is thought that they may be a good “alternative” food source whose protein content and bioavailability are compared to other plant/animal protein sources. In addition to their anti-carcinogenic, anti-bacterial, anti-microbial, anti-inflammatory, immunomodulatory activities; it is claimed in many clinical studies that they can be used to improve blood glucose levels and blood lipid profile. Authorities and current studies expressing opinions in this field draw attention to the fact that edible insects can be an “alternative” to meet the daily requirements of the human body and may become part of human nutrition more widely in the future. There is a need to establish guidelines for comprehensive, safe and hygienic practices to ensure the safety of the consumption of edible insects.

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Protective Mechanical Ventilation Modes in the ICU: Contemporary Approaches, Clinical Applications and their Association with Infections

Yoğun Bakım Ünitesinde Koruyucu Mekanik Ventilasyon Modları: Çağdaş Yaklaşımlar, Klinik Uygulamalar ve Enfeksiyonlarla İlişkisi

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ABSTRACT

In patients with respiratory failure, mechanical ventilation is the most basic treatment for intensive care patients. This treatment management is indispensable to ensure normal oxygen and carbon dioxide levels. If appropriate ventilation parameters are not used, possible lung damage is an inevitable consequence. Protective mechanical ventilation strategies have been developed to prevent such damage. Despite all these strategic approaches, the risk of lung infection may increase. This article seeks to explore how protective ventilation strategies influence infection risks and discusses the optimal application of these strategies in clinical practice.

Keywords: Protective mechanical ventilation, Intensive care ünit, Ventilator-induced lung injury

ÖZ

Solunum yetmezliği olan hastalarda mekanik ventilasyon yoğun bakım hastalarına yönelik en temel tedavi yönetimidir. Bu tedavi yönetimi normal oksijen ve karbondioksit düzeylerinin sağlanması için vazgeçilmezdir. Eğer uygun ventilasyon parametreleri kullanılmazsa olası akciğer hasarı kaçınılmaz bir sonuçtur. Bu hasarları önlemek için koruyucu mekanik ventilasyon stratejileri geliştirilmiştir. Tüm bu stratejik yaklaşımlara rağmen akciğer enfeksiyonu riski artabilmektedir. Bu makale, koruyucu ventilasyon stratejilerinin enfeksiyon risklerini nasıl etkilediğini araştırmayı amaçlamakta ve bu stratejilerin klinik uygulamada en uygun şekilde uygulanmasını tartışmaktadır.

Anahtar Kelimeler: Koruyucu mejanik ventilasyon, Yoğun bakım ünitesi, Ventilasyon ilişkili akciğer hasarı

Introduction

Mechanical ventilation (MV) is a crucial intervention within intensive care units (ICUs) for patients experiencing critical respiratory failure. This therapeutic modality is paramount in ensuring adequate oxygenation as well as facilitating carbon dioxide removal. Nonetheless, it is important to recognize that mechanical ventilation carries the potential risk of lung injury, particularly when inappropriate ventilatory parameters are employed. This can result in ventilator-induced lung injury (VILI), and the prolonged use of mechanical ventilation may further complicate a patient's recovery (Pearson et al., 2022).

To mitigate such risks, protective ventilation strategies have been formulated. These strategies are designed to safeguard lung function by implementing lower tidal volumes, judicious application of positive end-expiratory pressure (PEEP), and maintaining plateau pressure within safe limits. However, the utilization of these ventilation strategies may also have implications for infection risk. The procedures associated with mechanical ventilation can elevate the likelihood of infections, leading to significant complications during patient management. This article seeks to explore how protective ventilation strategies influence infection risks and discusses the optimal application of these strategies in clinical practice (Banavasi et al., 2020; Pearson et al., 2022) .

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Mechanical Ventilation Modes and Protective Strategies

Mechanical ventilation is a therapeutic intervention aimed at supporting the respiratory functions of critically ill patients. This treatment must not only assist respiratory function but also minimize potential lung harm. Among the most commonly utilized ventilation modes and protective strategies in intensive care today are (Banavasi et al., 2020; Pearson et al., 2022; Van Kaam et al., 2021).

Low tidal volume ventilation

Low tidal volume ventilation has become a standard approach for managing patients with acute respiratory distress syndrome (ARDS) and other lung conditions. Setting tidal volumes at 6 mL/kg of ideal body weight limits excessive lung expansion and decreases the risk of ventilator-induced lung injury (VILI). This method enhances oxygenation and is associated with reduced mortality in ARDS patients (Banavasi et al. 2020; Kadado et al., 2022; Van Kaam et al., 2021).

Positive end-expiratory pressure (PEEP)

PEEP plays a vital role in preventing the collapse of alveoli, which in turn improves oxygenation. By maintaining open alveoli, PEEP facilitates more effective oxygen transfer into the bloodstream. However, it is important to carefully regulate PEEP levels, as excessive use can lead to cardiovascular complications or lung distension (Santa Cruz et al., 2021).

Plateau pressure and lung protection

Plateau pressure, defined as the maximum pressure exerted during mechanical ventilation, should be maintained below 30 cm H_2O to avert barotrauma or volutrauma. Protective ventilation strategies aim to restrict plateau pressure to prevent lung damage (Diaz et al., 2024).

The Relationship Between Protective Ventilation and Infection Risk

In the context of mechanical ventilation, the risk of infection is predominantly associated with ventilator-associated pneumonia (VAP) and other respiratory complications. VAP is a common and serious infection in critically ill patients reliant on prolonged mechanical ventilation, typically arising when microorganisms infiltrate the lungs via endotracheal tubes (Diaz et al., 2024; Papazian et al., 2020).

VAP and protective ventilation strategies

The management of ventilator-associated pneumonia (VAP) poses a significant challenge in the application of protective ventilation strategies. While PEEP is effective in preventing alveolar collapse, elevated levels of PEEP can increase the potential for microaspiration, thereby raising VAP risk. Consequently, it is crucial to carefully adjust PEEP settings to mitigate infection risks. Furthermore, maintaining low tidal volumes aids in minimizing VAP risk by preventing lung over-distension (Diaz et al., 2024; Papazian et al., 2020).

Mechanical ventilation and other infection risks

Mechanical ventilation can serve as a conduit for microorganisms to enter the respiratory tract. Invasive devices, such as endotracheal tubes and tracheostomy cannulas, when utilized for extended periods, significantly heighten the risk of infections. Therefore, regular maintenance and stringent hygiene practices for these devices are imperative in preventing such infections (Diaz et al., 2024; Papazian et al., 2020).

Antibiotic resistance and infections

The increasing prevalence of antibiotic resistance in intensive care units has become a concerning issue. Resistant bacterial strains are more likely to be encountered during prolonged mechanical ventilation, complicating infection management and extending recovery times. Addressing the rise of antibiotic resistance calls for careful infection management and prompt treatment initiation. Early diagnosis and appropriate antibiotic therapy are crucial elements in preventing severe complications (Despatovic et al., 2020; Vincent et al., 2020).

Infection Management through Enhanced Ventilation Techniques

As the risk of infections rises, it is crucial to modify ventilation strategies to enhance patient care. The management of conditions such as ventilator-associated pneumonia (VAP) requires precise adjustments to ventilator settings (Despatovic et al., 2020; Vincent et al., 2020).

Implementing early mobilization and preventive measures

Early mobilization serves as an effective technique to minimize the duration of mechanical ventilation, thereby lowering the risk of developing infections. Reducing the time on ventilation can enhance patient outcomes and diminish infection rates. Furthermore, as patients show signs of recovery, transitioning to non-invasive ventilation methods should be considered to further mitigate infection risks (Despotovic et al., 2020; Diaz et al., 2024; Papazian et al., 2020).

Protocols for hygiene and ınfection prevention

The enforcement of strict hygiene protocols in intensive care settings is crucial in combating infections, particularly VAP. Proper care and sanitation of ventilators and endotracheal tubes play a significant role in lowering infection prevalence. It is important to routinely replace tracheal tubes and cannulas, and to provide healthcare workers with thorough training in infection

prevention techniques (Despatovic et al., 2020; Vincent et al., 2020).

Timely intervention and management of infection risks

The swift identification of infections can greatly enhance patient recovery times. Ventilator-associated pneumonia (VAP) and similar infections need to be recognized and treated without delay. In certain instances, preemptive antibiotic regimens may be employed to stave off infections. The prompt initiation of antibiotic treatment can help avert complications that may arise from extended periods of ventilation (Diaz et al., 2024; Papazian et al., 2020).

Innovations and Future Directions

Recent technological advancements have yielded new ventilatory systems and monitoring equipment designed to enhance protective ventilation and minimize infection risks. Smart ventilation technologies enable continuous monitoring of patients, allowing for real-time adjustments to ventilatory parameters. Furthermore, the introduction of antimicrobial coatings on ventilator components aims to lower the likelihood of infections.

Conclusion

Protective mechanical ventilation in intensive care units is vital for supporting patient recovery while reducing the potential for ventilator-induced lung injury (VILI). However, extended mechanical ventilation raises the risk of infections, particularly VAP. It is essential to integrate protective ventilation strategies with robust infection control measures to improve patient outcomes. To mitigate infection risks, employing early mobilization practices, adhering to stringent hygiene protocols, and addressing antibiotic resistance are essential steps. Anticipated advancements in technology and more efficient ventilation techniques are likely to enhance both protective ventilation and infection management in the future, enabling faster and more effective patient recovery in intensive care environments. This article discusses the various protective mechanical ventilation approaches and their association with infection risks, highlighting contemporary practices and the impact of technological innovations on improving patient outcomes.

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