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ORIGINAL RESEARCH

The Effect of Bee Products on the Quality of Life, Covid-19 Anxiety, and Disease Prognosis in Young Adolescents with Covid-19

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Abstract
Objective: This study aimed to evaluate the effects of bee products on quality of life, anxiety, and the prognosis of Covid-19 in young adolescents who had contracted the virus.
Material-Method: The population of this cross-sectional descriptive study comprised university students residing in a city center in Eastern Turkey. Data collection was conducted from January 2023 to January 2024, using a convenience sampling method, and included 514 participants. Data collection tools consisted of a sociodemographic characteristics information form, an apitherapy and nutritional supplement use form, the Turkish version of the SF-12 Health Survey, and the Coronavirus Anxiety Scale (CAS). Additionally, a Structural Equation Model was developed and tested to explore the relationship between the use of apitherapy products, fear of Covid-19, and quality of life.
Results: The study found that 25.3% of students used apitherapy methods during their Covid-19 illness, with honey being the most commonly used product (33.8%), and 50% of those who used these methods reported recovery from the disease. A significant difference was observed between the use of apitherapy methods and CAS scores, with those who used pollen reporting higher mean CAS scores. Gender, smoking status, and the use of apitherapy methods during Covid-19 were associated with fear of the virus.
Conclusion: This study highlighted the potential benefits of bee products for young adolescents during and after the Covid-19 pandemic. It is recommended that future studies include larger, multicenter samples and provide comparative results for further validation.
Keywords: Anxiety, Apitherapy, Bee Products, Covid-19

INTRODUCTION

In recent years, the use of natural products for the prevention and treatment of various diseases has risen significantly.[2] Among these natural products, bee products stand out. Apitherapy, which involves the use of bees and their products for the prevention and treatment of illnesses, is recognized as part of complementary and integrative medicine in many countries and is becoming increasingly popular.[3] The health benefits of bee products have been known for thousands of years and have been a cornerstone of traditional medicine across different cultures. Evidence indicates that apitherapy plays a crucial role in maintaining homeostatic balance and enhancing the immune system.[4,5] This treatment has demonstrated its positive effects in areas such as inflammation, pain management, wound healing, and skin diseases.

Recently, the role of apitherapy in managing pandemic-related health challenges has come into focus.[6] During global health crises such as the Covid-19 pandemic, studies have investigated the potential of bee products to support immune function and boost the body's resilience.[7] Additionally, research is exploring the potential of apitherapy to inhibit viral replication and fortify the body's defenses against infections. While there is optimism about the future role of apitherapy in treatment protocols, further scientific studies and robust clinical data are essential to validate its efficacy and safety.

The Covid-19 pandemic, which began in late 2019, has posed significant challenges to public health. The escalating epidemic threat has led to a decline in the overall quality of life.[8] The pandemic's progression has been marked by various symptoms, including acute respiratory distress, high fever, and gastrointestinal and musculoskeletal issues, all of which have had a significant impact on individuals' quality of life during the illness.[9] Researches indicate that even after recovery, patients often experience a persistent reduction in their quality of life.[10,11] Studies have identified associations between decreased quality of life and factors such as anxiety, depression, and poor disease prognosis.[11] A longitudinal study demonstrated that complications from Covid-19 could persist for up to a year after recovery.[12] Additionally, a systematic review evaluating Covid-19's impact on quality of life reported substantial reductions during infection and ongoing impairment post-recovery.[13] Supporting this, a study conducted in Iran found significantly lower health-related quality of life among Covid-19 patients.[14]

Further research has underscored the continued decline in quality-of-life following Covid-19. A meta-analysis revealed that 59% of patients experienced poor quality of life post-recovery, with 42% reporting pain or discomfort, 38% suffering from anxiety or depression, 36% facing mobility issues, 28% experiencing difficulties with daily activities, and 8% struggling with personal care.[15] In another study assessing quality of life after Covid-19, most participants reported significant physical and emotional limitations that impaired functionality.[16]

The pandemic's impact extended beyond physiological health, significantly affecting psychological well-being.[17] The widespread nature of the virus, rising infection rates, and high mortality contributed to severe psychological issues, with 'fear' emerging as a significant concern.[18] Numerous studies have shown that the fear of Covid-19 is closely associated with physical health problems and related symptoms.[19,20] Research has also indicated that quality of life post-recovery was markedly low, with patients facing challenges in achieving full recovery, which adversely affected their mental health.[21]

This study aimed to evaluate the effects of bee products on quality of life, anxiety, and the prognosis of Covid-19 in young adolescents who had contracted the virus.

Study questions

In this study, answers to the following questions were investigated:

Do the participants experience persistent problems after recovering from Covid-19?

Did the participants use apitherapy methods during their Covid-19 illness?

Do the participants perceive benefits from apitherapy in overcoming the disease?

MATERIALS AND METHODS

Type of study

This study used a cross-sectional-descriptive design.

Universe and sample

The study was conducted with university students residing in a city center in Eastern Turkey. Research data was collected between January 2023 and January 2024. Data collection took place from January 2023 to January 2024. A convenience sampling method, which relies on volunteer participation, was used for sample selection. Upon completion of the study, power analysis was conducted using G*Power 3.1 and OpenEpi Version 3. According to the literature, sample size determination for descriptive and cross-sectional studies typically involves an effect size of 0.5, an alpha level (α) of 0.05, and a power range of 0.80 ($1-\beta$).[22] The power analysis performed with 514 participants indicated a 95% confidence interval, an alpha level of 5%, and a power of 80%.

Inclusion criteria

The study included all students aged 18 years and above, who were capable of communication, had a confirmed diagnosis of Covid-19, and possessed the ability to complete questionnaires using electronic devices.

Exclusion criteria

Data from students who inconsistently or incompletely filled out the questionnaire were excluded from the analysis.

Data collection tools

The data collection utilized a set of tools including a "Sociodemographic Characteristics Information Form", "Apitherapy Method and Nutritional Supplement Use Form", the "Turkish Version of SF-12 Health Survey", and the "Coronavirus Anxiety Scale" (CAS).

Sociodemographic characteristics information form

Developed by the researchers, this form, drawing from the literature[19,20], encompasses questions aiming to ascertain various characteristics of the students. These include gender, grade, income level,

chronic disease, smoking, Covid-19 vaccination status, number of Covid-19 vaccine doses, frequency of Covid-19 occurrences, symptoms experienced during Covid-19.

Apitherapy method and nutritional supplement use form

This form, developed by researchers, gathers detailed information regarding the use of apitherapy, its application during the Covid-19 period, and the consumption of nutritional supplements during the illness.

Turkish version of Sf-12 health survey

This scale, which developed to assess quality of life comprises 12 questions and eight sub-dimensions: physical functioning (2 items), role-physical (2 items), bodily pain (1 item), general health (1 item), energy (1 item), social functioning (1 item), role-emotional (2 items), and mental health (2 items). Scores ranging from 0 to 100 can be obtained, with higher scores indicating a better quality of life. The scale, whose validity and reliability were confirmed by a study conducted in 2022, calculates physical and mental item scores separately.[23] In this study, Cronbach's alpha coefficient was found to be 0.81 for the physical item score and 0.85 for the mental item score.

Coronavirus anxiety scale (CAS)

This scale was developed to assess individuals' level of fear caused by Covid-19.[24] The scale's items were formulated through a comprehensive review of fear-related scales, expert evaluations, and participant interviews. The Turkish validity and reliability study of the scale was conducted in 2020.[25] Comprising a single dimension and 7 items, the scale does not include reverse items. The total score, ranging from 7 to 35, reflects the individual's level of fear regarding Covid-19. A higher score on the scale indicates a heightened level of fear related to the disease. In our study, Cronbach's alpha coefficient value of the scale was determined as 0.87.

Data collection process

To maximize participant reach, the questionnaire forms were created using Google Forms and adapted for online distribution. The links to the forms were shared with participants through multiple channels, including WhatsApp, Telegram, text messages, and emails. After obtaining consent through a voluntary participation form in the online survey, participants were instructed to complete the data collection tools. The entire questionnaire was designed to take approximately 5 minutes to complete.

Statistical analysis

Data evaluation utilized IBM SPSS Statistics 26.0. After establishing a database, a thorough error analysis was conducted. Descriptive statistics were employed to analyze the data. The normal distributions of variables were assessed using Skewness and Kurtosis tests.[26] Variables meeting the conditions for normal distribution underwent independent groups t-tests, analysis of variance (ANOVA), and correlation analyses. Non-parametric tests, including Mann-Whitney U test, Kruskal-Wallis H analysis, and Spearman tests, were applied to non-normally distributed variables. Additionally, a Structural Equation Model (SEM) was established and tested to discern the relationship between the use of apitherapy products and fear of Covid-19, as well as quality of life. In all analyses, a significant level of $p<0.05$ was considered statistically significant.

Ethical approval

Prior to initiating the study, approval was obtained from the Bingöl University Health Sciences Scientific Research and Publication Ethics Committee (15.12.2022-E.88524). Additionally, necessary permissions were secured from the relevant institution (23.12.2022-E.89519) for conducting research involving university students. All participants were informed about the study's purpose, and their consent was obtained before their inclusion. The entire study adhered to the principles outlined in the Declaration of Helsinki.

RESULTS

The mean age of the students was 21.01±2.43 years, with 52.5% identifying as male. Regarding education, 86.2% were high school graduates, 30.4% were in their first year of university, and 87.7% reported not having chronic diseases. It was observed that 70.2% of the participants did not smoke, while 76.8% had received the Covid-19 vaccine, and 72.6% had received two doses. Additionally, 80.5% of the participants had contracted Covid-19 once, with 43.2% reporting mild disease. Despite this, 70.6% expressed the belief that the vaccines were not effective in overcoming Covid-19. Furthermore, 72.8% of the students did not intend to receive another Covid-19 vaccine. It was also found that 37.7% of participants used antibiotics during their illness. Around 33.3% of the participants experienced loss of taste and smell during the Covid-19 disease process, and 30% continued to experience these symptoms after recovery (Table 1).

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Table 1. Sociodemographic characteristics of the participants according to the mean scale scores.

Variables	n	%	PF X̄±SD	RP X̄±SD	BP X̄±SD	GH X̄±SD	E X̄±SD	SF X̄±SD	RE X̄±SD	MH X̄±SD	CAS X̄±SD
Gender											
Female	244	47.5	134.4±29.4	62.2±11.3	89.7±41.1	95.0±28.5	78.2±33.9	83.8±36.7	60.2±10.7	82.8±27.6	25.5±7.5
Male	270	52.5	132.8±25.9	60.4±11.1	89.0±39.7	88.4±20.2	76.3±30.8	79.0±33.4	56.9±10.0	79.4±25.4	23.1±6.7
Test			t=0.636	t=1.835	t=0.192	t=3.070	t=0.661	t=1.530	t=0.083	t=0.191	t=3.979
Significance			p=0.525	p=0.067	p=0.848	p=0.002	p=0.509	p=0.127	p<0.001	p=0.142	p<0.001
Grade											
1st	156	30.4	133.6±26.8	60.3±10.9	88.3±38.7	94.3±23.1	74.1±30.9	79.8±34.0	59.4±10.2	8.5±29.1	23.1±7.1
2nd	145	28.2	137.1±28.8	62.5±11.9	92.5±40.1	91.5±26.8	78.6±35.9	88.2±37.9	59.3±11.0	84.1±24.7	25.6±7.4
3rd	86	16.7	135.1±30.7	61.4±10.8	89.5±41.0	93.1±25.0	80.5±31.4	81.1±34.7	59.7±11.0	83.5±26.2	22.9±7.0
4th	127	24.7	128.5±24.3	60.9±11.2	87.0±42.4	87.2±23.5	77.3±30.2	75.3±32.1	55.6±9.1	76.5±25.1	24.8±7.2
Test			F=2.285	F=0.977	F=0.486	F=2.105	F=0.843	F=3.245	F=4.483	F=2.146	F=4.390
Significance			p=0.078	p=0.403	p=0.692	p=0.099	p=0.471	p=0.022	p=0.004	p=0.093	p=0.005
Chronic disease											
Yes	63	12.3	132.7±26.7	56.3±10.1	80.1±37.6	91.0±25.1	76.6±32.4	79.7±30.7	54.9±8.5	80.2±26.8	23.6±6.7
No	451	87.7	140.1±33.1	61.9±11.3	90.6±40.6	95.6±21.7	81.7±31.1	81.5±35.6	59.1±10.6	86.9±23.6	24.3±7.3
Test			Z=-1.563	Z=-3.730	Z=-1.838	Z=-1.434	Z=-1.341	Z=-0.282	Z=-2.858	Z=-2.144	Z=-0.421
Significance			p=0.118	p<0.001	p=0.066	p=0.152	p=0.180	p=0.778	p=0.004	p=0.032	p=0.674
Type of chronic disease											
Cardiovascular	13	20.6	152.7±34.1	54.1±8.8	94.4±48.0	91.6±21.6	83.3±25.0	88.8±28.2	52.7±5.5	83.3±26.5	21.5±8.2
Metabolic	17	29.1	152.9±37.3	56.6±9.9	97.1±36.6	102.9±23.1	85.2±23.4	72.1±24.8	53.6±7.3	89.7±25.8	24.3±7.3
Respiratory system	33	50.3	135.6±27.2	57.9±10.7	71.2±33.1	95.4±21.1	83.3±36.2	84.8±33.6	54.9±9.8	85.2±24.6	25.1±7.6
Test			X^2=4.672	X^2=3.532	X^2=2.580	X^2=1.591	X^2=0.943	X^2=0.644	X^2=3.918	X^2=0.982	X^2=0.625
Significance			p=0.003	p=0.015	p=0.053	p=0.191	p=0.420	p=0.587	p=0.009	p=0.401	p=0.599
Smoking											
Yes	153	29.8	132.5±29.2	60.2±11.3	86.2±38.4	91.3±26.4	81.8±35.6	87.9±37.6	58.8±11.1	84.9±29.4	26.4±8.2
No	361	70.2	134.1±26.9	61.7±11.2	90.7±41.2	91.6±24.0	75.3±30.7	78.5±33.6	58.4±10.2	79.4±25.1	23.2±6.6
Test			Z=-0.858	Z=-1.315	Z=-1.167	Z=-0.505	Z=-1.637	Z=-2.635	Z=-0.154	Z=-1.661	Z=-4.513
Significance			p=0.391	p=0.189	p=0.243	p=0.614	p=0.102	p=0.008	p=0.878	p=0.097	p<0.001
Covid-19 vaccination status											
Yes	395	76.8	134.3±28.4	61.5±11.3	90.1±40.1	90.6±24.1	78.2±32.8	82.2±35.1	58.6±10.5	82.2±26.2	23.9±7.2
No	119	23.2	131.1±24.9	60.5±11.2	87.1±41.4	94.7±26.4	74.1±30.5	78.1±34.8	58.1±10.3	77.3±27.2	25.2±7.1
Test			Z=-0.822	Z=-0.865	Z=-0.687	Z=-1.665	Z=-0.967	Z=-1.434	Z=-0.527	Z=-2.002	Z=-1.493
Significance			p=0.411	p=0.387	p=0.492	p=0.096	p=0.334	p=0.152	p=0.598	p=0.045	p=0.135
Number of Covid-19 vaccine doses											
1	66	16.7	127.2±27.1	61.1±11.1	84.2±41.3	88.7±22.6	69.2±26.3	75.6±33.7	56.4±9.1	80.7±27.5	26.1±7.6
2	287	72.6	134.1±29.1	61.1±11.2	89.8±39.9	91.7±24.8	80.7±33.6	81.7±34.3	58.1±10.5	83.1±26.3	23.1±7.1
3	42	10.7	139.5±26.9	61.1±12.5	90.2±39.7	93.7±26.3	72.9±34.5	97.2±40.4	64.2±9.5	74.3±26.1	28.1±7.3
Test			X^2=6.457	X^2=0.535	X^2=1.463	X^2=1.095	X^2=8.176	X^2=9.664	X^2=16.724	X^2=6.301	X^2=22.053
Significance			p=0.091	p=0.911	p=0.691	p=0.778	p=0.041	p=0.022	p=0.001	p=0.098	p<0.001
Frequency of Covid-19 occurrences											
1	414	80.5	132.8±27.2	61.6±11.3	90.3±40.7	91.1±25.7	76.6±32.4	82.3±35.9	59.1±10.4	79.5±26.3	24.3±7.3
2	78	15.2	136.8±30.1	60.4±11.1	85.8±40.1	91.1±18.4	78.2±31.0	75.6±30.1	56.5±10.4	86.6±25.8	25.1±6.8
3 and over	22	4.3	136.6±27.5	56.8±10.1	82.9±34.8	104.5±22.6	85.2±35.9	81.8±35.5	55.1±9.9	89.2±30.9	19.8±6.9
Test			X^2=1.679	X^2=4.358	X^2=1.304	X^2=6.875	X^2=1.442	X^2=1.442	X^2=7.956	X^2=6.796	X^2=9.377
Significance			p=0.432	p=0.113	p=0.521	p=0.032	p=0.486	p=0.486	p=0.019	p=0.033	p=0.009
How did the Covid-19 process go?											
Bad	86	16.7	139.5±31.4	58.7±10.5	94.4±38.2	90.4±25.1	81.6±30.5	77.3±29.1	56.1±9.9	83.5±29.1	23.5±7.1
Middle	206	40.1	128.3±24.5	64.2±11.6	94.7±41.3	95.2±27.6	78.1±34.2	83.8±38.4	60.5±10.9	81.6±26.1	25.7±7.5
Good	222	43.2	136.1±28.1	59.5±10.7	82.4±39.5	88.6±21.0	74.7±31.1	80.5±33.8	57.6±9.9	79.5±25.9	23.1±6.8
Test			X^2=9.441	X^2=23.162	X^2=11.234	X^2=3.606	X^2=3.390	X^2=0.478	X^2=14.445	X^2=1.281	X^2=15.241
Significance			p=0.009	p<0.001	p=0.004	p=0.165	p=0.184	p=0.788	p=0.001	p=0.527	p<0.001
Are vaccinations effective in Covid-19?											
Yes	151	29.4	136.2±25.6	63.2±11.5	86.2±38.1	94.7±23.9	78.6±34.8	88.5±36.3	61.2±10.3	81.3±24.2	23.9±7.3
No	363	70.6	132.5±28.4	60.4±11.0	90.7±41.3	90.2±25.0	76.7±31.2	78.3±34.1	57.4±10.3	80.9±27.5	24.3±7.2
Test			Z=-1.940	Z=-2.493	Z=-1.382	Z=-2.310	Z=-0.302	Z=-3.288	Z=-4.165	Z=-0.606	Z=-0.514
Significance			p=0.052	p=0.013	p=0.167	p=0.021	p=0.763	p<0.001	p<0.001	p=0.545	p=0.607
Would you consider getting vaccinated against Covid-19 again?											
Yes	140	27.2	136.1±24.5	63.1±11.4	90.1±38.8	93.7±27.9	77.8±34.9	92.1±38.6	60.1±10.7	83.8±24.3	25.2±7.4
No	374	72.8	132.6±28.7	60.5±11.1	89.1±41.1	90.7±23.4	77.1±31.3	77.2±32.8	57.9±10.3	80.1±27.3	23.8±7.1
Test			Z=-1.884	Z=-2.241	Z=-0.058	Z=-1.393	Z=-0.106	Z=-4.080	Z=-2.243	Z=-1.865	Z=-2.101
Significance			p=0.060	p=0.025	p=0.953	p=0.164	p=0.916	p<0.001	p=0.025	p=0.062	p=0.036
Use of antibiotics during Covid-19 treatment											
Yes	194	37.7	134.7±29.4	58.6±10.8	87.5±40.6	90.4±23.7	79.7±33.8	82.6±33.4	56.7±9.1	79.7±25.1	23.6±6.5
No	320	62.3	132.8±26.5	62.8±11.3	90.5±40.3	92.2±25.3	75.7±31.3	80.5±36.1	59.6±11.1	81.8±27.4	24.5±7.6

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Test			t=0.756	t=-4.101	t=-0.828	t=-0.800	t=1.356	t=0.643	t=-3.003	t=-0.871	t=-1.374
Significance			p=0.450	p<0.001	p=0.408	p=0.424	p=0.176	p=0.520	p=0.003	p=0.384	p=0.170
Symptoms experienced during Covid-19											
Musculoskeletal disorders	134	26.1	136.1±26.4	61.4±11.3	86.7±39.4	92.5±25.6	79.6±35.2	85.1±33.8	59.3±10.3	84.1±23.8	23.9±6.7
Loss of taste, smell	171	33.3	133.3±26.8	60.4±11.4	89.7±41.1	87.4±21.4	72.2±30.8	76.1±33.1	57.3±10.1	75.8±24.5	24.6±7.6
Palpitations	27	5.3	141.6±32.5	61.5±10.9	87.9±41.2	93.5±23.6	79.6±33.9	82.4±37.8	59.2±11.2	87.9±29.3	25.7±7.6
Diarrhea	40	7.8	130.6±26.2	61.2±10.5	98.1±40.9	96.8±20.5	70.6±26.4	86.8±37.9	56.8±9.7	80.6±26.3	22.7±6.7
Headache	57	11.1	132.8±28.4	62.9±11.3	89.9±38.6	91.6±27.6	81.5±31.8	78.5±36.1	61.4±11.3	82.4±27.3	25.1±7.6
Weakness/fatigue	52	10.1	137.1±30.7	62.7±11.4	90.3±38.7	101.9±27.4	86.5±34.1	94.2±39.1	60.5±11.1	93.7±32.8	23.2±7.8
Sleep problems	33	6.4	117.4±22.9	59.4±11.7	86.3±46.3	84.8±27.9	78.1±27.1	70.4±29.6	54.5±8.7	68.9±22.9	24.3±5.7
Test			X^2=17.811	X^2=4.088	X^2=2.741	X^2=17.027	X^2=13.384	X^2=17.678	X^2=14.940	X^2=26.145	X^2=5.994
Significance			p=0.007	p=0.665	p=0.841	p=0.009	p=0.037	p=0.007	p=0.021	p<0.001	p=0.424
Loss of working capacity after Covid-19											
Yes	248	48.2	137.1±29.4	59.1±10.7	81.9±37.3	91.8±25.1	80.1±33.4	81.5±32.5	57.2±9.8	84.2±25.4	23.7±6.8
No	266	51.8	130.2±25.5	63.3±11.4	96.3±41.9	91.3±24.3	74.6±31.1	81.1±37.3	59.7±10.2	78.1±27.2	24.6±7.6
Test			t=2.860	t=-4.359	t=-4.091	t=0.220	t=1.938	t=0.143	t=-2.683	t=2.647	t=-1.370
Significance			p=0.004	p<0.001	p<0.001	p=0.826	p=0.053	p=0.886	p=0.008	p=0.008	p=0.171
Persistence of problems after Covid-19											
Yes	80	15.6	141.5±34.2	58.1±9.7	91.2±37.9	90.9±22.5	85.9±33.9	83.4±34.6	54.5±8.1	84.1±25.1	22.9±5.9
No	434	84.4	132.1±26.1	61.8±11.4	89.1±40.8	91.7±25.1	75.6±31.8	80.9±35.2	59.2±10.7	80.5±26.8	24.4±7.4
Test			Z=-2.200	Z=-2.626	Z=-0.515	Z=-0.058	Z=-2.656	Z=-0.763	Z=-3.631	Z=-1.333	Z=-1.773
Significance			p=0.028	p=0.009	p=0.607	p=0.954	p=0.008	p=0.445	p<0.001	p=0.183	p=0.076
	X̄±SD		**Min.**	**Max.**							
Age	21.01±2.43		18	38							

PF: Physical functioning, RP: Role-physical, BP: Bodily pain, GH: General health, E: Energy, SF: Social functioning, RE: Role-emotional, MH: Mental health, CAS: Coronavirus anxiety scale.

It was reported that 58.4% of the students were unaware of apitherapy, 25.3% used some form of apitherapy during the Covid-19 process, and 33.8% used honey as the most commonly applied apitherapy method. Additionally, 50% of participants reported that these methods were very useful in overcoming the disease. In terms of nutritional supplements, 37.9% of students used supplements during illness, with 37.4% specifically taking vitamin supplements (Table 2).

According to the Turkish version of the SF-12 Health Survey, it was found that the mean scores for bodily pain (t=-2.968; p=0.003) and social functioning (t=-2.312; p=0.021) sub-dimensions were lower in those who were familiar with apitherapy during the Covid-19 process compared to those who were not, with statistically significant differences (Table 2).

It was also found that the mean pain sub-dimension scores of those who used apitherapy during the Covid-19 process were lower than those who did not (Z=-3.768; p<0.001). A significant difference was observed between the apitherapy methods used and the bodily pain sub-dimension scores. The mean score for the physiological function sub-dimension was higher in honey users (X^2=14.274; p=0.014), showing a significant difference between the apitherapy method used and the bodily pain sub-dimension scores. The lowest mean bodily pain sub-dimension score was found in those who used a mixture of bee products (X^2=25.659; p<0.001). A significant difference was also found between the apitherapy methods used and the Coronavirus Anxiety Scale (CAS) scores. The mean score for pollen users was higher (X^2=14.696; p=0.005) (Table 2).

A significant difference was observed between the perceived benefit of the apitherapy method used in overcoming the disease and the mean score of the role-physical sub-dimension. Those who rated the benefits of apitherapy as low had higher mean scores for role-physical difficulty (X^2=7.845; p=0.049). The difference between the perceived benefits of apitherapy methods and CAS scores was also statistically significant. Participants who reported that the benefit of the apitherapy methods was low had higher CAS scores (X^2=9.668; p=0.022) (Table 2).

A structural equation model (SEM) was established to determine the relationship between the use of apitherapy products, fear of Covid-19, and quality of life. Upon examining the fit values in the model, it was determined that the established model ($\chi 2$/df=1.823, RMSEA=0.123, CFI=0.983, GFI=0.982) met the required criteria, indicating that the model was appropriately fitted, and significant results were obtained. The standardized regression (beta) coefficient for the SEM in this study was β=-0.205; p=0.036 (Figure 1).

Table 2. Distribution of apitherapy and dietary supplement use according to scale mean scores.

Variables	n	%	PF X̄±SD	RP X̄±SD	BP X̄±SD	GH X̄±SD	E X̄±SD	SF X̄±SD	RE X̄±SD	MH X̄±SD	CAS X̄±SD
Knowledge about apitherapy											
Yes	214	41.6	133.1±27.1	60.5±11.1	83.1±39.2	91.2±21.4	76.5±30.4	77.1±30.5	58.5±10.2	81.1±24.5	24.2±6.6
No	300	58.4	134.1±28.1	61.8±11.4	93.8±40.7	91.8±26.8	77.8±33.6	84.3±37.7	58.5±10.6	81.1±27.9	24.2±7.6
Test			t=-0.379	t=-1.306	t=-2.968	t=-0.268	t=-0.454	t=-2.312	t=-0.015	t=0.038	t=-0.081
Significance			p=0.705	p=0.192	p=0.003	p=0.788	p=0.650	p=0.021	p=0.988	p=0.969	p=0.935
Use of apitherapy method during Covid-19											
Yes	130	25.3	131.9±28.1	60.1±10.5	77.5±38.4	91.1±21.7	74.2±30.1	79.8±31.7	57.8±9.9	82.2±24.9	23.6±7.3
No	384	74.7	134.1±27.4	61.6±11.5	93.4±40.3	91.7±25.7	78.3±33.1	81.8±36.1	58.7±10.6	80.6±27.1	25.9±6.6
Test			Z=-0.618	Z=-1.167	Z=-3.768	Z=-3.352	Z=-1.028	Z=-0.181	Z=-0.656	Z=-0.792	Z=-1.773
Significance			p=0.537	p=0.243	p<0.001	p=0.725	p=0.304	p=0.857	p=0.512	p=0.428	p=0.076
Product of apitherapy used											
Honey	44	33.8	142.1±26.9	62.5±10.1	84.2±39.3	85.7±26.6	71.4±21.1	84.2±32.7	57.5±10.1	82.1±28.6	23.6±7.3
Propolis	33	25.3	131.1±27.2	58.3±10.2	77.2±38.7	92.4±18.2	78.1±31.7	75.7±32.1	56.4±10.4	79.1±25.5	24.8±6.1
Pollen	10	7.6	141.1±29.3	61.2±10.9	105.1±45.3	82.5±16.8	87.5±29.4	80.1±32.9	56.2±8.8	90.1±20.2	28.2±6.3
Royal jelly	35	26.9	126.7±28.2	59.9±11.3	71.1±35.7	97.1±20.3	71.1±34.9	80.6±32.7	59.9±10.2	83.5±23.3	22.1±6.4
Mixture	8	4.8	109.3±18.6	57.8±9.3	50.1±0.1	87.5±18.8	71.8±28.1	71.8±20.8	56.2±6.6	78.1±21.9	26.2±6.7
Test			X^2=14.274	X^2=4.328	X^2=25.659	X^2=7.139	X^2=5.126	X^2=1.479	X^2=3.679	X^2=2.619	X^2=14.696
Significance			p=0.014	p=0.503	p<0.001	p=0.210	p=0.401	p=0.915	p=0.596	p=0.758	p=0.005
The benefits of apitherapy in overcoming the disease											
Less	15	11.5	133.3±22.4	68.3±9.2	103.3±41.0	86.6±28.1	86.6±36.4	78.3±33.8	61.6±12.1	75.8±21.3	28.8±7.5
Middle	41	31.5	135.9±31.6	60.6±10.2	82.3±39.2	91.4±21.3	71.3±29.8	79.8±33.1	59.4±9.9	82.9±27.2	22.1±7.3
Much	65	50.0	129.2±29.1	60.1±11.5	75.3±37.1	92.3±19.7	76.5±32.1	71.1±28.7	55.9±9.1	80.5±23.9	25.4±7.4
Undecided	9	7.0	134.7±27.2	61.4±11.5	93.8±40.3	91.7±26.1	78.3±32.3	83.4±36.5	58.8±10.7	80.9±27.1	23.8±7.1
Test			X^2=2.092	X^2=7.845	X^2=7.087	X^2=0.781	X^2=3.119	X^2=3.520	X^2=5.535	X^2=0.751	X^2=9.668
Significance			p=0.553	p=0.049	p=0.069	p=0.854	p=0.374	p=0.318	p=0.137	p=0.861	p=0.022
Use of nutritional supplements											
Yes	195	37.9	133.5±29.1	58.9±10.5	81.1±40.6	88.9±20.7	77.5±30.9	75.3±28.7	56.3±9.2	80.8±24.2	23.7±6.7
No	319	62.1	133.6±26.8	62.6±11.5	94.5±39.5	93.1±26.7	77.1±33.1	84.9±38.1	59.8±10.9	81.1±27.9	24.5±7.5
Test			t=-0.012	t=-3.667	t=-3.716	t=-1.875	t=0.152	t=-3.023	t=-3.747	t=-0.122	t=-1.125
Significance			p=0.990	p<0.001	p<0.001	p=0.061	p=0.879	p=0.003	p<0.001	p=0.903	p=0.261
Type of dietary supplement used											
Vitamin	73	37.4	133.7±27.7	59.4±10.3	73.7±38.4	87.3±20.1	81.8±32.1	79.2±29.6	56.9±8.7	78.5±21.2	22.9±6.2
Mineral	41	21.0	137.1±29.1	56.4±10.5	97.5±41.3	91.4±22.1	80.4±33.3	91.4±33.8	56.6±6.9	74.3±23.1	23.1±5.6
Extract	27	13.8	133.3±30.2	61.5±10.9	77.7±40.6	91.6±16.9	85.1±34.8	68.5±26.4	57.8±10.4	83.3±25.7	24.5±7.7
Soups	17	8.7	127.9±23.1	60.2±10.1	80.8±40.1	82.3±26.1	79.4±35.6	69.1±24.2	58.8±11.4	83.8±26.4	24.5±6.8
Herbal teas	18	9.2	125.1±22.6	63.1±12.4	94.4±42.4	90.2±25.9	69.4±27.8	70.8±31.2	58.3±10.5	75.1±25.3	24.2±8.9
Local food and/or products	19	9.9	127.6±37.1	57.2±9.6	73.6±37.7	96.1±17.2	69.7±31.8	65.7±31.4	53.2±7.0	94.1±28.3	26.1±7.7
Test			F=0.702	F=2.852	F=4.151	F=1.041	F=1.049	F=2.766	F=3.593	F=1.572	F=0.854
Significance			p=0.648	p=0.010	p<0.001	p=0.398	p=0.392	p=0.012	p=0.002	p=0.153	p=0.529

PF: Physical functioning, RP: Role-physical, BP: Bodily pain, GH: General health, E: Energy, SF: Social functioning, RE: Role-emotional, MH: Mental health, CAS: Coronavirus anxiety scale.

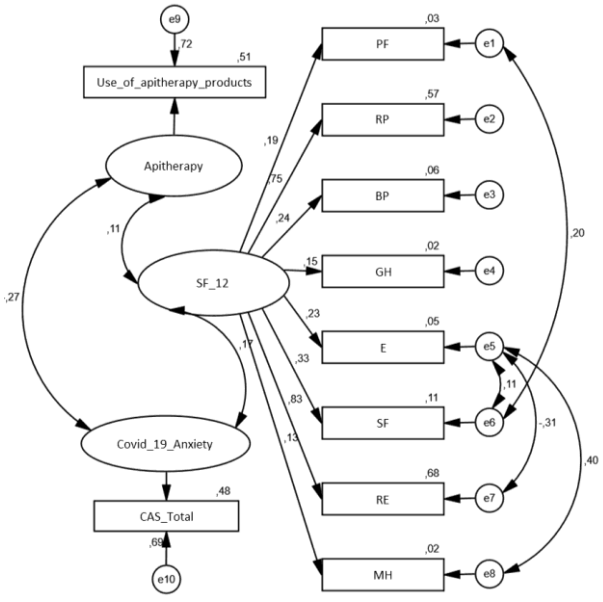

Figure 1. Standardized path coefficients.

Abbreviations: PF: Physical functioning, RP: Role-physical, BP: Bodily pain, GH: General health, E: Energy, SF: Social functioning, RE: Role-emotional, MH: Mental health, CAS: Coronavirus anxiety scale.

DISCUSSION

In this study, we investigated the impact of bee products on quality of life, anxiety, and Covid-19 prognosis in young adolescents who experienced Covid-19. Bee products, including propolis, pollen, and royal jelly, have a history of use in traditional medicine, known for their various benefits. The antioxidant, anti-inflammatory, and antimicrobial properties of these bee products, as well as natural remedies used in alternative medicine, may play a crucial role in alleviating symptoms and improving overall health status following Covid-19.[27] In this study, we found that the use of natural products during pandemics such as Covid-19 has positive effects on general health status, the fear of Covid-19, and the prognosis of the disease. Previous a research emphasized the antioxidant and anti-inflammatory properties of bee products, highlighting their positive effects on general health and quality of life.[27] In addition, another study reported that bee products positively affect the immune system and may have healing properties for general health.[28] A study has shown that bee products have the potential to improve quality of life by positively affecting general health.[29] In addition, a study published in 2019 reported that natural treatment methods positively affect the physical and mental health of young adolescents.[30]

The results of this study indicate that bee products have positive effects on Covid-19-related fear in young adolescents. These findings are consistent with a study published in 2020, which showed that natural therapeutic methods can effectively reduce anxiety and stress during the pandemic.[31] It has also been reported that natural products can improve the psychological health of young adolescents. Natural products can have effective results in alleviating anxiety and depression, which are common problems among young people during the pandemic period.[32,33,34]

One of the most important results of this study is that the use of bee products in Covid-19 disease is effective in mild disease and acceleration of the healing process. Previous studies have reported that bee products can strengthen the immune system and positively impact the recovery of Covid-19 patients.[35,36] A study published in 2021 stated that natural products can be effective in strengthening the immune system that protects the body against viral infections and alleviating the infection process.[37] Natural treatment methods have been reported to be useful in the management of chronic diseases and in improving quality of life.[38] In another study, it was reported that the use of natural products by young people during the pandemic had a positive effect on the recovery process.[39] This suggests that natural products could be used effectively in treating viral infections, particularly during pandemic situations.[40] Some studies further indicate that alternative treatments can play a significant role in managing epidemics such as Covid-19.[41]

CONCLUSION

The results of this study indicate that bee products, known for their antioxidant, anti-inflammatory, and antimicrobial properties, positively impact the general health and quality of life of young adolescents. Natural bee products were observed to have a favorable effect on the overall well-being of young individuals, reducing fear associated with Covid-19. These results underscore the effectiveness of natural therapeutic methods in enhancing quality of life and alleviating Covid-19-related fears during the pandemic. Furthermore, the study suggests an association between the use of bee products and a milder course of Covid-19, contributing to a faster recovery process. These results highlight the potential benefits of incorporating bee products into therapeutic approaches for managing Covid-19 in young individuals. In summary, this study sheds light on the potential benefits of bee products for young adolescents during and after the Covid-19 pandemic. To enhance our understanding of the effectiveness of bee products in epidemic diseases, it is advisable that future studies adopt larger sample sizes, a multicenter approach, and present results in a comparative manner. Furthermore, the conduct of long-term follow-up studies is essential to investigate the sustained impact of bee products. This will enable a deeper exploration of the prolonged effects of Covid-19, and the role of bee products in mitigating these effects can be elucidated. Although not addressed in this study, it is worthwhile for future research to compare the efficacy of bee products with standard treatment methods. Such comparisons will contribute to determining whether bee products serve as alternatives or complementary approaches to traditional treatment methods.

Limitations

This study was conducted to evaluate the effect of bee products on the quality of life, Covid-19 anxiety, and disease prognosis in young adolescents with Covid-19, has several limitations. Firstly, the

cross-sectional design of the study hinders the determination of causality. Additionally, the reliance on data from a single province restricts the generalizability of the results to the broader population. Furthermore, the assessment of Covid-19 fears and quality of life relied on self-report scales, which introduces the possibility of bias, and other factors influencing these aspects couldn't be comprehensively determined. Despite these limitations, the study's strengths include the utilization of a large sample group and the innovative use of structural equation modeling, providing a novel approach to understanding the relationship between the use of apitherapy products and fear of Covid-19, as well as the quality of life. These methodological choices enhance the reliability of the study's results.

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ORIGINAL RESEARCH

The Effects of Physiotherapists' Attitudes Towards Complementary and Alternative Medicine on Their Perspectives to Chronic Low Back Pain

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Abstract

Objective: This study aims to investigate how physiotherapists' perspectives on complementary and alternative medicine practices influence their strategies for managing chronic low back pain.

Material-Method: The study included 162 physiotherapists. Participants' attitudes toward complementary and alternative medicine were assessed using the Complementary, Alternative, and Traditional Medicine Attitude Scale. Physiotherapists' attitudes and beliefs related to chronic low back pain were evaluated using the Pain Attitudes and Beliefs Scale.

Results: Positive significant correlations were observed between Complementary, Alternative, and Traditional Medicine Attitude Scale-Total Score and Pain Attitudes and Beliefs Scale Factor 1 (r=0.204, p=0.009) and Factor 2 (r=0.174, p=0.027). Significant correlation was found between Complementary, Alternative, and Traditional Medicine Attitude Scale-Philosophical congruence with complementary and alternative medicine subscore and Pain Attitudes and Beliefs Scale Factor 1 (r=0.319, p=0.000) and Factor 2 (r=0.286, p=0.000). Complementary, Alternative, and Traditional Medicine Attitude Scale-Holistic balance subscores showed significant correlation with Pain Attitudes and Beliefs Scale Factor 1 (r=0.222, p=0.005) and Factor 2 (r=0.155, p=0.049).

Conclusion: Physiotherapists with a more positive attitude towards complementary and alternative medicine practices tend to lean towards a biomedical approach to chronic low back pain. Increasing physiotherapists' knowledge about complementary and alternative medicine practices and evidence-based interventions may influence their approaches to chronic low back pain.

Keywords: Chronic Low Back Pain, Complementary and Alternative Medicine, Physiotherapist

INTRODUCTION

Low back pain (LBP) is characterized by discomfort and pain typically situated between the lower rib margins and buttock creases, with or without lower extremity symptoms. Chronic low back pain is identified as enduring the condition for more than three months.[1] It is projected that by 2050, there will be an estimated 800 million cases of common low back pain globally, a notable increase from the 500 million cases reported in 2020.[2]

Globally, a substantial gap exists between evidence-based recommendations and actual practices in managing low back pain despite the presence of multiple clinical guidelines offering similar advice.[3] The attitudes and beliefs of healthcare professionals play a crucial role in the clinical treatment of patients with LBP, and they need to be aware of this aspect during patient consultations.[4] Physiotherapists, as healthcare professionals, play a vital role in the management of chronic low back pain.[5]

Complementary and alternative medicine (CAM), as defined, encompasses various medical care systems, products, and practices that deviate from conventional medicine. CAM practices are classified into five categories, including body-based and manipulative therapies (e.g., osteopathy, yoga, acupuncture, hydrotherapy, chiropractic), alternative medical systems (e.g., homeopathic approaches, ayurveda), energy healing therapies, nutritional therapies, and mind-body therapies (e.g., hypnosis, yoga).[6] Due to the variable outcomes of traditional treatments, there has been a growing inclination among both LBP patients and healthcare providers to explore CAM options.[7]

A study investigating the utilization of CAM methods by physiotherapists in the treatment of lower back pain revealed that while physiotherapists were aware of their patient's use of CAM, their understanding of the effectiveness of these practices was deemed insufficient.[8]

The primary objective of this study is to examine how physiotherapists' perspectives on CAM

practices influence their approach to managing chronic low back pain.

MATERIALS AND METHODS

Study design

This research was conducted as a prospective observational study involving physiotherapists in Turkey. Approval for the study was obtained from the Ethics Committee of a State University (2023/11-16). Before commencing the survey, participants were required to sign a declaration expressing their willingness to take part in the study, and adherence to the Declaration of Helsinki guidelines was ensured.

Study population

The study encompassed physiotherapists in Turkey, and a web-based survey created in the Turkish language using Google Forms was distributed to participants through social media and email channels. The survey comprised four sections and took approximately ten minutes to complete.

Inclusion and exclusion criteria

Physiotherapists in Turkey who willingly participated in the study were included, while physiotherapy and rehabilitation department students who had not yet graduated, as well as other healthcare professionals, were excluded.

Measures

Demographics

The survey included questions about participants' gender, age, university graduation status (private/public), and their current workplace.

Complementary, alternative and conventional medicine attitude scale (CACMAS)

Köse et al. (2018) conducted a validity and reliability study of this scale, originally developed by McFadden et al. (2010), in Turkey.[9,10] The scale, consisting of 27 questions, comprises three subscales: "Holistic Balance," "Dissatisfaction with Conventional Medicine," and "Philosophical Congruence with CAM." Responses are scored on a Likert-type scale ranging from "1 = Strongly Disagree" to "7 = Strongly Agree." Positive and negative expressions are evaluated separately, and an overall higher score indicates a more positive attitude toward traditional complementary medicine.

The pain attitudes and beliefs scale for physiotherapists (PABS-PT)

Dalkılınç et al. conducted the Turkish validity and reliability study for this scale, which assesses health professionals' attitudes and beliefs regarding pain based on biomedical and biopsychosocial approaches.[11,12] The survey consists of 13 items, with 7 covering biomedical approaches (Factor 1) and 5 covering biopsychosocial approaches (Factor 2). Participants rate statements on a scale from "1 = Completely Disagree" to "6 = Completely Agree," and higher scores on each subscale indicate a stronger inclination toward the respective treatment approach.

Statistical analysis

The sample size was determined using the G*Power program (version 3.1), considering a power ratio of 80%, a correlation of $\rho H1=0.2$, and a significance level of $\alpha=0.05$ based on a pilot study. The calculated sample size was 153 physiotherapists, but accounting for potential data loss, 162 physiotherapists were included. Data were analyzed using the statistical program JASP, with the Shapiro-Wilk test assessing a normal distribution. Categorical variables were presented as frequencies (percentages) using the chi-square test, and Spearman's rank correlation analysis was employed for parameters lacking normality, with $p<0.05$ considered statistically significant.

RESULTS

The study comprised 162 physiotherapists, with an average age of 29.38 ± 5.48. Table 1 details the participants' descriptive information, while Table 2 presents the mean and standard deviation values for the participants' total scores and sub-scores on the CACMAS and PABS-PT.

The association between physiotherapists' attitudes toward complementary, alternative, and conventional medicine and their attitudes and beliefs concerning chronic low back pain is outlined in Table 3. A positive and statistically significant correlation was observed between the CACMAS Total Score and PABS-PT Factor 1 (r=0.204, p=0.009) and PABS-PT Factor 2 (r=0.174, p=0.027). Similarly, a positive and statistically significant relationship was found between the CACMAS-Philosophical congruence with CAM subscore and PABS-PT Factor 1 (r=0.319, p=0.000) and PABS-PT Factor 2 (r=0.286, p=0.000). A statistically significant and positive relationship was identified between the CACMAS-Holistic balance subscore and PABS-PT Factor 1 (r=0.222, p=0.005) and PABS-PT Factor 2 (r=0.155, p=0.049). No significant relationship was observed between CACMAS-Dissatisfaction with conventional medicine subscore and PABS-PT Factor 1 (r=-0.098, p=0.213) and PABS-PT Factor 2 (r=-0.027, p=0.735).

Table 1. Descriptive Characteristics of Participants

Variables	***N=162***
Age (years) (X ± SD)	29.38 ± 5.48
Gender n (%)	
Female	106 (65.4%)
Male	56 (34.6%)
Graduated University n (%)	
State University	146 (90.1%)
Private University	16 (9.9%)
Where does he/she work? n (%)	
Public Hospital	71 (43.8%)
Special Education And Rehabilitation Center	18 (11.1%)
Healthy Lifestyle Center	24 (14.8%)
Academical Personal	15 (9.3%)
Private Hospital	15 (9.3%)
Not Working	19 (11.7%)

X:mean, SD: standard deviation, N: number of total participants, n: number of participants, %: percent

Table 2. Mean Scores of Scales

	X ± SD
CACMAS Total Score	116.78 ± 19.94
CACMAS-Philosophical congruence with CAM	33.30 ± 10.20
CACMAS- Holistic balance	48.81 ± 7.50
CACMAS- Dissatisfaction with conventional medicine	34.64 ± 9.30
PABS-PT Factor 1	28.43 ± 6.23
PABS-PT Factor 2	21.52 ± 4.95
PABS-PT Total Score	49.97 ± 10.21

X:mean, SD: standard deviation, CACMAS: Complementary, Alternative and Conventional Medicine Attitude Scale, CAM; complementary and alternative medicine, PABS-PT: The Pain Attitudes and Beliefs Scale for Physiotherapists

Table 3. The Relationship between the Complementary, Alternative, and Conventional Medicine Attitude Scale and The Pain Attitudes and Beliefs Scale for Physiotherapists

		PABS-PT Factor 1	**PABS-PT Factor 2**
CACMAS Total Score	**Spearman's Correlation**	0.204	0.174
	Sig. (2-tailed)	0.009**	0.027*
	N	162	162
CACMAS-Philosophical congruence with CAM	**Spearman's Correlation**	0.319	0.286
	Sig. (2-tailed)	0.000**	0.000**
	N	162	162
CACMAS- Holistic balance	**Spearman's Correlation**	0.222	0.155
	Sig. (2-tailed)	0.005**	0.049*
	N	162	162
CACMAS- Dissatisfaction with conventional medicine	**Spearman's Correlation**	-0.098	-0.027
	Sig. (2-tailed)	0.213	0.735
	N	162	162

CACMAS: Complementary, Alternative and Conventional Medicine Attitude Scale, CAM; complementary and alternative medicine, PABS-PT: The Pain Attitudes and Beliefs Scale for Physiotherapists, N: number of participants, *:$p<0.05$

DISCUSSION

This study aimed to explore the connection between physiotherapists' attitudes towards CAM and their approach to managing chronic low back pain. The findings highlighted a significant correlation between physiotherapists exhibiting positive attitudes towards CAM practices and their inclination towards adopting biomedical approaches to addressing chronic low back pain. However, the correlation with the biopsychosocial approach was noteworthy but relatively lower.

Two models describe physiotherapists' attitudes and beliefs about chronic low back pain: the biomedical model, which emphasizes physical pathology as the cause of pain and disability, and the biopsychosocial model, which emphasizes the role of psychological and social factors.[11,13] Healthcare professionals' attitudes and beliefs about health and illness play a crucial role in patient treatment decisions. The Theory of Planned Behavior suggests that individual behavior is influenced by attitudes and beliefs regarding the potential outcomes of their actions.[14]

In chronic low back pain cases, various factors impact physiotherapists' decision-making, including clinical experience, patient expectations, socio-cultural factors, perceptions of pain by both the physiotherapist and patient, and the physiotherapist's knowledge level.[15] The increasing use of CAM methods globally and in Turkey

suggests a growing interest among physiotherapists.[16–18] The results indicated a significant correlation between positive attitudes towards CAM and a biomedical perspective on addressing chronic low back pain. This suggests that physiotherapists may be inclined to use CAM methods targeting symptomatic or pathologically related areas.

Studies show physiotherapists' interest in CAM methods, with acupuncture and massage being commonly utilized.[19,20] However, knowledge gaps about the evidence base for these methods persist. Enhancing physiotherapists' knowledge about CAM practices is crucial to influencing their attitudes and behaviors towards patients with chronic low back pain.

While our study focused on assessing attitudes, it did not delve into physiotherapists' knowledge and behaviors, limiting the exploration of the relationship between attitude, knowledge, and behavior.

CONCLUSION

To our knowledge, this study is the first to unveil the link between physiotherapists' attitudes towards CAM and their approaches to chronic low back pain. The findings suggest that a positive attitude towards CAM aligns with a more biomedical approach to managing chronic low back pain. Improving physiotherapists' knowledge about CAM practices and evidence-based applications may reshape their approaches to chronic low back pain. Practices aimed at enhancing the knowledge level of physiotherapists who frequently play a role in managing patients with chronic low back pain can lead to changes in their attitudes and behaviors, thereby potentially improving patient management clinically. It is recommended that future studies evaluate physiotherapists' attitudes and behaviors towards chronic low back pain and complementary and alternative medicine, as well as assess professional autonomy and satisfaction.

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Author contributions: Conceptualization: HK; Design: HK; Writing: HK; Investigation/Data collection: HK

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ORIGINAL RESEARCH

Protecting Spermatogenesis from Doxorubicin-Induced Damage: The Effects of *Prunus laurocerasus* on Oxidative Stress in an Animal Model

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Abstract

Objective: A side effect of chemotherapy is its tendency to increase oxidative stress, which can lead to infertility. This study examines the potential of *Prunus laurocerasus* fruit extract (PLFE) to reduce Doxorubicin (DOX)-induced oxidative stress and preserve sperm production.

Material-Method: Rats received daily doses of PLFE for 15 days, with DOX administered on day 13. Control groups had no treatment, while damage groups received only DOX. The protective effects of high (HD) and low (LD) doses of PLFE against DOX-induced damage were assessed by evaluating sperm count, motility, dead sperm ratio, and oxidative stress markers.

Results: PLFE significantly improved sperm concentration and reduced dead sperm percentage in both LD and HD groups compared to the DOX group ($p<0.05$), with no significant difference between LD and HD ($p>0.05$). Superoxide dismutase (SOD) activities and glutathione increased while malondialdehyde (MDA) decreased in PLFE groups compared to DOX ($p<0.05$) though these markers remained and couldn't reach the control levels ($p<0.05$). Catalase (CAT) activities did not differ between the HD+DOX and DOX groups ($p>0.05$), but the LD+DOX group showed an increase ($p < 0.05$ vs DOX), though lower than controls ($p<0.05$).

Conclusion: *P. laurocerasus* and its active compounds could be targets for developing treatments to reduce oxidative stress, preserve fertility, and improve reproductive success post-chemotherapy. Further studies are needed to adjust the application duration and dose, understand the mechanism of action, and clarify the limited impact on CAT activity, which suggests selective pathway activation in antioxidant defense.

Keywords: Doxorobucin, Phytotherapy, Spermatogenesis, Oxidative Stress, Chemotherapy, Infertility

INTRODUCTION

Male infertility has many physiological, genetic, and environmental causes[1,2]. Oxidative stress is a key factor in most cases of idiopathic male infertility. It plays a vital role in redox metabolism, which underpins essential processes from bioenergetics to metabolism, with mild oxidative stress being crucial for sperm cell capacitation[3–6]. Various environmental toxins and pathological processes can accumulate reactive oxygen species (ROS) at levels detrimental to general health and fertility[2,6]. Subfertility, defined as reduced fertility or subsequent secondary infertility, is also largely associated with oxidative stress and living conditions[3,7]. Sperm cell membranes contain a high concentration of unsaturated fatty acids[8], making them particularly susceptible to the harmful effects of ROS. Chemotherapeutic agents can negatively affect spermatogenesis by causing increased oxidative stress, impacting patients' quality of life post-treatment[9,10].

Doxorubicin (DOX) has been extensively used for the treatment of various tumors over the past 40 years. Despite its effectiveness in combating tumors, DOX induces significant oxidative stress in non-

target tissues, including the testes, making them susceptible to damage and highlighting the need to consider potential complications when administering this medication[11–15]. DOX can dramatically suppress spermatogenesis, though its precise impact on testicular tissue remains unclear[16–19]. Several studies have suggested that lipid peroxidation and apoptosis may play crucial roles in this toxicity[20,21]. High doses of DOX are used in experiments to replicate oxidative stress-induced damage in tissues[22,23]. The impact of various antioxidants in preventing this damage has been extensively researched[23–26].

Prunus laurocerasus (PL), also known as *Laurocerasus* officinalis, is a perennial evergreen tree from the Rosaceae family[27]. Found in the Black Sea region of Turkiye, this fruit is commonly referred to as "Taflan" and "Karayemiş". Although it is commonly eaten as a fresh fruit, it is also dried or boiled and consumed as jam or molasses. Its fruits, seeds, and leaves are used in traditional medicine, especially for diabetes[28]. Various extracts of Prunus are rich in phenolic and polyphenolic compounds, such as chlorogenic, coumaric, gallic, benzoic, and caffeic acids, and demonstrate significant antioxidant capacity, enhancing their pharmaceutical value[27,29–34].

Previous studies have shown the effects of different Prunus species on testicular tissue[35,36] and cancer cells[37,38]. Limited research exists on the protective effects of PL against chemotherapy side effects and its specific influence on spermatogenesis. This study addresses the existing research gap by examining the potential protective effects of PLFE on DOX-induced testicular toxicity.

MATERIALS AND METHODS

Extracts

Fruits harvested from the Central Black Sea region of Turkiye were extracted in ethanol, and the antioxidant capacity and reducing power were determined, as described in our previous study[12]. The PLFE was diluted to two different concentrations suitable for low-dose (LD) and high-dose (HD) applications and administered to the experimental animals as described below.

Animal model and experimental procedures

The Animal Ethics Committee at Ordu University approved all experimental protocols and animal procedures (decision dated 30th March 2020, reference number 14). The study strictly adhered to the established guidelines for the Care and Use of Laboratory Animals. Twenty-eight male Sprague-Dawley rats weighing 280±30 grams were acquired from OMÜ DEHAM. The animals were acclimatized for one week at the Ordu University Experimental Animal Research and Application Laboratory under controlled conditions (25±1°C temperature, 55% relative humidity, and a 12-hour light/dark cycle) to ensure their health and adaptation to the environment and randomly assigned to four distinct groups. Each group, comprising seven rats (n=7), received a specific pharmacological treatment. We provided the rats with routine care and feeding for 15 days with ad libitum access to food and water. Application doses of DOX and PLFE were selected based on previous studies[22,28,31,39], with the high dose (HD) confirmed as non-toxic in our earlier paper[12].

Group-1 (CTR): A healthy control group was given intragastric (I.G.) water for 15 days.

Group-2 (DOX): The damaged group was given water (I.G.) for 15 days and administered 15 mg/kg DOX intraperitoneally (I.P.) on the 13th day of the experiment.

Group-3 (LD+DOX): The treatment group was given a low dose (500 mg/kg) PLFE (I.G.) for 15 days and administered 15 mg/kg DOX (I.P.) on the 13th day of the experiment.

Group-4 (HD+DOX): The treatment group was given a high dose (1000 mg/kg) PLFE (I.G.) for 15 days and administered 15 mg/kg DOX (I.P.) on the 13th day of the experiment.

We administered gavage applications of 2-3 ml per dose to the animals at the same time every morning. On the 15th day of the experiment, the animals were euthanized under general anesthesia with intraperitoneal injections of 80 mg/kg Ketamine and 10 mg/kg Xylazine. Tissue samples were then collected for analysis.

Tissue collection and preparation

Following a thorough rinse with phosphate-buffered saline (PBS) to remove contaminants. Tissue samples were weighed using a precision balance, and the measurements were carefully documented. The cauda epididymis and testes were collected in a separate dish for sperm analysis (Figure 1). Testes were stored at -80°C in a deep freezer to evaluate oxidative stress and molecular studies. Relative testis weights (RTW) were calculated as a ratio to the final body weights.

Sperm analysis

Sperm count in the epididymis was determined using a modified version of the method developed by Kenjale et al.[40]. Briefly, the unilateral cauda epididymis was minced in 10 ml PBS in glass Petri

dishes using anatomical scissors and incubated at 37°C for 10 minutes with gentle shaking. The supernatant was diluted 10 times with PBS at room temperature. A volume of 10 microliters of the diluted sperm sample was carefully pipetted onto a hemocytometer. After allowing it to settle for 5 minutes, the sperm cells were counted using a light microscope. During the total count, the motile and stationary sperm were recorded. The percentage of motile sperm was then calculated by dividing the number of motile sperm by the total number of sperm and multiplying by 100 to obtain the percentage.

The Eosin-Nigrosin method [41] was employed to perform the dead sperm ratios. Accordingly, 20 µL of sperm suspension was treated with a standard solution containing 1% eosin Y and 5% nigrosin (GBL, Turkiye) and then dropped onto microscope slides and covered with a coverslip. The prepared slides were subsequently examined using light microscopy. In total, at least 200 sperm were counted on each slide, and the ratios of live (transparent) and dead (purple-stained) spermatozoa were calculated as percentages (%).

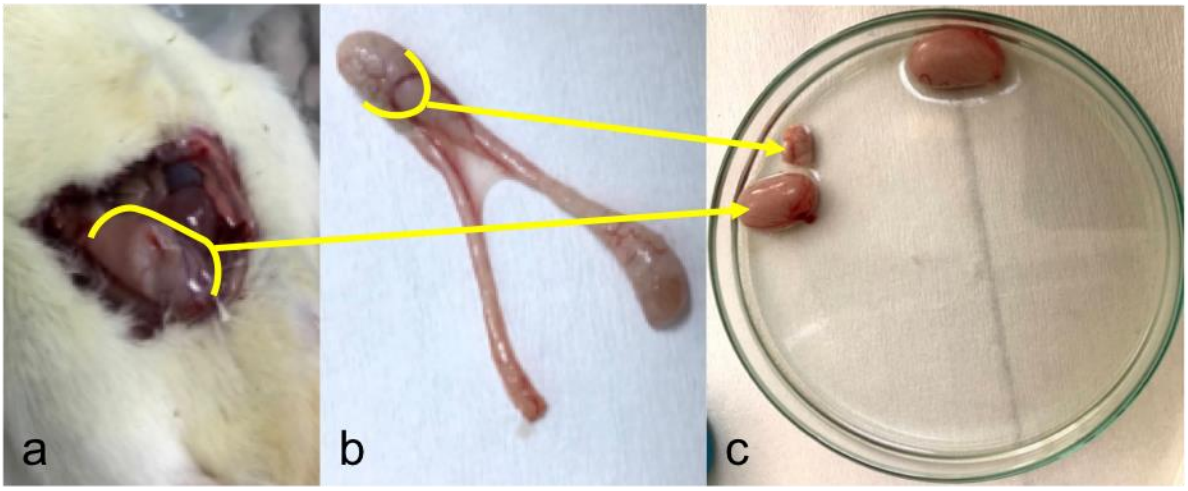

Figure 1. (a) Removal of tissues from a rat under anesthesia, (b) caput, corpus, and cauda epididymis with vas deferens, (c) tissue preparation on a 10 mm petri dish.

Assessment of oxidative stress in tissues

To quantify the extent of oxidative stress in the tissue, we conducted analyses for malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities using the prescribed methodologies.

MDA assay

The quantification of malondialdehyde (MDA) in the tissue was conducted using the thiobarbituric acid reactive substances (TBARS) method, a widely utilized technique for assessing lipid peroxidation and oxidative stress[42]. The tissue samples were weighed and homogenized in trichloroacetic acid (TCA) using a homogenizer. The supernatant was collected, followed by adding thiobarbituric acid (TBA) and butylated hydroxytoluene (BHT). The samples' optical density was measured at 535 nm using a spectrophotometer against a blank.

GSH assay

GSH in the tissue was measured using the modified Ellman method[43]. Tissue samples were efficiently homogenized and centrifuged. The supernatant was then combined with a solution of NaH2PO4 and 5,5′-dithiobis-(2-nitrobenzoic acid), followed by an incubation at room temperature for 5-10 minutes. The absorbance of the solution was quantified at a specific wavelength of 412 nm using a spectrophotometer, while a blank solution was used as the reference for calibration[44].

SOD activity

The method outlined by Sun et al. was employed to measure the superoxide dismutase (SOD) activity in the tissue[45]. The process entails generating the superoxide radical by utilizing xanthine and xanthine oxidase. This radical then transforms nitroblue tetrazolium into a red-colored formazan chromogen. The analysis was conducted using a spectrophotometer. The activity level was determined by measuring the intensity of the red color produced in the solution at a wavelength of 505 nm[45].

CAT activity

The classical method developed by Aebi[46] was used to determine CAT activity in this study. CAT is a

vital enzyme that catalyzes the breakdown of hydrogen peroxide (H2O2) into water and molecular oxygen, which is crucial in protecting cells from oxidative damage. The principle is based on monitoring the decrease in H2O2 concentration over time at 240 nm spectrophotometrically [46].

Statistical analysis

We conducted several statistical analyses to evaluate the data. Initially, the sample size in each group was determined through power analysis to ensure adequate statistical power for detecting significant effects. Data normality was assessed using the Kolmogorov-Smirnov test. Subsequently, a one-way analysis of variance (ANOVA) was employed to compare the means across the groups. Post-hoc comparisons between treatment groups were performed using Tukey's test to identify specific group differences. Pearson's correlation coefficient was calculated to examine the linear relationships between oxidative stress and sperm parameters, with corresponding p-values indicating statistical significance. A threshold of $p < 0.05$ was set to denote significance. All data are expressed as mean ± standard deviation. Statistical analyses were carried out using SPSS software (version 25), and results were visualized with Python (version 13.12.4).

RESULTS

Body and reproductive organs weights

The body weights of the experimental animals showed no significant differences between the groups ($p>0.05$) at the beginning and end of the experiment. The DOX group had a significantly lower relative testis weight (RTW) of 0.84 ± 0.58 compared to the control group's Relative testis weight (RTW)of 1.01 ± 0.05 ($p<0.05$). The groups receiving extracts (LD+DOX and HD+DOX) had RTWs of 1.05 ± 0.16 and 0.99 ± 0.11, respectively, both significantly higher than the DOX group ($p<0.05$). There was no significant difference between the RTWs of the extract and the control groups ($p>0.05$) (Table 1).

Table1. Animal's initial body weight (IBW), final body weight (FBW), and relative testis weight (RTW)

Group	IBW	FBW	RTW (%)
Control	259.67 ± 7.39	278.17 ± 14.19	1.01 ± 0.05 #
DOX	283.29 ± 16.79	273.00 ± 18.31	0.84 ± 0.58*
LD+DOX	283.33 ± 6.05	271.67 ± 16.52	1.05 ± 0.16#
HD+DOX	284.17 ± 23.96	280.67 ± 29.1	0.99 ± 0.11#

(n=7 for all groups; one-way ANOVA followed by Tukey's post-hoc test, values are means ± SDs; statistically significant differences *p<0.05 vs. the control; # p<0.05 vs. the DOX). Formula of RTW= [(Left testis+ Right testis weigts)/FBW]X100)

Sperm parameters

The sperm parameters for the control, DOX, LD+DOX, and HD+DOX groups were assessed. The control group exhibited the highest sperm concentration, motility, and the lowest percentage of dead sperm. In contrast, the DOX group showed significantly reduced sperm concentration and motility, along with a significantly higher percentage of dead sperm ($p<0.05$ for both parameters compared to the control group).

The groups receiving extracts (LD+DOX and HD+DOX) showed varied improvements in sperm parameters compared to the DOX group. Both extract groups had higher sperm concentration and motility ($p<0.001$ for both) and a lower percentage of dead sperm ($p<0.05$) than the DOX group. However, these values were still significantly different from the control group ($p<0.05$). The detailed values and statistical significance are presented in Table 2.

Table 2. Sperm parameters. Epididymal sperm concentration, motility, and dead sperm rates among the groups.

Group	Epidydimal Sperm Concentration (mil/ml)	Motility (%)	Dead Sperm (%)
Control	65.30 ± 2.9#	68.92 ± 12.12#	29.58 ± 4.22 #
DOX	23.98 ± 2.17*	41.08 ± 8.75*	58.17 ± 8.06 *
LD+DOX	41.85 ± 3.3*#	49.25 ± 4.17*	39.5 ± 1.64*#
HD+DOX	43.00 ± 4.3*#	51.58 ± 4.04*	39.08 ± 2.08*#

(n=7 for all groups; one-way ANOVA with Tukey's post-hoc test, values are means ± SDs; statistically significant differences *p<0.05, **p<0.01 vs. the control; and # p<0.01 vs. the DOX).

Oxidative stress parameters

As shown in Figure 2, the parameters assessed for evaluating oxidative stress in testicular tissue indicated that GSH, SOD, and CAT activities were significantly lower in the DOX group compared to the control group ($p<0.05$). At the same time, MDA levels were significantly higher ($p<0.05$), highlighting the testicular damage induced by our

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experimental model.

MDA levels were lowest in the control group (249.30 nmol/g) and highest in the DOX group (471.6 nmol/g) ($p < 0.01$). The MDA levels in the LD+DOX and HD+DOX groups were 376.87 nmol/g and 365.05 nmol/g, respectively. These levels were significantly lower than the DOX group ($p<0.05$) but higher than the control group ($p<0.05$ for both) (Figure 2a).

The control group had the highest average GSH level at 6.58 µmol/g, while the DOX group had the lowest at 1.58 µmol/g ($p<0.05$). The LD+DOX and HD+DOX groups exhibited GSH levels of 3.53 µmol/g and 3.73 µmol/g, respectively. The GSH level in the extract groups was significantly different from the control and DOX groups ($p<0.05$ for both). However, there was no significant difference between the two extract groups ($p>0.05$) (Figure 2b).

Similar to GSH, SOD activities were highest in the control group at 4.87 U/mg and lowest in the DOX group at 1.49 U/mg ($p<0.05$ control vs DOX). For LD+DOX, this value was 2.35 U/mg, while for HD+DOX, it was 2.57 U/mg. The LD+DOX group showed a significantly higher outcome than the DOX group ($p<0.05$). Additionally, the HD+DOX group also exhibited a significant increase relative to the DOX group ($p<0.05$) (Figure 2c).

The DOX group also had significantly lower CAT activity than the control group ($p<0.05$). The HD+DOX group did not show a significant difference compared to the DOX group ($p>0.05$) but remained lower than the control group ($p<0.05$). The LD+DOX resulted in a significant increase compared to the DOX group ($p<0.05$). The highest CAT activity was observed in the control group at 5.21 µmol H2O2/min/mg, decreasing progressively in the LD+DOX, HD+DOX, and DOX groups (3.67, 3.47, and 2.90 µmol H2O2/min/mg, respectively). No dose-dependent differences were observed between the extract groups ($p > 0.05$; Figure 2d).

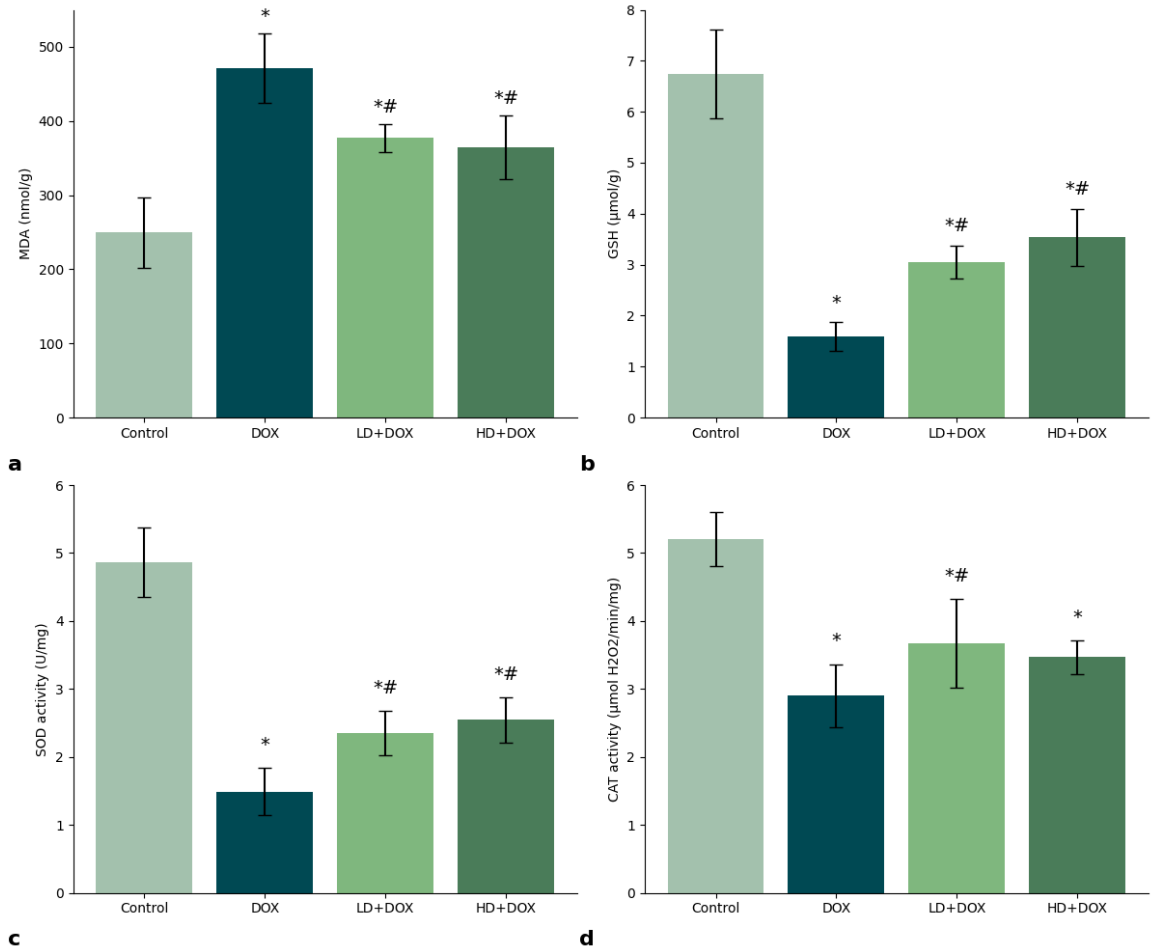

Figure 2. Testicular tissue levels of MDA (a), GSH (b), SOD activity (c), and CAT activity (d) in Control, DOX, LD+DOX, and HD+DOX groups. Data are mean ± SD (n=7). Statistical significance was determined using one-way ANOVA, followed by Tukey's post-hoc test (* $p<0.05$ vs. Control; # $p<0.05$ vs. DOX).

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Correlations with oxidative stress markers and sperm parameters

The correlation analysis revealed significant relationships between oxidative stress markers and sperm parameters. MDA showed a strong negative correlation with sperm count ($r = -0.93$, $p < 0.001$), suggesting that higher MDA levels are linked to lower sperm counts. GSH showed a positive correlation with sperm motility ($r = 0.71$, $p < 0.001$), while SOD was positively correlated with sperm vitality ($r = 0.73$, $p < 0.001$). Additionally, CAT demonstrated a slight positive correlation with sperm count ($r = 0.84$, $p < 0.001$) (Figure 3).

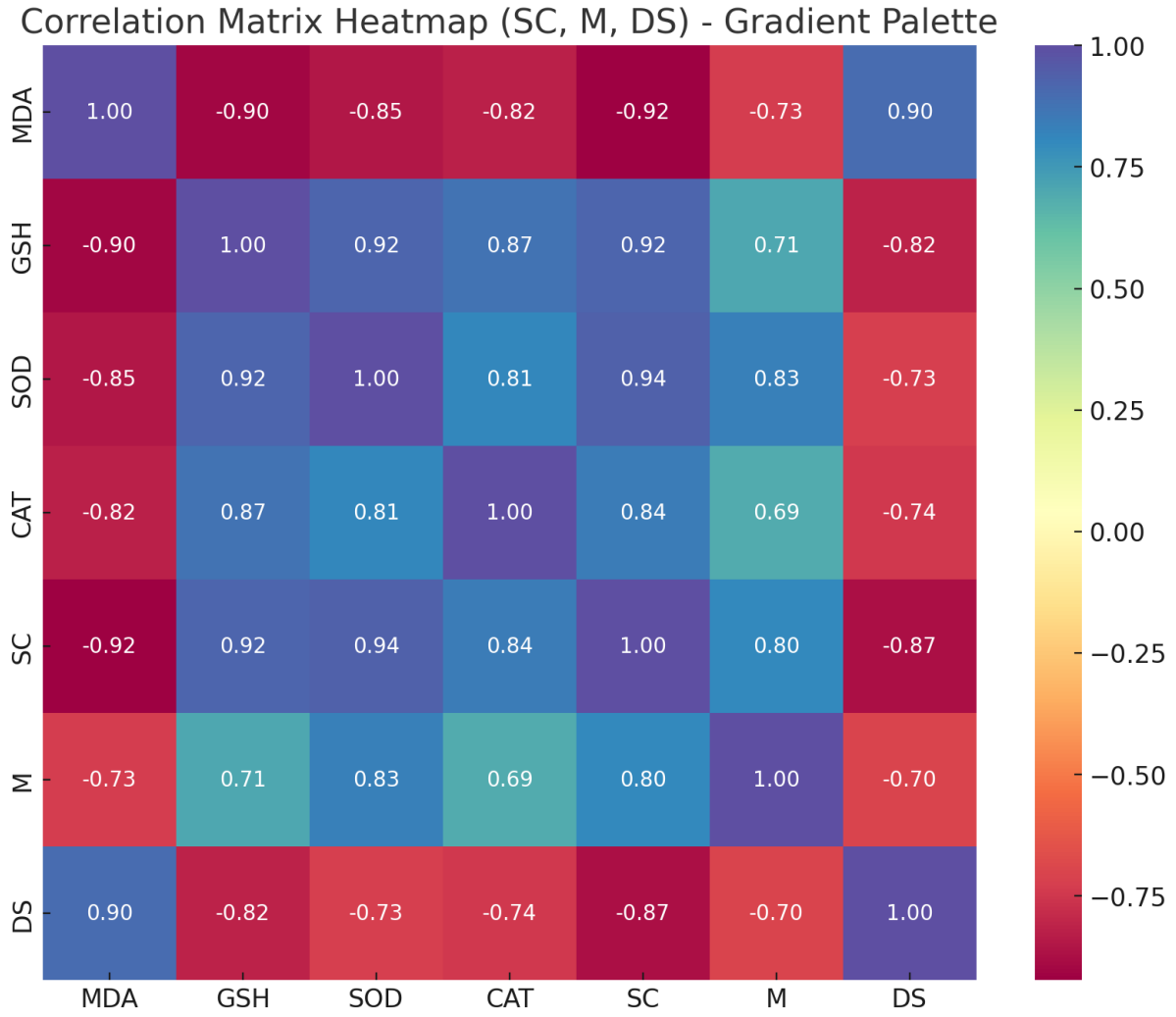

Figure 3. Heatmap showing the correlation matrix between oxidative stress markers (MDA, GSH, SOD activity, CAT activity) and sperm parameters (SC: Epididymal Sperm Count, M: Motility, DS: Dead sperm ratio). The color gradient ranges from cooler tones (blue) to warmer tones (burgundy), providing a clear visual representation of positive and negative correlations (Pearson's correlation coefficient).

DISCUSSION

Chemotherapeutic agents, such as doxorubicin (DOX), are known to induce oxidative stress, which disrupts spermatogenesis and can lead to secondary infertility, a significant concern for young men undergoing cancer treatment[9]. This study investigates the potential therapeutic impact of PLFE on DOX-induced reproductive toxicity, specifically focusing on oxidative stress and spermatogenesis.

This study underscores the significant reproductive toxicity induced by the chemotherapeutic agent DOX, emphasizing its potential to disrupt spermatogenesis, reduce sperm quality, and induce secondary infertility. Specifically, DOX exposure at 15 mg/kg was shown to cause testicular weight reduction, decreased sperm parameters, and increased oxidative stress markers within just two days post-administration, consistent with prior

research findings[17,24,25]. These detrimental effects highlight an urgent need to identify supportive therapies to protect reproductive function during chemotherapy, especially for younger male cancer patients at risk of infertility. The experimental outcomes demonstrated a promising protective effect of PLFE against DOX-induced oxidative stress.

Flavonoids, phenolic acids, and chlorogenic acids are powerful plant-based antioxidants that counteract oxidative stress in infertility[47,48]. By scavenging free radicals and reducing lipid peroxidation, phytochemicals help preserve sperm DNA integrity and cell membrane stability, offering therapeutic benefits against DOX toxicity[14,15,22,25,49].

Various PL extracts, well-recognized for their polyphenolic compounds, have demonstrated organ-protective effects across multiple studies, including against hepatic[12,50], renal[12,51], cardiac[13], and gastric[52] damages. Additionally, only one study specifically investigated Prunus laurocerasus fruit extract (PLFE) in relation to spermatogenesis[53]. They reported that a 7 mg/kg dose of PLFE administered over 8 weeks reduced pesticide-induced reproductive damage, improving sperm parameters by mitigating DNA damage and apoptosis. Nevertheless, full recovery in sperm concentration and motility was not achieved, which is similar to our findings. In our study, higher PLFE doses showed significant improvements in DOX-induced damage but did not normalize MDA levels, unlike Bakır et al.'s results with dimethoate[53].

The results of our study reveal that PLFE positively impacted sperm parameters by improving sperm concentration, motility, and vitality (Table 2). The administration of PLFE led to significant improvements in oxidative stress markers (Figure 2), with increases in glutathione (GSH) and superoxide dismutase (SOD) activities and reductions in malondialdehyde (MDA) levels. As indicated by MDA, the notable reduction in lipid peroxidation and a boost in key antioxidant enzymes for spermatogenesis[3–5] suggests PLFE's potential in counteracting ROS-induced cellular damage in reproductive tissues.

Interestingly, while PLFE was effective in enhancing GSH and SOD levels ($p<0.05$ LD+DOX and HD+DOX vs DOX for both parameters), it did not produce a dose-dependent increase in CAT activity ($p>0.05$ between LD+DOX and HD+DOX; Figure 2). This suggests that PLFE may exert selective antioxidant effects, primarily supporting the GSH and SOD pathways while having a more limited impact on the CAT pathway. This raises the question of whether PLFE's active compounds reach a functional threshold for CAT activity. Aitken and Drevet[6] emphasized catalase's essential role in preserving sperm motility by scavenging hydrogen peroxide, which supports our findings. However, in our study, the lower CAT activity in the PLFE-treated groups may have contributed to motility not reaching the control level.

The study's correlation analysis reinforces the role of oxidative stress in male fertility. A strong negative correlation between lipid peroxidation and sperm parameters—specifically, sperm count and vitality—highlights the detrimental impact of ROS on sperm cell membrane integrity. In contrast, the positive correlations of GSH and SOD with sperm count and motility reflect their protective roles against oxidative stress (Figure 3). These findings emphasize the importance of a balanced antioxidant environment in protecting spermatogenesis and overall sperm quality. Elevated ROS levels can impair the fluidity and permeability of sperm cell membranes[6], leading to decreased functionality. Additionally, these ROS levels disrupt energetic and mitochondrial processes[7,15] through genetic[15,18] and epigenetic[19] mechanisms, contributing to increased cell death[21]. DOX and natural compounds are known to modulate numerous molecular mechanisms[39,54,55], including oxidative stress response pathways such as NF-κB[56] and Nrf2[57–59], thereby enhancing antioxidant defenses and regulating inflammatory and apoptotic signals related to reproduction. In this context, it can be said that PLFE exerts its protective effects through these molecular pathways. However, the application of PLFE for a specific time interval (matching the duration of spermiogenesis) and the absence of molecular or histological analysis in our study limits our ability to elucidate the protective mechanisms of PLFE on spermatogenesis fully. Future studies should incorporate molecular-level investigations to identify the specific pathways targeted by PLFE's active compounds, allowing for a more comprehensive understanding of its protective effects.

Despite these encouraging results, several key points warrant further research. Notably, while PLFE improved oxidative stress markers and sperm parameters, it did not fully restore them to control levels. This partial improvement suggests that while PLFE has protective properties, it may not be a standalone therapeutic approach for mitigating chemotherapy-induced damage. Instead, optimizing

the dose and duration of PLFE treatment may be critical in maximizing its therapeutic benefits. Hormesis, wherein moderate stress levels induce protective effects but higher doses yield diminishing returns[60], might explain the observed plateau in PLFE efficacy at higher doses, particularly concerning CAT activity. Lower doses could activate endogenous antioxidant responses more effectively, while higher doses might lead to enzyme saturation or limited additional benefits. It should also be noted that certain flavonoids may inhibit CAT activity[61], which could explain the reduction in CAT activity observed at higher doses of PLFE.

Our findings indicate that higher doses of PLFE can significantly improve oxidative markers and sperm quality. This study is the first to explore PLFE's protective effects against chemotherapy-induced sperm damage, highlighting the varying impacts of different doses on testicular damage caused by DOX. Such experimental studies in rat models offer valuable insights into potential therapeutic approaches for human health. One of the strengths of our study is the systematic examination of different doses of PLFE, allowing us to assess its dose-response relationship.

In summary, this study is the first to systematically examine the protective effects of PLFE against chemotherapy-induced testicular toxicity. PLFE is a promising candidate for preserving male fertility by enhancing antioxidant defenses and reducing DOX-induced reproductive damage. However, further research is needed to optimize its therapeutic use, investigate its biochemical mechanisms, and determine its long-term effects on spermatogenesis. This research provides a foundation for developing phytotherapeutic interventions aimed at reducing the reproductive side effects of chemotherapy and underscores the importance of strengthening antioxidant defenses in male reproductive health. Future studies should examine the roles of PLFE's active compounds, particularly flavonoids and phenolic acids, in spermatogenesis at molecular and histological levels.

CONCLUSION

This study underscores the potential of PLFE as a supportive therapy for reducing DOX-induced oxidative damage in testicular tissue, suggesting its value in fertility preservation during chemotherapy. PLFE showed encouraging effects by lowering oxidative stress and enhancing sperm parameters. However, complete restoration to control levels was not achieved, highlighting the need for further research to refine the composition of extracts, dosage, and treatment duration. While PLFE activated several antioxidant pathways, its limited impact on catalase activity and the partial restoration of oxidative markers indicate that dosage optimization and further studies are essential.

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ORIGINAL RESEARCH

Applications of Traditional Medicine Among Patients Attending the Chest Disease Clinic

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Abstract

Objective: Among various traditional practices for respiratory complaints, the most common one involves the consumption of herbs in different forms. This study was conducted to detect the use of herbs in a particular region, Duzce.

Material-Method: Patients who visited the chest diseases clinic (n=204) were asked whether they used herbal remedies as a part of traditional medicine, aside from the treatments provided at the clinic.

Results: The rate of traditional medicine application for their health complaints among the patients was 39.7% (81/204). The most frequently used applications were ginger tea (18.5%), lemon juice (18.5%), pine cone syrup (16%), and carob molasses (14.8%). Patients mainly used traditional medicine for chest pain (50%), phlegm (47.1%), cough (43.1%), and shortness of breath (35.4%). Traditional medicine was significantly lower in patients experiencing shortness of breath (35.4% of those who used traditional medicine compared to 64.6% of those who did not, p=0.001). The frequency of traditional medicine use was 50% in sarcoidosis patients, 43.1% in asthma patients, and 30% in COPD patients. The rate of traditional medicine use was 41.5% among regular medication users.

Conclusion: In addition to regular medication for respiratory complaints and diseases, patients used traditional medicine at an average rate of 40%. When patients' use of regular and/or traditional medicine is questioned, valuable information is obtained regarding their compliance with treatment and the possible benefits and harms of their traditional medicine products.

Keywords: Health, Traditional Medicine, Respiratory Complaints

INTRODUCTION

Culture is defined as the values, beliefs, attitudes, behaviors, customs, and traditions learned, shared, and transmitted across generations by a group of people. Health and disease are also concepts shaped within cultural structures, which may vary from each culture. Therefore, beliefs about illness and health in societies and healing methods continue to be transmitted to the present as a part of traditional culture and maintain their impact.[1]

Turkey's geographical location has brought together various cultures, resulting in the formation of diverse syntheses. The interactions of these cultures have diversified customs and traditions while enriching the remedies for the public's health issues. Especially practices and beliefs concerning sociocultural and even biological phenomena such as birth, newborn care, postpartum period, illness, and death have persisted to the present day.[1]

Studies on the use of traditional medicine methods in chest diseases involve various practices, such as brewing and using leaves and roots of numerous plants, as well as using their powder forms. Cough, asthma, common cold, and bronchitis were the most common conditions for which herbal remedies were used. A wide range of plants, from daisy family members to root vegetables, has been utilized in these practices.[2]

Düzce, a province in Turkey with its diverse cultural heritage, also boasts a rich herbal flora. This study aims to gather information about traditional treatment methods applied by the people of Düzce for complaints related to chest diseases, such as cough, phlegm, and shortness of breath. Additionally, the research aims to discuss the most frequently used herbal products in traditional medicine for chest diseases and examine data related to their place in evidence-based medicine.

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MATERIALS AND METHODS

Materials

The study was conducted with 204 patients who presented to the Chest Diseases Clinic at Düzce University Faculty of Medicine with various chest disease complaints between September and December 2021 and completed routine medical examinations and controls.

Methods

A questionnaire form was presented to the participants through face-to-face interviews. The first part of the form, which we developed, included questions about participants' sociodemographic characteristics (gender, age, place of birth, education level, marital status, and number of children). The second part of the form consisted of questions related to the reasons for patients' clinic visits (their diseases and/or complaints), whether they had tried any traditional medicine application for these reasons, whether they experienced any side effects from the applied traditional remedy, and the source from which they learned about this traditional medical practice. An informed consent was derived from all participants. Patients with communication problems due to impaired intellectual and/or physical capacity were not included. The study obtained Ethical approval from the Non-Interventional Research Ethics Committee of Düzce University Faculty of Medicine (Decision No: 2022-43).

Statistical analysis

Statistical analyses were performed using SPSS version 21. Descriptive statistics, including the mean and standard deviation, were calculated for all data types in the study. For group comparisons, the Independent Samples t-test was used for variables meeting parametric test assumptions, while the Mann-Whitney U test was applied for variables that did not meet these assumptions. Fisher's Exact and Pearson Chi-square tests were employed to compare categorical variables. A p-value of <0.05 was considered statistically significant.

RESULTS

Among the 204 patients included in the study, 51% (n=104) were female. The ages of the participants ranged from 19 to 84 years, with the youngest being 19 and the oldest being 84 years old. The average age of the participants was 53.2 ± 15.5, and approximately half (47.5%) had completed primary education. Most (83.8%) were married, and the average number of children among them was 3 (min: 1, max: 11). Nearly 70% of the patients were born in Düzce and its districts, and 30% were employed (Table 1).

Table 1. Demographic characteristics of patients

	n	%
Gender		
Male	100	49.0
Female	104	51.0
Marital Status		
Married	171	83.8
Single/Other	33	16.2
Education		
Illiterate	13	6.4
Literate	6	2.9
Primary School	97	47.5
Middle School	15	7.4
High School	42	20.6
College/University	31	15.2
Birthplace		
Düzce	140	68.6
Other Cities	63	30.9
Foreign	1	0.5
Employment		
Yes	143	70.1
No	61	29.9
Age Groups		
0-39	42	20.6
40-59	77	37.7
60 and above	85	41.7

The most common reasons for patients' visits to the chest disease clinic were shortness of breath (88.7%), cough (70.6%), phlegm (34.3%), and chest pain (23.5%). Their current diseases included

asthma (25%), COPD (9.8%), sarcoidosis (8.8%), and lung cancer (1.5%). Among the participants, 40.2% (82) used regular medication for their disease or complaint. The most commonly used medication forms were inhalers, aerosolizers, and nebulizers.

The rate of using traditional medicine for their health complaints among the patients was 39.7% (81/204). The most frequently used applications were ginger tea (18.5%), lemon juice (18.5%), pine cone syrup (16%), and carob molasses (14.8%). Other herbs, such as turmeric, mint, linden, thyme, cinnamon, mulberry, garlic, and fennel, were used less frequently (Table 2).

Table 2. Used herbs

	n	%
Ginger	15	18.5
Lemon	15	18.5
Pine cone	13	16.0
Carob molasses	12	14.8
Turmeric	8	9.8
Mint	8	9.8
Linden	8	9.8
Thyme	6	7.4
Cinnamon	3	3.7
Mulberry	3	3.7
Garlic	3	3.7
Fennel	2	2.4
Others*	17	20.9

* Onion, black pepper, mushroom, corn, cherry, apple, green tea, rose hip, radish, cherry laurel, juniper, cat's tail, St. John's wort, laurel, mastic, capon, sage

Most of the used products were in the form of fruit (35.8%), leaves (24.7%), and roots (19.7%) of the plants. They were commonly used with honey, molasses, or by brewing them.

It was observed that the herbs were used both individually and in combination, and several application methods were used simultaneously. Ginger (used alone at a rate of 6.7%, and in combination at a rate of 93.3%, p=0.007) and lemon (not used individually, all usage combined, p=0.001) were used statistically significantly in combination. Sour or bitter products (lemon, ginger, pine cone) were especially used with honey or molasses to sweeten their taste. When looking at the most common complaints for which traditional remedies were used, patients primarily resorted to them for chest pain (50%), phlegm (47.1%), cough (43.1%), and shortness of breath (35.4%). The rate of using traditional medicine was significantly lower in patients experiencing shortness of breath (35.4% of those who used traditional medicine compared to 64.6% of those who did not, p=0.001). The frequency of traditional medicine use was 50% in sarcoidosis patients, 43.1% in asthma patients, and 30% in COPD patients. Among regular medication users, the overall rate of using traditional medicine was 41.5%, 41.2% for inhaler users, 37.0% for aerosolizer users, 52.4% for nebulizer users, and 50.0% for oral medication users.

The relationship between patients' traditional medicine methods and demographic characteristics was tested. Gender, age, education, employment status, birthplace, and working status did not affect the rates of traditional medicine usage (Table 3).

According to the responses to the question of which plant they used for which complaints or clinical conditions, ginger was found to be used for cough (8.3%), expectoration (11.4%), shortness of breath (7.7%), and relieving chest pain (16.7%). The rate of using ginger was significantly higher in patients with chest pain than those without (16.7% vs. 4.5%, p=0.009). The rate of using ginger for asthma was 15.7%, and for COPD, it was 10%. The rate of using ginger in patients diagnosed with asthma was significantly higher than in non-asthmatic patients (15.7% vs. 4.6%, p=0.014). Lemon, as the second most common herb, was used for all complaints related to chest diseases. It was mainly used for cough (8.3%), shortness of breath (6.6%), and asthma (7.8%). Pine cone, the third most common herb, was used for cough (7.6%), phlegm (8.6%), asthma (7.8%), shortness of breath (6.6%), and COPD (10%). Lastly, carob was used for chest pain (6.3%), cough (7.6%), and asthma (7.8%).

When asked about the source from which they learned traditional medicine practices, participants reported primarily from their close environment (26.5%), followed by the internet (3.9%), herbalists (3.4%), relatives (2%), television (0.5%), and other sources.

Table 3. Use of traditional medicine by demographic characteristics

	Using traditional medicine		Not using traditional medicine		p
Gender	n	%	n	%	
Male	40	40.0	60	60.0	0.933
Female	41	39.4	63	60.6	
Marital Status					
Married	69	40.4	102	59.6	0.668
Single, other	12	36.4	21	63.6	
Education					
Primary and Pre-middle school	48	41.4	68	58.6	0.665
High School, College/University	33	37.5	55	62.5	
Birthplace					
Düzce	55	39.3	85	60.7	0.878
Other Cities	26	40.6	38	59.4	
Employment					
Yes	26	42.6	35	57.4	0.640
No	55	38.5	88	61.5	
Age Groups					
0-39	13	31.0	29	69.0	0.141
40-59	37	48.1	40	51.9	
60 and above	31	36.5	54	63.5	

DISCUSSION

Due to the richness of its vegetation, Turkey is one of the leading countries in the use of traditional herbal medicine. In our study conducted in Duzce province, where rural life characteristics are still dominant, the rate of herbal product use for various respiratory complaints was around 40%, and there were no significant differences in gender, education, marital status, and birthplace. Although herbal product use was 48% in the age group of 40-59, there was no significant difference compared to other age groups. Patients mainly obtained herbal medicine recommendations from their close circles. In our study, ginger, lemon, pine cone, and carob were the most preferred herbs. In Erarslan et al.'s survey-based research on individuals with lung disease, it was observed that women and urban residents used herbal products significantly more. The most preferred herbs in their study were mint lemon, licorice root, linden, and rosehip.[3] Gülhan et al.'s study, which included 94 COPD patients, observed a herbal product use rate of 73%, which was significantly higher in severe COPD cases.[4]

Our study discussed the use of the four most common plants in traditional medicine and scientific studies in detail. We observed their place in traditional medicine in respiratory complaints.

Ginger (Zingiber officinale Roscoe): Ginger is a perennial plant from the Zingiberaceae family, capable of growing up to one meter in height, with slender, elongated leaves and flowers that exhibit yellow-red hues. Its root tubers are used as both a spice and medicine, and it thrives in tropical or subtropical climates. In terms of appearance, it closely resembles a sweet potato. The irregular, fragmented, peeled, or unpeeled dried roots of the ginger plant, either in whole or ground form, find widespread culinary use across the globe.

Fresh ginger is richer in active substances; it contains 80% water, 2% protein, 1% fat, 12% starch, calcium, phosphorus, iron, and vitamins B and C. The water content in dried ginger is 10%. One teaspoon of ginger, which can be used as a spice, contains negligible nutrients except for manganese. This amount of ginger meets 79% of the daily manganese requirement.[5]

Usage in traditional medicine: In Ayurveda, ginger has been used to treat throat disorders, headaches, chest ailments, hemorrhoids, and rheumatism. It is also utilized for its carminative and digestive properties.[5] In traditional and non-evidence-based medical practices, ginger is believed to be beneficial for stomach discomfort and dizziness. Additionally, it is mentioned to have appetizing, antiseptic, carminative, digestive regulatory, respiratory tract opening, and detoxifying effects. It is also claimed to dilate blood vessels, induce sweating and warmth, and invigorate the heart. Moderate amounts of ginger are generally considered safe by the FDA (Food and Drug Administration). However, it can interact with medications such as Coumadin and Warfarin, which are used regularly, and alter their safe levels.[6] In powdered form, ginger may cause allergic rashes and, despite being considered safe, may lead to symptoms such as heartburn, gas, and nausea. It can also have adverse effects on individuals with gallstones.

Medical research involving its use in chest

diseases: Ginger oil has been proven to cause bronchodilation by stimulating B2 adrenergic receptors in experiments with mice, due to its citral, eucalyptol, and camphor content.[7] Other animal studies have also shown that it reduces airway hyperreactivity and inflammation.[8]

In a study by Rouhi et al. involving 92 patients diagnosed with asthma who took ginger (150 mg/day), significant reductions were noted in wheezing, chest tightness, nocturnal cough attacks, daily inhaler use, and weekly dyspnea attacks in the ginger group; however, there was no significant difference in respiratory functions between the groups. In one of the 109 randomized controlled trials examining the effects of ginger on humans, ginger was found to alleviate asthma symptoms, and in another study, it was observed to reduce the mechanical ventilation time in patients with Acute Respiratory Distress Syndrome (ARDS).[10]

In our study, ginger was the most commonly used herbal product, and it was used primarily to suppress cough and phlegm, open the airways, and alleviate chest pain. The use of ginger by patients diagnosed with asthma, at a rate of 15.7%, also drew attention. The improvement in symptoms observed in the ginger-using group in Rouhi et al.'s studies may also apply to our study group. However, the fact that the daily ginger intake of the individuals included in our study was not standardized weakens our inference. In our group, ginger was more frequently observed than in Erarslan et al.'s survey studies. Since patients in our group were most influenced by their close circles (26.5%) regarding herbal medicine, it is appropriate to investigate the process that made ginger popular.

Lemon (Citrus genus, Rutaceae family): It contains various citrus fruits (orange, mandarin, sweet lemon, lemon, grapefruit, loquat). They are mainly consumed as fresh or raw materials for fruit juices or in canned form. They are also used as additives in food, beverages, cosmetics, medicines, spices, and cosmetic components. Apart from vitamin C, they contain sugar, fiber, potassium, folate, calcium, thiamine, niacin, vitamin B6, phosphorus, magnesium, copper, riboflavin, and pantothenic acid. Citrus fruits contain active metabolites such as flavonoids, alkaloids, coumarins, limonoids, carotenoids, phenolic acids, and volatile oils. These metabolites have various bioactivities, including antioxidant, anti-inflammatory, anticancer, cardiovascular protective, and neuroprotective effects.[11]

Usage in traditional medicine: Lemon has been described as a powerful antidote to potent poisons since ancient Greek times.[12] In Ayurveda, different citrus fruits have been used as appetite stimulants, cardiotonic, antiemetics, and for regulating digestion.[13]

Medical research involving its use in chest diseases: Giving citrus fruits in different doses to mice exposed to cigarette smoke and developing chronic neutrophilic inflammation suppressed inflammation. The intake of citrus fruits reduced neutrophil, myeloperoxidase, and matrix metalloproteinase-9 levels in the bronchoalveolar lavage fluids of mice.[14] Important flavonoids in lemon, such as quercetin, have been shown to block histamine and other allergy mediators, making it potentially effective in treating asthma.[15] In our study, lemon was used for cough, shortness of breath, and asthma. However, it was used less frequently for asthma (7.8%) than ginger (15.7%).

Pine Cone [Pinus pinea L.]: Terpenoids, steroids, proanthocyanidins, and flavonoids within the Pinus species have drawn the attention of both traditional and modern medicine. Various studies have shown these compounds to have antibacterial, antifungal, and antioxidant activities.[16, 17]

Usage in traditional medicine: In ancient Chinese medicine, pine cones were used to moisturize the lungs, eliminate cough, and reduce fever.[18] Studies in Artvin and Çankırı showed that people used pine cones to relieve various complaints. For example, the green cones of Scots Pine (Pinus sylvestris) were used as a decoction for coughs, and the cones of Corsican Pine (Pinus nigra) were boiled in water and mixed with honey for the treatment of asthma.[19, 20]

Considering that Düzce is rich in pine forests, it is unsurprising that pine cones were our study's third most commonly used herbal product. Pine cones alleviate cough, phlegm, and shortness of breath and were preferred by 10% of COPD patients and 7.8% of asthma patients.

Medical research involving its use in chest diseases: The French Maritime Pine (Pinus Maritime) extract from the pine species was used in a mouse model developed for asthma. The oral administration of Pycnogenol in mice reduced inflammatory cytokines and cells such as IL-4, IL-5, and IL-13 in bronchoalveolar secretions. This suggests that Pycnogenol could be used in asthma treatment.[21]

In a study where Pycnogenol was combined with inhaled corticosteroids in asthmatic patients, a daily dose of 100 mg was administered, resulting in

reduced steroid dosage, decreased nighttime awakenings, and improved asthma control.[22] Additionally, in a mouse model of Chronic Obstructive Pulmonary Disease (COPD), Pycnogenol demonstrated the ability to reduce airway inflammation.[23]

Carob [Ceratonia siliqua **L.**]: Carob is a widely used plant in the food industry due to its rich content of fiber, sugar (especially sucrose, fructose, and glucose), and minerals (sodium, potassium, iron, copper, manganese, and zinc). It is abundant in the Mediterranean region and is rich in polyphenols and flavonoids.[24]

Usage in traditional medicine: One of the observed historical medical uses is treating mouth ulcers.[25] In Southern Italy, it has been used to alleviate digestive system inflammation and as an expectorant.[26] In Iraq and Morocco, it is known that carob powder is used against diarrhea and abdominal pain.[27, 28]

In studies conducted in our country, it has been observed that carob is used through infusion for the treatment of urinary system diseases, in the form of molasses for the treatment of anemia, and in the treatment of liver diseases. It has also been used for the treatment of prostatitis and anemia.[29, 30, 31]

Medical research involving its use in chest diseases: In a model of isolated tracheal bronchoconstriction developed in rabbits, carob has been observed to induce dilation through its antimuscarinic activity.[32] In another study conducted on mice, carob extract reduced myeloperoxidase activity, hydroxyproline content, and nitric oxide levels, which were increased by shisha smoke in the lungs.[33] In our study, carob was used for chest pain (6.3%), cough (7.6%), and asthma (7.8%).

When searching the scientific literature, it has been observed that there are very few randomized controlled clinical trials for this study's most commonly used four plants for alleviating respiratory complaints. However, animal studies have revealed some therapeutic mechanisms of action. Ginger has been shown to induce bronchodilation by stimulating β2 receptors, lemon inhibits neutrophils, myeloperoxidase, and matrix metalloproteinase-9 while reducing histamine release, pine cone reduces inflammatory cytokines such as IL-4, IL-5, and IL-13, and carob creates bronchodilation through antimuscarinic activity in animal studies.

However, all these studies have not yet reached a channel where appropriate, effective, and reliable dosage ranges provided by randomized controlled human studies, which are the basis of evidence-based medicine, are determined. The results of ongoing randomized controlled trials related to ginger and similar plants in asthmatic patients are yet to be published. In our study, the rate of using traditional medicine practices among patients receiving standard bronchodilator treatment ranged from 37% to 52%. The significant use of traditional medicine practices by patients with respiratory complaints, alongside modern medical approaches, may be scientifically justified as randomized controlled studies involving medicinal products increase.

The widespread use of traditional medicine, including herbs, has prompted the WHO to study and track data on traditional medicine for years. To this end, the WHO has developed a "traditional medicine strategy" to help member states research, integrate, and regulate traditional medicine in their national health systems. Monitoring its safety is an essential and prioritized area of work for traditional medicine. The WHO encourages Member States to establish an integrated pharmacovigilance system for both conventional pharmaceuticals and traditional products.

Traditional products and practices are subjected to the same scrutiny (regulation, safety, and quality control) as pharmaceuticals; 124 WHO Member States (including Turkey) have passed laws or regulations for herbal medicines.[34, 35]

CONCLUSION

Besides significant improvements in the therapeutic interventions for respiratory diseases, traditional medicine methods have remained. Cough, asthma, common cold, and bronchitis were the most common conditions for which herbal remedies were used. A wide range of plants has still been used to heal respiratory complaints. Patients with respiratory complaints living in Duzce mostly use ginger, lemon, pine cone, and carob. Those four plants have been used in animal and human research studies, and effects such as bronchodilation, inhibition of neutrophils, myeloperoxidase, and reduction of histamine release were observed.

Suppose all patients who apply to the Chest Diseases Clinic for respiratory complaints are questioned whether they use traditional medicine and/or modern medical practices. In that case, valuable information will be obtained regarding their compliance with treatment and the possible benefits and harms of the traditional medicine

products they use.

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ORIGINAL RESEARCH

Evaluation of Knowledge and Opinions on Apitherapy Products Among 1st and 4th Year Nutrition and Dietetics Department Students

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Abstract

Objective: Apitherapy is a traditional treatment method in which honey bee products are used for therapeutic purposes. Students studying in the Department of Nutrition and Dietetics are not heavily involved in the application of apitherapy.

Material-Method: This study investigates the knowledge and opinions of 1st- and 4th-year students studying in the Department of Nutrition and Dietetics regarding apitherapy. A survey was administered to students who were randomly selected from the 1st-year (n=168) and 4th-year (n=241) students. The survey addressed descriptive characteristics of the students, their knowledge, experience, and expectations regarding apitherapy, as well as the use and frequency of apitherapy products.

Results: The most preferred bee product among both 1st- and 4th-year students was honey, followed by bee pollen. The primary source of information about apitherapy for 1st-year students was the internet (63.5%), while for 4th-year students, it was the media (46.1%). When asked who should be the source of information about apitherapy, the majority of 1st-year students (64.0%) and 4th-year students (67.2%) responded that dietitians should be the source. The majority of students in both groups reported not having sufficient knowledge or opinions about bee products.

Conclusion: This study shows that knowledge and opinions about apitherapy among future dietitians are limited. Including apitherapy in the curriculum and creating and promoting the identity of the "apitherapist dietitian" will increase the likelihood of dietitians using apitherapy.

Keywords: Apitherapy, Nutrition, Dietitian, Curriculum

INTRODUCTION

Apitherapy is a traditional treatment method that uses honey bee (Apis mellifera) products (such as pollen, royal jelly, propolis, honey, bee bread, bee venom, etc.). It can be applied in both acute and chronic conditions and has been practiced since the ancient Indian, Sumerian, Egyptian, Greek, and Chinese periods. It is still widely used around the world, from Germany to India and from Venezuela to Nigeria.[1,2]

Apitherapy has two distinct aspects. One is holistic apitherapy, which is a part of alternative medicine and is widely discussed at congresses. Personal experience and treatment methods serve as a guide for other practitioners. The other is scientific apitherapy, which focuses on the medical value of bee products.[3] Apitherapy has also been extensively used in Islamic medicine and Anatolian history. The healing properties of honey were mentioned in medical texts from the Ottoman period.[4]

In 2014, Turkey enacted traditional and complementary medicine legislation, which regulates the use of these practices in clinics. Apitherapy is one of these practices and can be applied in certified centers. Currently, approved apitherapy products in Turkey, such as honey, bee venom, royal jelly, propolis, and bee pollen, are used following the Traditional, Complementary, and Integrative Medicine (TCIM) regulation.[5] Other hive products, such as apiair (the use of hive air) and drone larvae (apilarnil), are considered potential apitherapy products that may be approved in the future.[6]

The nutritional, digestive, anti-inflammatory, antimicrobial, and antitumor properties of honey; the immunostimulating, antiallergic, antioxidant, antiulcer, and antidepressant properties of bee pollen; the cell-protective, antioxidant, antimicrobial, anti-inflammatory, hypoglycemic, and liver-protective properties of royal jelly; the antioxidant, neuroprotective, and hypoglycemic

properties of propolis; and the analgesic, immunomodulatory, neuroprotective, and antirheumatic properties of bee venom are the prominent features of these products. In addition, it is reported that all hive products have positive healing effects on wounds and burns.[7,8]

People who are allergic to bees and their products, as well as babies under one year of age, should avoid apitherapy applications.[5] Although the ready-made drugs used in apitherapy are approved by the U.S. Food and Drug Administration (FDA),[9] it is recommended to perform an allergy test before application and adjust the dose according to the patient's age, weight, application time, and condition.[10]

There is currently insufficient education in apitherapy within the field of Health Sciences. Nutrition and Dietetics students learn about this discipline with special efforts, mainly through the internet, media, and their environment. This study aimed to investigate the knowledge and opinions of first-year and fourth-year students who have just entered the Faculty of Health Sciences regarding apitherapy and to bridge the gap between clinical research, apitherapists, and nutritionists.

MATERIALS AND METHODS

Study population

This cross-sectional and descriptive study included 409 students: 168 first-year students and 241 fourth-year students, all studying in the Department of Nutrition and Dietetics at Firat University in Elazig, Turkey. All students who volunteered to participate in the study were included by signing the "Voluntary Consent Form" without the need for a sample selection method. The research was conducted between September 2022 and May 2024. The ethical compliance of the study was approved by the Firat University Non-Interventional Research Ethics Committee under decision number 2022/03-38. Volunteer participants who were 18 years of age or older, open to communication, and enrolled in either the first or fourth year were included in the study.

Data collection tools

The survey was developed based on a literature review and consists of three sections. The first section includes information on the students' descriptive characteristics (grade, gender, age, body weight, height, and place of residence). Body weight and height measurements were based on self-reported statements. The second section assessed the students' knowledge, experience, and expectations regarding apitherapy. This section evaluated whether certain information was correctly known and for which disease apitherapy products could be used. Additionally, students were asked to respond to statements regarding apitherapy products and their applications using the following Likert scale options: "strongly agree," "agree," "undecided," "disagree," and "strongly disagree." The third section addressed the status and frequency of use of apitherapy products. Previous articles were used to prepare the information and opinion questions.[11,12] No special training was provided on apitherapy before or after the survey. During the survey, students were instructed not to communicate with each other to avoid influencing one another's responses.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0. Descriptive data were presented as frequency and percentage distributions. Normally distributed data were expressed as mean ± standard deviation (X ± SD). The Mann-Whitney U test was used to compare the measurement values of two independent groups. Statistically significant results were considered at $p < 0.05$.

RESULTS

The participants in the study consisted of 89.5% female and 10.5% male. The average age was 21.5 ± 2.95 years. The study included 409 participants, 168 (41.1%) of whom were first-year students and 241 (58.9%) of whom were fourth-year students. Of the participants, 84.4% lived in urban areas, while 15.6% lived in rural areas. Additionally, 14.2% of the participants had someone in their family involved in beekeeping, and 4.2% expressed an interest in working in beekeeping in the future. The average body weight of the participants was 58.9 ± 10.53 kg, and the average height was 165.5 ± 6.89 cm.

The daily consumption of bee products by students is shown in Table 1. No significant differences were found between the classes in terms of the consumption of honey, bee pollen, propolis, bee bread, and royal jelly ($p > 0.05$). The most preferred bee product among both first-year and fourth-year students was honey (5.48 ± 7.06 g and 4.61 ± 9.31 g, respectively), followed by bee pollen (0.26 ± 1.88 g and 0.05 ± 0.29 g, respectively).

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Table 1. Daily usage amount of bee products (g)

Bee Product	First-Year students	Fourth-Year students	p-value
Honey	5.48 ± 7.06	4.61 ± 9.31	0.308
Bee Pollen	0.26 ± 1.88	0.05 ± 0.29	0.095
Propolis	0.22 ± 0.98	0.24 ± 1.08	0.895
Bee Bread	0.09 ± 0.60	0.06 ± 0.60	0.070
Royal Jelly	0.13 ± 0.78	0.03 ± 0.23	0.536

The students' knowledge and opinions about apitherapy are presented in Table 2. The primary source of information about apitherapy for first-year students was mostly internet sources (63.5%), while fourth-year students primarily used media (46.1%). When asked who should be the source of information about apitherapy, the majority of both first-year (64.0%) and fourth-year (67.2%) students responded that dietitians should be the source. Regarding which groups should not use bee products, both first-year and fourth-year students most commonly selected allergic diseases (75.3% and 74.0%, respectively), followed by diabetes patients (53.8% and 58.1%, respectively). When asked about the possible side effects of bee products, the majority of both first-year (98.8%) and fourth-year (95.1%) students identified allergies as a possible side effect.

Table 2. Students' knowledge and opinions about apitherapy

	First-Year Students (%)	Fourth-Year Students (%)	Total (%)
Source of Information about Apitherapy*			
Parents/grandparents	21.5	15.5	18.1
Friends/community members	14.4	11.8	12.9
Magazines	8.4	12.6	11.0
Internet sources	63.5	37.4	65.2
Other healthcare professionals	10.8	10.1	10.8
Media	41.2	46.1	44.3
Training	18.0	16.8	17.9
I do not know	1.8	2.5	2.2
Who Should Be Your Source of Information about Apitherapy*			
Doctor	53.1	45.2	50.1
Pharmacist	22.2	30.8	27.4
Dietitian	64.0	67.2	64.8
Apitherapists	55.5	71.4	68.5
Beekeepers	33.2	27.9	31.8
Traditional healers	10.2	6.5	8.3
Scientists	19.2	25.5	23.7
Which Groups Should Not Use Bee Products*			
Diabetes patients	53.8	58.1	57.0
Pregnant women	27.0	31.2	29.8
Children under 4 years old	38.8	56.9	51.8
Oncology patients	25.8	32.7	30.1
Teenagers	16.8	11.9	13.2
Allergic diseases	75.3	74.0	74.8
Individuals aged 65 and over	24.0	17.1	20.0
I do not know	0.6	1.2	1.0
Possible Side Effects Of Bee Products*			
Allergy	98.8	95.1	96.5
Bleeding	7.8	6.8	7.3
Headache	27.5	23.7	25.4
Unintentional weight loss	8.4	8.0	8.3
Body weight gain	22.1	24.3	23.5
Vomiting	38.8	41.2	40.3
Visual impairment	14.4	14.8	14.7
I do not know	0.0	0.8	0.5

** More than one option was selected.*

The level of knowledge of students about apitherapy is presented in Table 3. The questions "There is no genetic difference between queen bees and worker bees" and "Bees fed too much royal jelly turn into queen bees" were answered "true" more frequently by first-year students (33.3% and 72.6%) than by fourth-year students (26.6% and 58.1%). The questions "Due to hormonal properties, royal jelly is not recommended during adolescence" and "Honey is not recommended for babies under one year of age due to botulism" were answered "true" more frequently by first-year students (59.5% and 90.5%) than by fourth-year students (55.6% and 88.8%). The questions "There is no need to assess the risk of

allergy to bee products before using apitherapy", "There is an inverse relationship between the amount of fructose and the glycemic index", and "80% of honey is glucose" were answered incorrectly most often by fourth-year students (89.6%, 61.8%, and 39.4%, respectively). The question "The science of apitherapy dates back to 4000 BC" was answered incorrectly most often by first-year students (84.5%).

Table 3. Daily usage amount of bee products (g)

Statement	First-Year Students		Fourth-Year Students	
	True (%)	False (%)	True (%)	False (%)
There is no genetic difference between queen bees and worker bees. (T)	33.3	66.7	26.6	73.4
Bees fed too much royal jelly turn into queen bees. (T)	72.6	27.4	58.1	41.9
Apitherapy science dates back to 4000 BC. (F)	84.5	15.5	85.1	14.9
There is no need to evaluate the risk of allergy to bee products before using apitherapy. (F)	12.5	87.5	10.4	89.6
There is an inverse relationship between the amount of fructose and the glycemic index. (F)	61.3	38.7	38.2	61.8
80% of honey is glucose. (F)	64.9	35.1	60.6	39.4
Royal jelly is not recommended for adolescents due to its hormonal properties. (F)	59.5	40.5	55.6	44.4
Honey is not recommended for babies under one year of age due to botulism. (F)	90.5	9.5	88.8	11.2

**T: True; F: False*

The students' views on apitherapy are presented in Table 4. The majority of first-year students (42.9%, 47.0%, and 58.3%, respectively) and fourth-year students (43.2%, 45.6%, and 58.5%, respectively) responded "undecided" to the statements: "Apitherapy use is very popular in our country today," "Healthcare professionals have sufficient knowledge about apitherapy," and "Apitherapy has fewer side effects than other drugs".

Additionally, the majority of first-year students (40.5% and 40.5%, respectively) and fourth-year students (52.3% and 44.8%, respectively) responded "undecided" to the statements: "Apitherapy products should be encouraged" and "I use apitherapy products because they are good for me/my health.

Regarding the opinion that "Apitherapy is a part of traditional medicine," the majority of first-year students (44.6%) and fourth-year students (45.6%) agreed. However, regarding the statement "Apitherapy products should be available in every pharmacy," the majority of first-year students (33.3%) were undecided, while the majority of fourth-year students (37.8%) disagreed.

Table 4. Daily usage amount of bee products (g)

	I strongly agree (%)		**I agree (%)**		**Undecided (%)**		**I disagree (%)**		**I strongly disagree (%)**	
Grade	1st	4th	1st	4th	1st	4th	1st	4th	1st	4th
Apitherapy is a part of traditional medicine.	23.8	22.0	44.6	45.6	28.6	29.0	3.0	2.1	0.0	1.2
The use of apitherapy is very popular in our country today.	8.3	7.9	28.0	31.5	42.9	43.2	18.5	14.9	2.4	2.5
Healthcare workers have sufficient knowledge about apitherapy.	7.7	4.6	19.6	14.1	47.0	45.6	22.0	29.5	3.7	6.2
As a future healthcare professional, I have sufficient knowledge about apitherapy.	7.7	5.8	10.7	8.7	33.3	32.8	24.4	37.8	23.8	14.9
Apitherapy has fewer side effects than other drugs.	11.9	5.8	23.2	23.2	58.3	58.5	4.8	10.8	1.8	1.7
The use of apitherapy products should be encouraged.	19.0	10.4	39.3	29.9	40.5	52.3	1.2	6.6	0.0	0.8
Apitherapy products should be available in every pharmacy.	23.2	11.6	39.3	32.0	32.7	46.1	4.8	8.7	0.0	1.7
I use apitherapy products because they are good for me/my health.	13.1	7.1	28.6	26.1	40.5	44.8	8.9	12.9	8.9	9.1

**1st: First-year students, 4th: Fourth-year students*

DISCUSSION

This study aims to investigate the knowledge levels of 1st- and 4th-year students who have just started at the Faculty of Health Sciences regarding apitherapy, their views on apitherapy, the use of apitherapy products, and their inclusion in the education and training curriculum. Honey has been described as safe, natural, and traditional among complementary traditional approaches.[13,14] In a study conducted in

Turkey on bee products, it was found that 39.6% of participants consumed 0–500 grams of honey per month.[15] Furthermore, studies have shown that the most well-known bee product in Turkey is honey.[16,17] Additionally, a study found a significant relationship between honey consumption and education level.[18] In this study, the most consumed bee product among 1st- and 4th-year students is honey. Although honey is preferred as a source of health and energy due to its composition[19], caution should be exercised when using it for apitherapy purposes.

One study found that propolis and bee venom were the least known apitherapy products, while honey, beeswax, royal jelly, and pollen were the most recognized.[20] Another study also reported that propolis (8.9%) and bee venom (16.3%) were less known.[16] Sener and Karaca (2020) determined that the sources of information affecting the use of alternative and complementary medicine were friends/neighbors and family members.[21] Another study stated that consumers were most influenced by "Promotional Sales," "Friends/Relatives/Neighbors," and "Discount Days," and least influenced by "Newspaper/Magazine Advertisements" when choosing apitherapy products.[22] In this study, most students were aware of apitherapy products. The majority of 4th-year students reported that they received information from the media, while the majority of 1st-year students stated they obtained information from internet sources. The minimal difference in the responses of 1st- and 4th-year students to many of the questions suggests that apitherapy may not have been included in the curriculum.

Honey should not be given to babies due to the risk of botulism.[23,24] In this study, most of the 1st- and 4th-year students knew that this information was correct. Royal jelly and propolis should also be used with caution, considering their daily doses and allergenic effects.[25] In this study, the majority of participants believed that the risk of allergy should be evaluated before using apitherapy products. While foods with allergenic effects are known, nutrition and dietetics students need to be more comprehensively informed.

Therapeutically, honey is used in the treatment of bedsores, ulcers, and skin infections.[26] It also has protective properties against cancer and metastasis and activates the immune system.[27] Royal jelly has properties such as antioxidative,[28] antihypertensive,[29] antidiabetic,[30] and cell-renewing effects.[31] Propolis has antioxidant[32] and immune-boosting effects.[33] Pollen[34] and bee bread[35] also have antioxidant properties. When the knowledge (Table 3) and opinions (Table 4) of the students about apitherapy were examined in this study, it was found that most of the information about apitherapy was not well known, and most of the opinions were undecided.

Informing the public about the health benefits of apitherapy products is important for both public health and the bee economy.[20] In the United States, alternative and complementary medicine education is provided for reasons such as being safer than other chemicals, its increasing use, growing clinical research, and government support.[36] In Germany, it has been included in medical education since 2003.[37] In Turkey, the title of apitherapist can only be obtained by doctors, and the number of apitherapists is very low.[11] Dietitians need to be given more space in the educational curriculum. Additionally, dietitians, apitherapists, and beekeepers need to find common ground and raise public awareness on this issue.

Limitations

Apitherapy is not taught in many universities in the Nutrition and Dietetics department. An attempt was made to reach all students without calculating the sample size, and the study could have been applied to a larger sample. The fact that this survey was conducted at only one university is a limitation. However, the study is significant because it shows that this topic is not included in the curriculum and that students obtain information from unreliable sources.

CONCLUSION

Dietitians need to understand evidence-based apitherapy products, be involved in interdisciplinary collaboration, and make public recommendations. This study has shown that future dietitians have insufficient experience and knowledge of apitherapy. The widespread use of bee products among the public and the increase in scientific studies on this topic highlight the need for nutritionists to receive training. It is important to include this subject in the nutrition and dietetics curriculum, to accelerate studies on the topic in our country, to discuss it in national and international congresses, and to raise public awareness to prevent the indiscriminate use of apitherapy products.

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ORIGINAL RESEARCH

Investigation of Antiproliferative and Apoptotic Effects of Ethanol Extract of *Viscum album L.* on A549 Cell Line

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Abstract
Objective: This study aimed to investigate the effect of *Viscum album L.* extract on apoptosis and cell proliferation in the A549 and HEK-293 cell lines.
Material-Method: The cell proliferation test was conducted using the MTT method, and apoptosis analysis was performed using the Annexin V method with a Muse flow cytometer. *Viscum album* L. ethanol extract was applied to A549 and HEK-293 cells at concentrations of 1600, 1400, 1200, and 1000 µg/mL, and proliferation analysis was carried out using the MTT method.
Results: The IC_{50} value for A549 cells was 1090 µg/ml at the 72nd hour and 413.6 µg/ml for the HEK-293 cells at the 48th hour. Apoptosis analyses of A549 cells were performed with the AnnexinV method in a Muse/Flow cytometry device. A549 cells, treated with *Viscum album L.* extract at the IC_{50} concentration obtained, underwent late apoptosis at approximately 80%.
Conclusion: *Viscum album L.* methanol extract had antiproliferative and apoptotic effects on A549 cells. In addition, it had an antiproliferative effect on HEK-293 cells, a kidney epithelial cell line. Other studies have reported that *Viscum album L.* has an anticancer effect in different cell lines and can be used as a therapeutic agent. In most studies, its impact on cancer cells has only been investigated, and its effect on healthy cells has not been investigated. Our study shows that *Viscum album L.* extract also showed an antiproliferative effect on healthy cells. The mechanism of action of *Viscum album L.* should be elucidated with further molecular studies.
Keywords: *Viscum album L.*, A549, HEK-293, Proliferation, Apoptosis

INTRODUCTION

Lung cancer is the most common cancer worldwide and the leading cause of cancer death. Lung cancer has a survival rate of 5 years, but this rate is increasing due to not smoking and reducing smoking, early diagnosis, and targeted treatments[1]. Chemotherapy is the standard first-line treatment for lung cancer. Resistance to chemotherapeutic agents reduces the therapeutic effectiveness of these drugs. For this reason, targeted and combined treatments have been used as an alternative to chemotherapy. In traditional medicine, various plants have been used to treat many diseases[2]. Integrative treatments, which also use phytotherapeutic agents, aim to reduce physical and emotional symptoms and improve the quality of life in cancer patients[3].

Viscum album L.(VA) is a semi-parasitic plant that grows on various host tree species[4]. VA has been used for medicinal purposes since ancient Greek and Roman times[5]. In 1917, patients were given mistletoe preparations for cancer treatment[6]. The chemical content of VA varies depending on the species of the host tree, the harvest period, and the treatment of the plant before use[7]. Studies have shown that VA extracts have many biological activities, such as stimulating various cytokines, increasing the activity of natural killer (NK) cells, immunoadjuvant, anticancer, antimycotic, antibacterial, antiviral, and immunomodulatory activities[8,9]. Nowadays, VA preparations are used in addition to existing treatments to reduce the side effects of cancer treatment or to help patients recover[10]. Many chemical components of the VA

plant have been isolated in many studies. Among these chemical components, viscotoxins, lectins, alkaloids, amines, flavonoids, organic acids, terpenoids, phenols, lecithins, triterpenes, polypeptides, saponins, caffeic acid, acetylcholine derivatives, vitamin C, histamine, resins, thionins and cardenolides have been found[5,11] VA lectins play a growth inhibitory role on tumor cells[12-15]. Cell culture studies conducted with VA have shown that it has an anticancer effect. However, most studies have not investigated its impact on healthy and cancer cells. While the therapeutic effects of plants containing many active substances are being investigated, it is important also to investigate their impact on healthy cells. This study investigated the effect of VA extract on the apoptosis and proliferation of A549 cells, a lung cancer cell line. In addition to A549 cells, HEK-293 cells, a renal epithelial cell line, were used as a healthy control.

MATERIALS AND METHODS

Plant extraction

It was collected from *Salix alba L.* (willow tree), spreading on the 50th kilometer of the Konya-Seydişehir highway outside the city center in April-June. After the VA plant was dried in a closed and ventilated environment without exposure to direct sunlight for approximately one month, the trunk, branch, and leaf parts were ground into powder in a stainless steel chamber plant grinder (9000 rpm).

30 g of VA collected from the *Salix alba L.* plant and 300 mL of ethanol (solvent) were extracted in a volumetric flask at 50-55 oC in a Soxhlet extraction device for 8 hours. After extraction, the solvents in the extracts were removed with a Rotary Evaporator device at 45 ^{o}C. The extracts remaining in the flasks were sonicated with a minimal amount of solvent (4-5 mL ethanol) for 5 minutes at 37 °C using an ultrasonicator and transferred to vials. The vials were kept in a closed water bath at 55 °C for 1 week to allow the solvents to evaporate and the extracts to dry.

Preparation of VA concentrations

4 g of VA extract was dissolved in 25 mL of ethyl alcohol suitable for cell culture. Doses were prepared at concentrations of 1600 µg/mL, 1400 µg/mL, 1200 µg/mL, and 1000 µg/mL, not exceeding 1% ethyl alcohol.

Cell culture

The medium of A549 cells incubated at 37 ^{o}C was removed and washed with PBS. Trypsin was added to the petri dish containing the cells and incubated at 37 ^{o}C for 5 minutes. Cells separated from the surface with trypsin were transferred to the vial and centrifuged. A homogeneous cell suspension was obtained by discarding the supernatant and adding medium to the pellet. Cell suspension and Trypan Blue mixture was obtained and transferred to the Thoma slide. This dye exclusion assay determines the number of viable and/or dead cells in a cell suspension. Trypan blue is a large, negatively charged molecule. Trypan blue dye exclusion assay is based on the principle that live cells possess intact cell membranes that exclude this dye, whereas dead cells do not. Then, cell counts were performed under the microscope. Cells were planted in 96-well plates at $5x10^3$ in each well and incubated for 24 hours in a CO_2 incubator at 37 ^{o}C. At the end of 24 hours, the cells' medium was removed. 100 µL of the prepared different VA doses were added to each well. A new medium was added to the control wells. A new medium was added to the blank wells containing no cells. Six replicates were studied for each dose of VA at different concentrations. Plates were incubated separately for 48, 72, and 96 hours. 50% of the dose-medium mixture in each well was removed and discarded into each well where VA doses were applied every 24 hours. Fresh extract-medium mixture was added. All procedures performed for A549 cells were also applied to HEK-293 cells, which is the kidney epithelial cell line we used as control.

Proliferation analysis with MTT method

To prepare 1 mL MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) solution, 5 mg MTT powder was dissolved in 1 mL PBS. Cells incubated for 48, 72, and 96 hours were subjected to MTT testing on three days. 20 µL of the prepared MTT solution was added to each well after the medium was Removed and incubated at 37 °C for 4 hours. 150 µL DMSO was added to dissolve the formazan crystals formed after incubation. It was wrapped in aluminum foil and kept in the mixer for 20 minutes to ensure homogeneity. Absorbance was measured at 540 nm by spectrophotometric method.

Apoptosis analysis with annexin V method

A549 cells taken from the incubation were washed with PBS, removed with Trypsin, and then centrifuged, and the supernatant was discarded. The pellet part was homogenized with the medium, stained with Trypan blue, and counted under a microscope using a Thoma slide. Planting was done in a 6-well petri dish with $5x10^4$ cells in each well. It was studied in 3 repetitions. It was incubated for 24 hours in a

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37°C incubator with CO_2. VA extract was applied to the cells at the IC_{50} concentration determined by the MTT method. The same amount of medium was added to control cells. As in the MTT test, 50% of the dose-medium mixture in the cells was removed every 24 hours. The same amount of fresh extract-medium mixture was added. Apoptosis analysis of cells at 72 hours. Annexin V & Dead Cell Reagent were added, and Annexin V was measured in the MUSE Cell Analyzer. The results were evaluated.

RESULTS

IC_{50} values of VA extract applied A549 and HEK-293 cells determined by MTT method

VA extract was applied to A549 cells at doses of 1000 µg/mL, 1200 µg/mL, 1400 µg/mL, and 1600 µg/mL. Figure 1 shows the dose- and time-dependent variation of proliferation analysis results with the MTT method. In the same dose range, the same proliferation analysis was also applied to HEK-293 cells, a healthy kidney epithelial cell line. The results are shown in Figure 2.

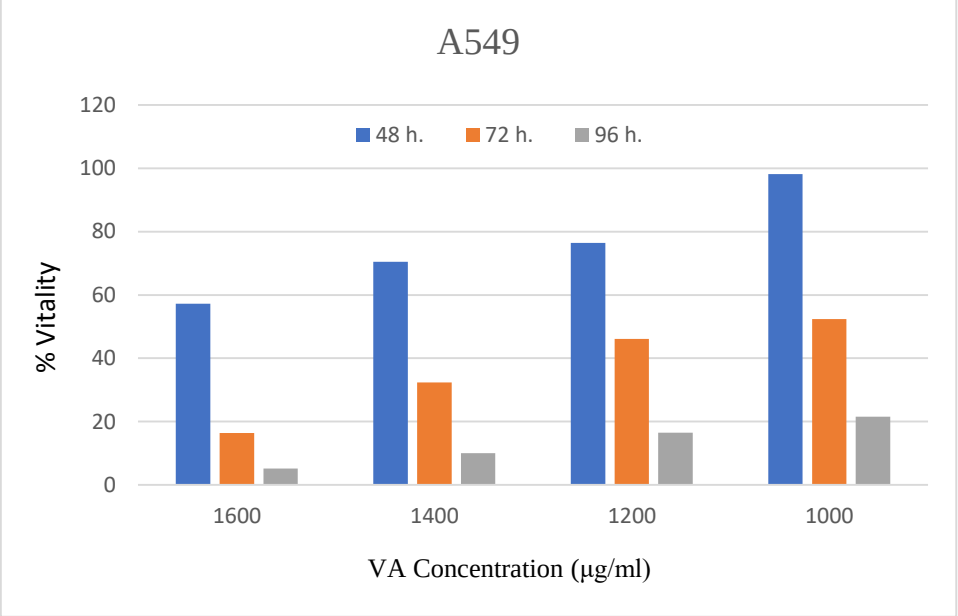

Figure 1. % viability of A549 cells applied with different concentrations of VA extract at 48, 72, and 96 hours

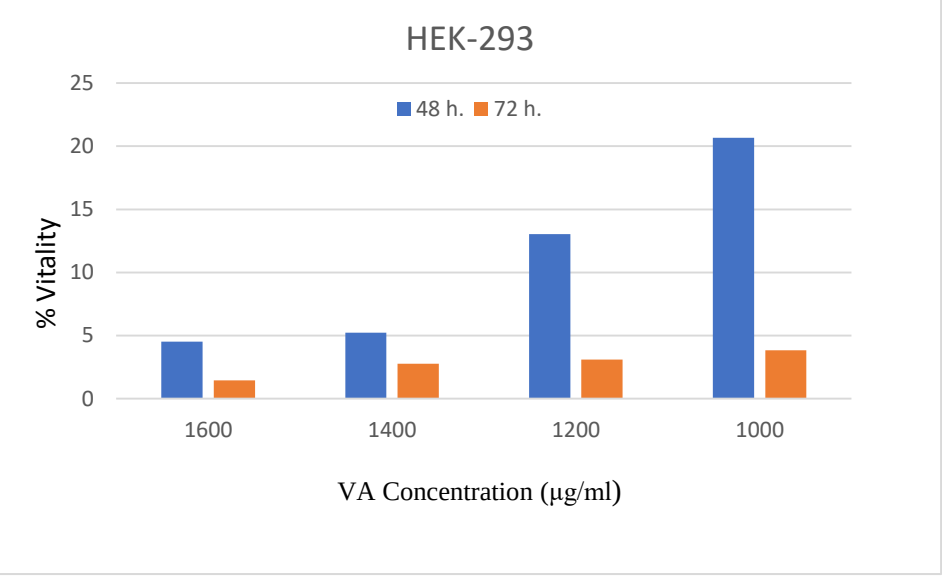

Figure 2. % viability of HEK-293 cells applied with different concentrations of VA extract at 48 and 72 hours

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The closest value to the IC_{50} value in A549 cells was obtained at a concentration of 1000 µg/mL at the 72nd hour, and the IC_{50} value was calculated using the equation in Figure 3.

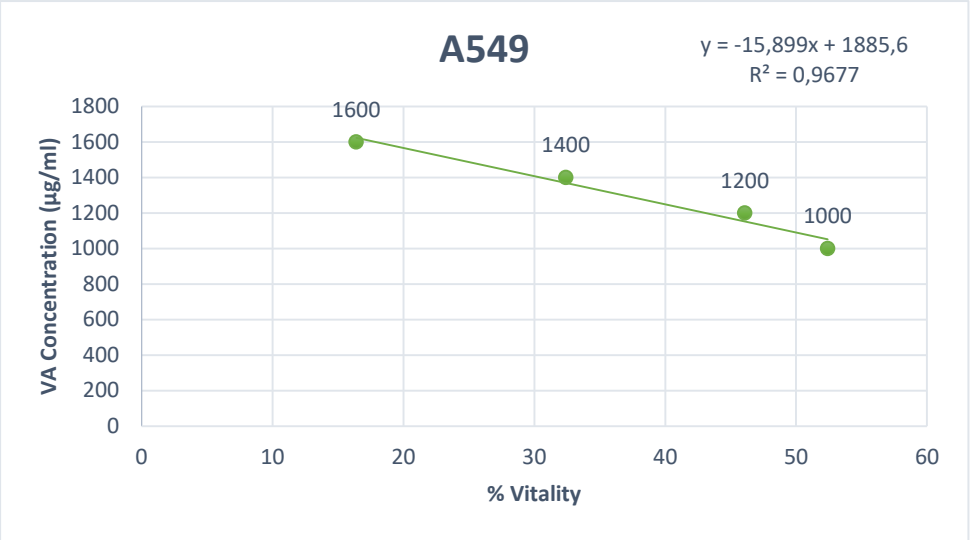

Figure 3. % viability, trend line, and line equation of A549 cells applied with different concentrations of VA extract at the 72nd hour (IC50: 1090 µg/mL).

Apoptosis analysis results with annexin V method on muse/flow cytometry device

A549 cells were seeded in 6-well plates at $5x10^4$ per well. Apoptosis analysis was performed using the Annexin V method on the Muse/Flow cytometry device. When the cell undergoes apoptosis, the phosphatidyl serine on the inner surface of the cell is translocated to the membrane's outer surface. Results were obtained according to the binding of Annexin V to phosphatidylserine translocated to the outer surface and the binding of 7AAD to the DNA in the cell nucleus. According to the results obtained from the Muse/Flow cytometry device (Figure 4, Figure 5, and Table 1), it was observed that A549 cells underwent late apoptosis at a rate of 80.60%.

Table 1. % viability and apoptosis table of VA extract-treated A549 cells and control cells at the 72nd hour

	% Viability	Early Apoptosis	Late Apoptosis	Debris	Total Apoptosis
Control	%93.9	%2.35	%2.25	%1.5	%4.6
A549	%2,25	%0.9	%80.6	%16.25	%81.5

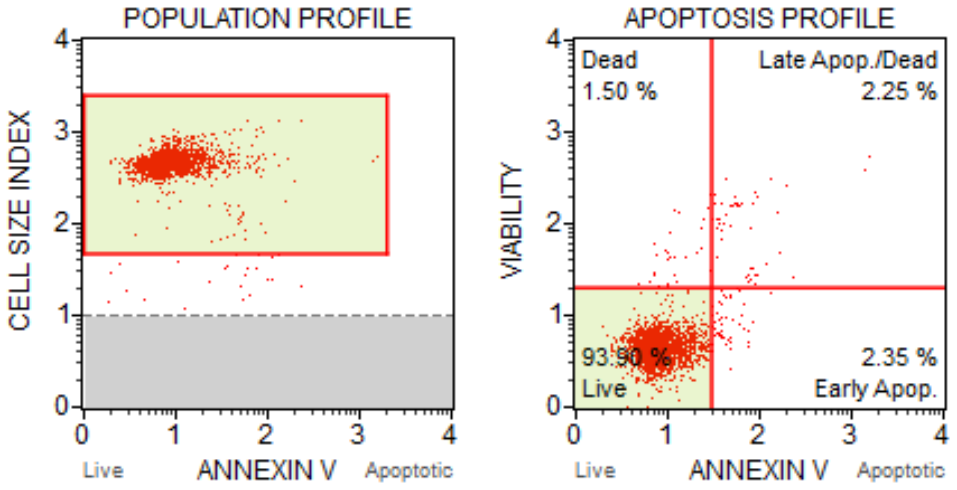

Figure 4. Apoptosis graphs of A549 control cells

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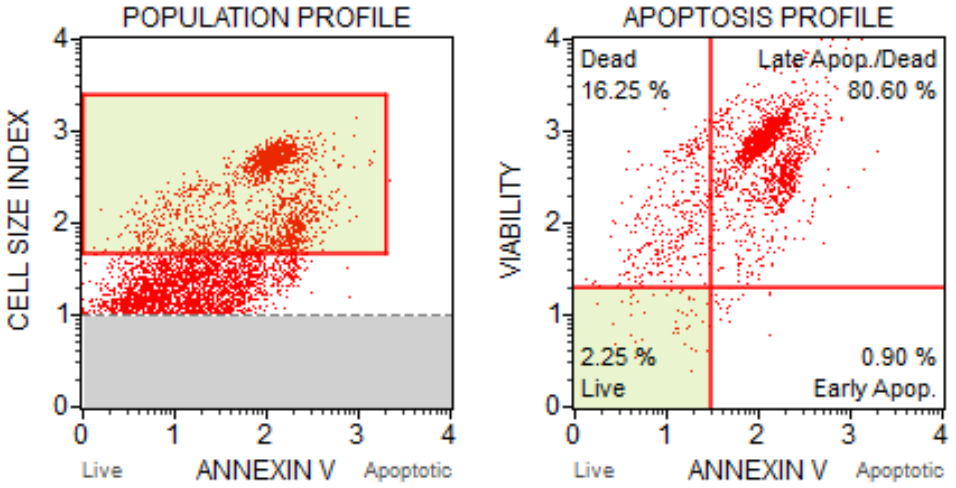

Figure 5. Apoptosis graphs of A549 cells were applied with an IC_{50} dose of *Viscum album* L. extract at the 72nd hour

DISCUSSION

Lung cancer is one of the most important and prevalent diseases worldwide. The prognosis for patients with advanced lung cancer is poor, with an average survival of one year. Lung cancer can be treated with surgery or chemotherapeutic drugs, depending on the type and stage of the disease. Due to the side effects of modern treatment methods such as chemotherapy, radiotherapy, and immunotherapy, studies have been conducted on the anticancer properties of medicinal plants traditionally used to treat many diseases, and it has been shown that they can be used in cancer treatment. VA has been used traditionally to treat many diseases for many years. Studies have proved the anticancer properties of the lectins and viscotoxins they contain.

In the phytochemical analysis of VA obtained from ethanol extract, flavonoid, and viscotoxin contents were the highest. Phytochemical compounds and viscotoxin contents are probably related to the antitumor effects of VA [16]. Cell death mechanisms include necrotic effects, depending on the impact of the host tree, time, and dose. Lectins in the *Viscum album*, especially viscotoxin and mistletoe lectins (ML-I, ML-II, ML-III), exhibit strong anticancer effects. These lectins can bind to the cell membrane, affect intracellular signaling pathways, and induce apoptosis. Lectins stop the growth of cancer cells by triggering apoptosis mechanisms leading to cell death.

Previous studies investigated the antiproliferative effect of VA extract in various cell lines (Table 2). Huyen et al. obtained an aqueous extract of VA cultivated on oak, apple, and pine trees, examined its cytotoxic effects on lymphoblastoid and monocytic cell lines, and reported its anticancer properties in these cell lines. Urech et al. demonstrated that viscin, betulinic acid, oleanolic acid, and ursolic acid inhibited growth and induced apoptotic cell death in Molt4, K562, and U937 leukemia cells. In the study by Pieme et al., the methanol extract of VA exhibited an antiproliferative effect in MB-MDA435 cells. Vlad et al. also indicated that ethanol and aqueous extracts of VA showed antiproliferative effects in HepG2 and MCF7 cells.Sarpataki et al. investigated the antiproliferative effect of ethanol extract of VA on normal human fibroblasts (Hfl) and a tumor cell line (Hela). Their results showed that ethanol extract of VA had minimal effect on the proliferation of normal fibroblasts (Hfl) while significantly reducing the proliferation of Hela cells. In the study by Urech et al. in 2006, the antiproliferative and apoptosis effects of VA extract were measured in four human bladder carcinoma cell lines (T24, TCCSUP, J82, and UM-UC3). According to the results, VA showed anticancer effects in all four different cell lines.

Different cell lines are genetically distinct, and these differences affect the response of the cells to the *Viscum album*. For example, some cell lines may have more active pathways that activate apoptosis mechanisms, while others may suppress these pathways. The rate of cell proliferation can also affect the IC_{50} value. Fast-proliferating cell lines are generally more sensitive to anticancer agents.

Table 2. Previous cell culture studies with VA extract

Researcher	Cell Line	IC_{50} Value
Huyen et al. (2003)[17]	Lenfoblastoid ve monositik hücre hattı	6,25-12,5 µg/mL
	K562	252 µg/mL
	Molt4	118 µg/mL
Urech et al. (2005)[18]	U937	138 µg/mL
Pieme et al. (2012)[19]	MB-MDA435	172 µg/mL
Vlad et al. (2016)[20]	MCF-7, HepG2	75 mg/mL
Sarpataki et al. (2015)[21]	Hela	5 µg/ml
	UM-UC3	380 µg/mL
	T24	1640 µg/mL
Urech et al. (2006)[22]	J82	1880 µg/mL
	TCCSUP	1920 µg/mL
Our study	A549	1090 µg/ml
	HEK-293	413,6 µg/ml

In our study, the IC50 value for A549 is compatible with the literature. The antiproliferative and apoptotic effect of VA extract on A549 cells was demonstrated in this study. However, the point that should be noted is this: VA extract did not only show an antiproliferative effect on A549 cells, which is a lung cancer cell line but also showed an antiproliferative effect on HEK-293 cells, a healthy epithelial cell line. In fact, as a result of applying the same VA concentration, HEK-293 cells showed more antiproliferative effects than A549 cells. In this study, which we conducted with the hypothesis that VA could be used alone or as a supportive treatment with existing drugs in lung cancer, the fact that healthy epithelial cell lines were also affected showed that VA could not be used for therapeutic purposes in lung cancer. It is seen that in the majority of other studies, healthy cell lines were not used and were not compared with cancer cells. The content of herbal extracts varies depending on the region where they grow, the collection period, and the extraction method. In addition to its effect on cancer cells, its effect on healthy cells should also be investigated. Having an antiproliferative or apoptotic effect on cancer cells without knowing its effect on healthy cells may not indicate that it can be used as a treatment tool. In this regard, while investigating herbal extracts' antiproliferative and apoptotic effects, their effects on healthy cells should also be evaluated.

CONCLUSION

Our study demonstrated that *Viscum album* L. extract exhibits anti-proliferative and pro-apoptotic effects on the A549 lung cancer cell line while inducing significant cytotoxicity and apoptosis in HEK-293 healthy cells. These findings suggest that *Viscum album* L. has potential as a therapeutic or adjuvant agent in cancer treatment. However, considering its adverse effects on healthy cells, further studies are required to enhance the selectivity of the extract. Future research should focus on testing different concentrations and formulations, investigating cell-type-specific effects, and conducting comprehensive toxicity analyses supported by in vivo models. Such investigations would provide a solid foundation for the safe and effective therapeutic use of *Viscum album* L.

Research Limitations: A limitation of this in vitro study is that there was no comparison with VA using commonly used chemotherapeutic agents for lung cancer, such as cisplatin or etoposide, in the same cell line. The synergetic effect of VA with chemotherapeutic agents can be investigated.

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Ethical Approval: Approved by the Local Ethics Committee of the Selcuk University Faculty of Medicine with decision number 2019/12

Disclosure statement: The authors have no conflicts of interest to declare.

Author contributions: Fadime Ovali; conceptualization, data curation, formal analysis, ınvestigation, methodology, project administration, resources, validation, software, visualization, writing-original draft, writing-review&editing. Husamettin Vatansev; supervision, conceptualization, project administration, resources,

validation, writing-review&editing. Hakan Vatansev; data curation, formal analysis, ınvestigation, methodology, writing-review&editing.

Conflict of interest: There is no potential conflict of interest relevant to this article.

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CASE REPORT

Pain Mesotherapy Adventure of Three Sisters Who Applied to a Traditional And Complementary Medicine Center

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Abstract
Today, musculoskeletal pain is frequently seen in the working population. Analgesic drugs are frequently used in the treatment of this chronic pain. However, analgesic drug treatment causes various side effects. Mesotherapy is an application method that aims to heal by injecting a small amount of medication under the skin. In this method, less medication is used compared to systemic treatment and a similar effect is achieved. Although mesotherapy has a wide range of uses, it does not yet have a standard protocol. For these reasons, every study conducted in the field of mesotherapy is quite valuable. It was aimed to contribute to the literature by evaluating the mesotherapy results of three sisters from the same family who applied to our center with various pain complaints. The pain of the sisters, aged 44, 47 and 50, was evaluated with the Visual Analog Scale (VAS) before and after mesotherapy. The pain levels, which were 10/10, 8/10 and 10/10, decreased to 7/10, 4/10 and 3/10 after three sessions of mesotherapy, respectively. In addition, all of them reported an increase in their quality of life. The decrease in pain in these patients with similar genetic and sociodemographic characteristics, consistent with previous studies, can be interpreted as significant.
Keywords: Pain, Mesotherapy, Siblings, Musculoskeletal

INTRODUCTION

In the twenty-first century, chronic musculoskeletal pain has become quite common, especially in the working population [1]. Studies such as the United States Bone and Joint Initiative in the USA and the Fit for Work Europe in Europe have shown that musculoskeletal disorders cause significant functional limitations in the adult population [2,3]. Musculoskeletal pain reduces the patient's quality of life by causing increased physical disability, decreased social functioning and mental health deterioration. Thus, it consumes a large amount of health and social care economic resources [4]. In addition, nonsteroidal anti-inflammatory (NSAID) and myorelaxant drugs, which are frequently used systemically in the treatment of these pains, have a very high side effect and drug interaction profile [5]. As an option, intra-articular injections and interventional spinal procedures are used. However, this should only be done under sterile conditions and by highly trained personnel due to the risk of infection and bleeding.

According to the Traditional and Complementary Medicine Regulation published in the Official Gazette on October 27, 2014 in Turkey, mesotherapy is the application of local, small doses, intradermal injection of herbal and pharmacological drugs with special needles and special techniques, aiming to improve organ pathologies of mesoderm origin. It can be applied by certified physicians and dentists as a complementary treatment for muscle, joint and skin pathologies. This method, which can also be defined as local intradermal treatment (LIT), is a technique used to slowly spread drugs to the tissues below the injection site in order to prolong the pharmacological effect compared to intramuscular injection or other systemic applications [6]. It is offered alone, alone or in combination with other pharmacological/non-pharmacological treatments. One of its greatest advantages and purposes of use is to obtain optimum benefit with lower drug doses and therefore fewer side effects and costs compared to other treatments. The "mesoderm layer" that gives the method its name is one of the three germ layers

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in which tissues develop during the embryonic period. It is assumed that the skin, muscle, bone, cartilage, ligament, fat and cardiovascular system tissues originating from the mesoderm layer are in communication with each other and that a substance applied to the skin, which is one of these tissues, will also affect the tissues in its projection [7]. Although this hypothesis has not yet been scientifically proven, its beneficial effects on patients have been shown by many studies. In addition, although mesotherapy has a wide range of uses such as pain relief, sports medicine, chronic venous disease, immune prophylaxis and cosmetics, it does not yet have a standard protocol. Application frequency, total number of sessions, drugs and doses used, application depth, etc. vary according to the practitioner and the patient [7,8]. For these reasons, increasing scientific studies on mesotherapy applications is of great importance both in terms of understanding the mechanism and in creating a standard protocol.

CASE PRESENTATION

Our case series includes three sibling patients who applied to Samsun Training and Research Hospital Traditional and Complementary Medicine (GETAT) Application Center. Patients applied to our center in February 2024.

The first case, a 44-year-old woman, presented with a pain in the lumbar region that had lasted for about 7 months, which eased somewhat with rest, but continued uninterruptedly throughout the day. On inspection, there was difficulty in walking. The patient could lie on the stretcher with the help of two people. There was serious tenderness in the lumbar region. The patient consulted Brain and Nerve Surgery before applying to our center, and no surgery was considered, and NSAID treatment and exercise were recommended. The previous lumbar Magnetic Resonance (MR) image is as shown in the figure (Figure 1). It was interpreted as *"L5-S1, L4-5 disc distances have narrowed. Signal changes compatible with type I degeneration were observed in the end-plates facing the L5-S1 disc, and type II degeneration was observed in the end-plates facing the L4-5 disc. Loss of intensity was observed in T2-weighted series due to degeneration in L5-S1, L4-5, L3-4 discs."* The patient defined his pain as 10/10 according to Visual Analogue Scale (VAS).

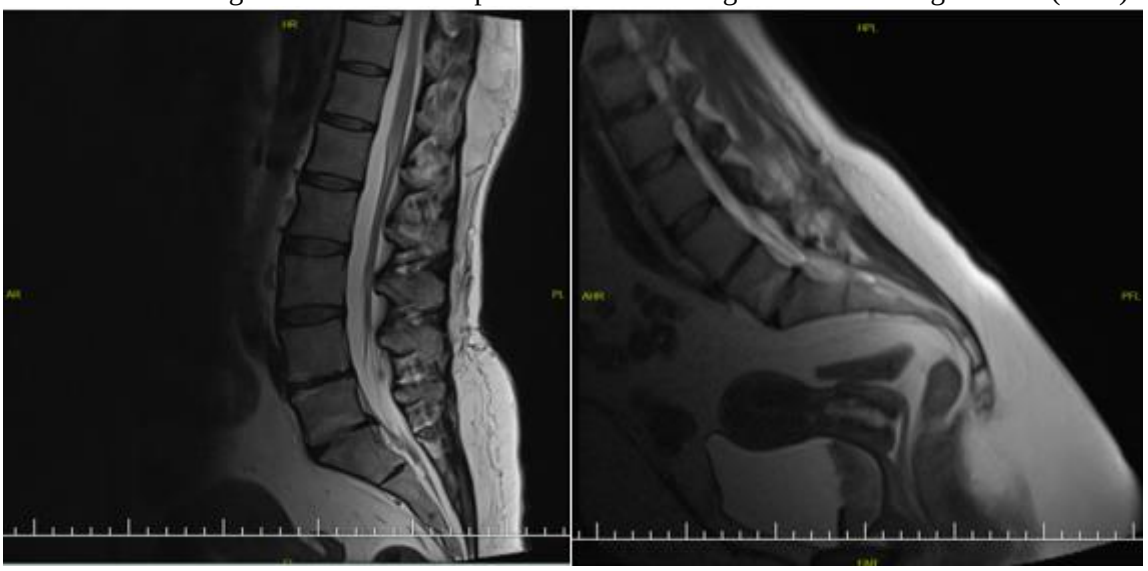

Figure 1. First case's lumbar MRI image

The second case, a 47-year-old woman, had pain in the interscapular and cervical areas that had been present for 1 year, varying depending on the position, and was described as having an intensity of 8/10 according to VAS. On physical examination, there was stiffness in the cervical area that could be felt by palpation. The third case was a 50-year-old woman. She had right knee pain that recurred from time to time and increased in the last month and a half, reaching a level of 10/10. The previous knee MRI image is as shown in the figure (Figure 2). It was interpreted as *"Grade II degeneration was observed in the anterior and posterior horns of the medial meniscus. A grade IV laceration was observed in the posterior horn of the lateral meniscus body. Linear signal increases in favor of interstitial rupture were noted between the anterior cruciate ligament fibers."* The second and third patients do not receive any treatment other than NSAIDs. No pathology was found in the vital signs and laboratory results of all three patients.

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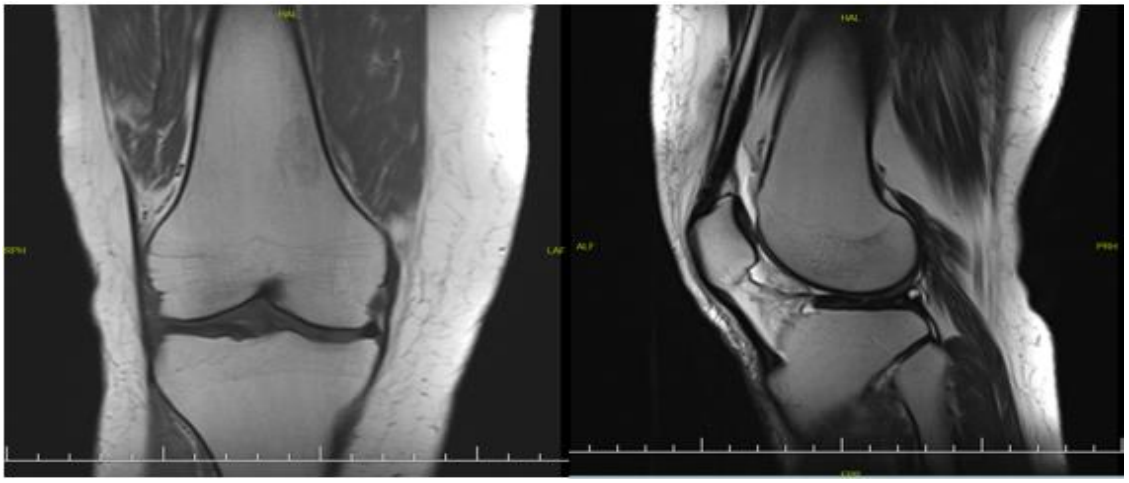

Figure 2. Third case's knee MRI image

Mesotherapy treatment was planned for all three patients once a week. The drugs used in the treatment protocol are a combination of lidocaine without adrenaline, thiocolchicoside and physiological saline. Although the total applied amounts vary depending on the size of the applied area, the application is made at approximately one centimeter intervals and at a depth of 2-4 millimeters into the skin, with 0.02-0.05 milliliters of medication applied in each injection. Point by point (Pbp), papule and napage techniques were used (Table 1).

Table 1. Features of cases

Cases	**1. Case**	**2. Case**	**3. Case**
Gender	F	F	F
Age	44	47	50
BMI	28,7	22,5	27,3
Number of applications	3 (1/w)	3 (1/w)	3 (1/w)
Administered drugs	L,T,PS	L,T,PS	L,T,PS
Amount applied	2 mL	1,5 mL	0,75 mL
Application method	Pbp, papule, napage	Pbp, papule, napage	Pbp, papule, napage

(BMI: body mass index, L: lidocain, T: thiocolchicoside, PS: physiological saline, Pbp: point by point)

According to the VAS results of the second week, the first case defined pain as 7/10, the second case as 5/10, and the third case as 7/10. They also stated that the frequency of pain decreased and their quality of life increased.
In the third week, they described pain as 7/10, 4/10, and 3/10, respectively (Figure 3).
Accordingly, there was a significant decrease in the pain level of the cases. This has led to an increase in their participation in business life and physical activity.

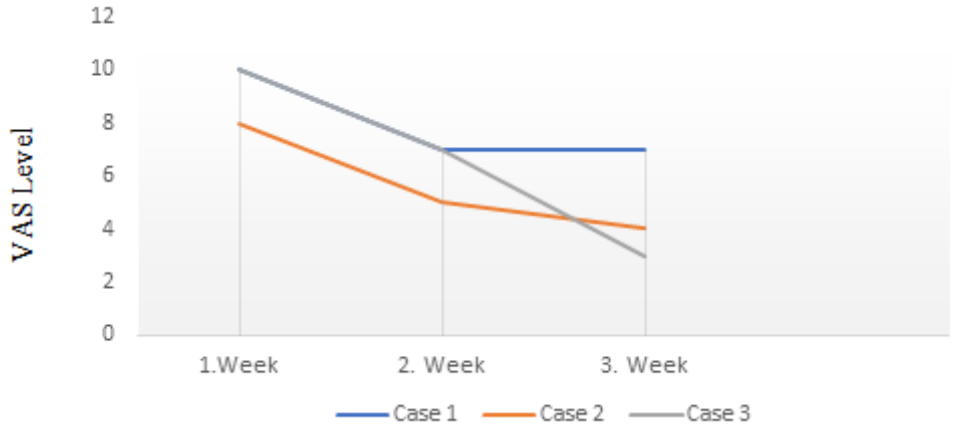

Figure 3. Pain level-Time graph

DISCUSSION

Compared to other GETAT applications, scientific studies on mesotherapy, which is a relatively new method, are insufficient. A meta-analysis published in 2021 examined mesotherapy studies conducted between 1999 and 2020 in musculoskeletal pathologies. It was shown that mesotherapy had the same or sometimes even higher effects with fewer side effects when compared to systemic treatment [3]. The VAS system was used for pain scoring in all known studies. The most important side effects obtained were short-term minimal bleeding, ecchymosis, and rarely allergic reactions.

Case-control studies on mesotherapy were mostly conducted in multiple sessions spread over several weeks. In a study conducted in our country in 2019, single-dose mesotherapy and parenteral treatment methods were compared in patients with extremity injuries who applied to the emergency room. According to this study, a significant decrease in VAS was observed at the 10th, 30th, 60th, and 120th minutes in patients who received mesotherapy compared to the systemic treatment group [9]. Another study comparing 5 sessions of mesotherapy with systemic administration of NSAIDs and corticosteroids in patients with acute low back pain used VAS and Roland-Morris disability questionnaire (RMDQ). No significant difference was found in 6-month results between mesotherapy and systemic treatment on either scale [10].

Another study compared 5 sessions of mesotherapy, systemic treatment and bee venom application in patients with low back pain of more than 3 months duration and recorded the results immediately after treatment and 6 months later. Here again, VAS and RMDQ values after treatment were similar in all three groups. In the measurements after 6 months, a significant decrease was observed in the mesotherapy group compared to the other two groups [11]. In a study on patients with knee osteoarthritis and pes anserine bursitis, oral and mesotherapeutic diclofenac were compared using VAS, Knee Injury and Osteoarthritis Outcome Score (KOOS) and ultrasound. Accordingly, significant decreases were detected in VAS and KOOS in both groups, and interestingly, a decrease in inflammation was observed in ultrasonography in the mesotherapy group [12]. In another study, mesotherapy and systemic treatment were compared in patients with neck pain according to Neck Disability Index (BDI) and VAS. According to the results of the 3rd hour, 1st day and 3rd day, positive results were obtained in the mesotherapy group compared to the systemic treatment group [13].

To our knowledge, there is no published study on the results of mesotherapy treatment in sibling patients. Although our case series had fewer sessions compared to other studies, an approximately 50% decrease in pain was observed according to VAS scoring and no side effects were observed. In these respects, it was seen that it was parallel to the results of other studies in the literature. It is important that our patients are similar in many aspects such as genetic and sociodemographic characteristics, physical activity and nutritional habits due to being siblings, and it differs from other studies in this respect. The fact that the quality of life of the patients could not be analyzed with a standard tool and therefore verbal comments were taken into consideration subjectively is a limitation of our study. Using standardized scales to measure quality of life may provide more objective results. The low number of cases is also an important limitation.

CONCLUSION

The responses of three siblings of the same gender, with similar genetic characteristics, who received the same frequency and the same number of mesotherapy sessions showed that pain decreased and quality of life increased according to VAS. One of the unique aspects of our study was that our patients were siblings. This situation emphasized the potential importance of genetic factors. It is a fact that our study needs to be improved due to reasons such as the small number of cases, the fact that the applied doses, application depth and application points were not fully standardized. The standard dose and number of sessions help to keep the amount of drug used constant. This will allow the measurement of the effect to be obtained with the fixed amount of drug and the comparison of the amount of side effects with the dose. The most common side effects obtained in mesotherapy are minimal bleeding and hematomas. Therefore, it is also important that the application depth and points are standard. It is possible that the applied drug doses and the number of sessions will be more standardized with future studies. It is thought that much lower doses of drugs are used to achieve the same effect as current treatments in mesotherapy. When the amount of drug used is standardized according to the dose and the number of sessions, drug savings can be measured by comparing it with systemic treatment. This will also contribute to

pharmacoeconomics.

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Author contributions: Conceptualization: [DEC]; Design: [DEC, OO]; Writing: [DEC, OO]; Investigation/Data collection: [DEC, OO]

Conflict of interest: There is no potential conflict of interest relevant to this article.

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REVIEW

Investigation of Current Approaches in Rehabilitation of Chronic Respiratory Diseases: Salt Therapy and Exercise

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Objective: This study aims to examine studies that evaluated salt therapy (speleotherapy and halotherapy) and exercise together in chronic respiratory diseases.
Material-Methods: The research is a descriptive screening study aiming to examine studies that address salt therapy (speleotherapy and halotherapy) and exercise together between 2020 and 2024. For this purpose, studies in Pubmed, Cochrane Library, Google Scholar, Reseachgate, and Scopus academic databases were examined. The keywords "Halotherapy", "Speleotherapy", "Salt therapy", and "Exercise" were used.
Results: The findings of the obtained studies showed that the combination of salt therapy (speleotherapy and halotherapy) and exercise increased the functionality, muscle strength (respiratory, upper extremity, and lower extremity), flexibility, balance, chest mobility, and endurance of individuals with chronic respiratory diseases. However, it is noteworthy that the studies addressing salt therapy and exercise together are quite limited.
Conclusion: Studies that examine the combination of salt therapy (speleotherapy and halotherapy) and exercise show that the combination of both types of treatment can further strengthen the therapeutic effect obtained. In this direction, more studies with high evidence are needed in which salt therapy, called speleotherapy and halotherapy, is considered together with exercise.
Keywords: Exercise, Halotherapy, Pulmonary Rehabilitation, Salt Therapy, Speleotherapy

INTRODUCTION

Chronic respiratory diseases represent a group of diseases characterized by multiple comorbidities resulting from lesions in the trachea, bronchi, alveoli, and chest cavity due to various causes, increasing the prevalence of morbidity and mortality. [1] Asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, and interstitial lung disease are the most common chronic respiratory diseases. These diseases cause a significant burden on the health ecosystem worldwide. Therefore, preventive and rehabilitative methods are needed to reduce the burden on the health systems and negatively affect patients' quality of life. This need can only be met with practical and economical interventions. Speleotherapy, halotherapy, and pulmonary rehabilitation based on exercise training are some of these methods. [2,3]

The pulmonary rehabilitation approach comes to the forefront for relieving symptoms and maintaining and increasing functionality in chronic respiratory diseases. Pulmonary rehabilitation, a comprehensive and multidisciplinary intervention, improves the quality of life by increasing the participation of patients in physical and social activities. It helps to reduce healthcare costs by stabilizing or reversing the systemic symptoms of respiratory diseases. [4,5] Pulmonary rehabilitation programs based on exercise training have a significant place in treating chronic respiratory diseases. Rehabilitation programs based on exercise training have a significant place in treating chronic respiratory diseases. Physical inactivity and exercise intolerance are common in these patients. While avoiding exercise and physical activity provides momentary

relief for these patients, in the long term, it becomes a situation where problems gradually increase. This situation does not only affect the lungs but also the entire body outside the lungs. [6] The underlying cause of physical inactivity and exercise intolerance often stems from physiological disorders caused by respiratory distress. In addition, the effects of peripheral muscle weakness are undeniable. [7] Exercise plays a key role in correcting respiratory distress and muscle strength disorders. With resistance exercises, exercise tolerance and muscle strength of chronic respiratory patients increase, and with aerobic exercises, patients' maximum oxygen consumption, heart rate control, and quality of life can be improved. [8-10]

Salt therapy is another non-pharmacological approach used to reduce chest disease symptoms. There are two types of salt therapy: speleotherapy (natural salt cave environment) and halotherapy. The climate of natural salt caves provides a therapeutic environment for patients with respiratory distress. This type of therapy is called speleotherapy. Being protective against air pollutants and pollen, having constant air temperature and medium-high humidity, and being rich in fine aerosol elements (sodium, potassium, magnesium, and calcium) strengthen the therapeutic effects of the cave environment. [11] Since the natural cave environment is not available everywhere, creating this environment artificially is called halotherapy. Halotherapy is a form of therapy that provides an effect by releasing small salt particles from a source into a room and causing individuals to inhale these salt particles. In other words, in halotherapy, the natural microclimate of a salt cave is created. [12] Salt therapy has antibacterial, antimycotic, and anti-inflammatory effects. It can provide effective results, especially in treating respiratory diseases such as asthma, COPD, and cystic fibrosis. It is also stated that the natural environment provided by the environment is also beneficial in psychological and emotional terms. [13]

Individuals with chronic respiratory disease may be severely affected by environmental factors, including tobacco smoke, pollen, molds, and other aeroallergens. Therefore, keeping them away from unfavorable environmental conditions is very important. Because unfavorable environmental conditions can exacerbate the diseases of individuals with this disease, various substances called bioaerosols (a wide variety of organisms and substances such as airborne particles of biological origin, bacteria, fungi, viruses, pollens, and spores) can cause respiratory diseases, inflammation, and allergic reactions when inhaled. [14,15] In this respect, environments such as speleotherapy and halotherapy offer significant opportunities for environmental modification in managing these diseases. This environment provides an isolated environment from particles that can exacerbate chronic respiratory diseases. [16] It has been reported that with speleotherapy, sputum discharge becomes easier, respiratory muscles relax, the self-cleaning function of the lungs is stimulated, and an anesthetic effect is provided. [11,17] In another study, it was stated that halotherapy could be used as an adjuvant treatment for respiratory diseases. Halotherapy has been shown to reduce airway inflammation and improve pulmonary function by improving mucociliary function. It has been emphasized that this treatment can be used as an adjuvant treatment because it is safe and does not cause serious side effects. [18]

The proven benefits of salt therapy (speleotherapy and halotherapy) and exercise in respiratory diseases can be further strengthened by considering both methods. Although the number of studies on this subject has recently increased, studies considering both therapy methods are limited. Therefore, this study aimed to examine the studies evaluating the effectiveness of studies that consider salt therapy (speleotherapy, halotherapy) and exercise together.

MATERIALS AND METHODS

This study is a systematic review that refers to the retrospective systematic scanning of articles on the subject. This study was completed according to the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) checklist, which guides authors in reporting systematic review and meta-analysis studies. PRISMA provides researchers with a roadmap for conducting systematic review analyses. It is stated that this method contributes to the creation and evaluation of studies included in the systematic review in a better quality. [19]

Eligibility criteria

Male and female participants aged 18 years and over with lower and upper respiratory tract diseases were included in the study. Supervised intervention studies that evaluated the combined effectiveness of salt therapy (speleotherapy and halotherapy) and exercise interventions designed for adults were included. Quantitative studies of these interventions were preferred. Retrospective studies, conference abstracts, theses, books, book chapters, and articles published in languages other than English were not

included. Pre-test-post-test without a control group, comparative studies with a control group, and comparative studies without a control group were considered. Main outcome measures included studies assessing quality of life, physical and functional capacity, cardiovascular capacity, pulmonary function testing, and chest mobility.

Study design

PICOS (Population, Intervention, Comparison, Outcomes, Study design) standards were used to determine the study design.[20] (Tablo 1).

Table 1. PICOS Method in Determining Study Design

P	Population	Participants with chronic respiratory diseases
I	Intervention	Salt therapy (speleotherapy, halotherapy), aerobic exercises, resistance exercises, breathing exercises
C	Comparison of Groups	Pre-test-post-test without a control group, comparative with a control group, comparative without a control group
O	Outcomes	Quality of life, physical and functional capacity, cardiovascular capacity, pulmonary function testing, chest mobility
S	Study design	Interventional study

Search strategy

An electronic search strategy was used to determine the studies targeted by the research. A search was conducted using five electronic databases (Pubmed, Cochrane Library, Google Scholar, Reseachgate, and Scopus). All studies conducted between 2020 and 2024 were included. The publication of these studies in the specified databases between 2020 and 2024 was taken as the basis. The keywords "Halotherapy," "Speleotherapy," "Salt therapy," and "Exercise" were used as keywords. The search terms were related to salt therapy, which was the main theme of the study. The second stage consisted of associating salt therapy with exercise. In these stages, the words AND, NOT, and OR were used to ensure that studies that combined the topics were addressed.

Study selection and data collection process

Three researchers (RCY, DDK, AP) independently reviewed the titles and abstracts of the studies to determine whether the studies met the inclusion criteria. The studies that met the criteria were recorded, and the full texts were evaluated. The obtained studies were prepared in the context of the title, journal, publication year, authors, purpose of the study, therapy method, and results. The researchers ensured that the form was completed after the evaluation using this form.

Assessing the risk of bias

The review authors, RCY, DDK, and AP, used the PEDro risk of bias tool to assess the risk of bias in the included studies. This tool assesses sources of bias such as inclusion criteria and source, random allocation, concealed allocation, comparability at baseline, blinded subjects, blinded therapists, blinded assessors, outcomes for more than 85%, intent-to-treat analysis, between-group comparisons, means, and variability data. The authors report that total PEDro scores of 0-3 are considered ‘poor’, 4-5 ‘fair’, 6-8 ‘good’, and 9-10 ‘excellent’. [21]

Assessment of methodological quality

Information on the authors, physical characteristics of the included participants (age and gender), methodological features of the included study (inclusion criteria, severity of disease, sample size), details of the interventions (supervised intervention, frequency, intensity, type, and duration), comparison groups, outcomes assessed, follow-up period and side effects were recorded. Quality assessment of the included studies was performed using the PEDro scale, which has been shown to have good validity and reliability. [21]

The ethical aspect of the research

The study was conducted in accordance with the systematic review design. Ethics committee approval is not required for systematic reviews. The studies included in the evaluation were cited in the article.

RESULTS

The results obtained from different databases revealed 277 studies. From these studies, those that were repetitive and did not meet the search criteria were eliminated. The initial screening, titles, and abstracts of potential studies suitable for the purpose of the study were read and reviewed by the researchers. Finally, a total of 5 studies were included in the review. Table 2 summarizes the reviewed studies on salt therapy and exercise.

Freidl et al. (2020) conducted randomized controlled studies investigating the effects of winter exercise and speleotherapy on adults with allergic rhinitis or asthma; the speleotherapy group (n=23) was given a ten-day winter exercise and speleotherapy program, and the exercise group (n=18) was given winter exercise. In the baseline evaluation, differences were observed in the age of the individuals and the six-minute walking test results. It was observed that the speleotherapy group was younger compared to the exercise group ($t_{(32.2)}$ =

2.22, p = 0.03), and the speleotherapy group had better six-minute walking test results ($t_{(37.1)} = -2.26$, p = 0.03). In this study, where allergic airway inflammation, quality of life, spirometry parameters, and cardiorespiratory fitness were evaluated, it was seen that winter exercise alone and combined with speleotherapy could improve quality of life and allergic symptoms. In this study, fractional exhaled nitric oxide (FeNO) and nasal nitric oxide (NO) parameters were examined to evaluate allergic airway inflammation. FeNO and NO values did not show any significant change due to the applied treatment. It was observed that changes in mucociliary clearance time, nasal eosinophilic cell count, and differential blood count were better in the speleotherapy group. It was stated that the lack of significant change in spirometry values was due to the participants' initial spirometry values not being too bad. The 6-minute walk test results also showed no difference between the two groups.

Table 2. Studies Reviewed on Salt Therapy and Exercise

Title	Winter Exercise and Speleotherapy for Allergy and Asthma: A Randomized Controlled Clinical Trial	The influence of speleotherapy combined with pulmonary rehabilitation on functional fitness in older adults – preliminary report	Dynamic Balance and Chest Mobility of Older Adults after Speleotherapy Combined with Pulmonary Rehabilitation, Endurance, and Strength Training—A Prospective Study in Chronic Respiratory Diseases	Pulmonary rehabilitation in subterranean chambers combined with neuro-orthopedic activity-dependent plasticity therapy influences patients' quality of life – A preliminary study	Respiratory Muscle Function in Older Adults with Chronic Respiratory Diseases after Pulmonary Rehabilitation in Subterranean Salt Chambers
Journal, year, authors	J Clin Med., 2020, Freidl et al.	Ther Adv Respir Dis.. 2020, Mętel et al.	Int J Environ Res Public Health, 2022a, Mętel et al.	Complementary Therapies in Clinical Practice, 2022b, Mętel et al.	J Clin Med., 2023, Mętel et al.
Aim of the study	Investigation of the specific effects of recreational winter exercises combined with speleotherapy	To determine the effect of pulmonary rehabilitation conducted in therapeutic salt mine chambers on the functional fitness of older adults.	To evaluate the dynamic balance and thoracic mobility of older adults participating in speleotherapy in combination with pulmonary rehabilitation, endurance, and strength training.	To evaluate whether Neuro-orthopedic Activity-Induced Plasticity therapy combined with standard underground pulmonary rehabilitation conducted in a salt mine affects health-related quality of life and thoracic mobility in patients with asthma or chronic upper respiratory tract disease.	To evaluate the function of respiratory muscles in the elderly before and after a period of pulmonary rehabilitation and treatment in the underground chambers of a salt mine.
Therapy program	Winter exercises and Speleotherapy	3-week outpatient pulmonary rehabilitation program (gait training, upper and lower extremity strength training, aerobic exercise with musical accompaniment, respiratory exercise), 6-hour daily treatment accommodation in an underground salt room 5 days a week (Monday-Friday)	Speleotherapy combined with pulmonary rehabilitation, endurance, and strength training	Neuro-orthopedic Activity-Induced Plasticity treatment combined with pulmonary rehabilitation (breathing exercises, aerobic and resistance exercises) in the setting of speleotherapy	Standing pulmonary rehabilitation (walking, aerobic and resistance exercises, breathing exercises) 135 m underground for 3 weeks (5 days a week, 6 hours a day)
Results	It has been found that winter exercise alone and combined with speleotherapy positively affect quality of life and allergic symptoms in individuals with allergic rhinitis and/or asthma.	Speleotherapy combined with pulmonary rehabilitation has been found to improve the functional fitness of older adults in terms of upper and lower body strength, lower body flexibility, and dynamic balance.	Speleotherapy combined with pulmonary rehabilitation, endurance, and strength training has been found to improve dynamic balance and thoracic mobility in older adults with chronic respiratory disease.	Neuro-orthopedic Activity-Induced Plasticity treatment to the pulmonary rehabilitation program in the underground section of the Salt Mine Health Center. The addition of therapy techniques resulted in a statistically significant and clinically important improvement in subjects' health-related quality of life	Speleotherapy combined with pulmonary rehabilitation has been shown to improve respiratory muscle function in elderly patients with chronic respiratory diseases. The improvement was reflected in maximal inspiratory pressure and nasal inspiratory pressure but was particularly evident in maximum expiratory pressure.

The main reason for this was shown to be that the initial values were not too bad, and the ten-day treatment period was short. It was emphasized that a more extended treatment program should be implemented for better cardiorespiratory results. It was observed that winter exercise alone and winter exercise combined with speleotherapy could improve quality of life and allergic symptoms. In addition, none of the participants in the study experienced any adverse events during winter sports and speleotherapy. Therefore, it was stated that recreational winter exercises and speleotherapy could be recommended for allergic rhinitis and asthma with reasonable disease control. [22]

Mętel et al. (2020) investigated the effectiveness of speleotherapy combined with pulmonary rehabilitation in their study involving 22 people over the age of 65 with chronic respiratory diseases. The 3-week rehabilitation program was carried out in an underground salt chamber for 5 days/6 hours per week. The pulmonary rehabilitation program was carried out by a physiotherapist and consisted of 15 treatment sessions with three sessions (90 minutes) of supervised group training. In this program, walking training, 30 minutes of lower and upper extremity strength training (gradual increase using dumbbells, elastic bands, gym ball, step platform, and body weight), aerobic exercise with music or a stationary bike for 30 minutes, fitness exercise including breath control (30 minutes), relaxation and postural control exercises using neuro-orthopedic activity-induced plasticity (N.A.P) therapy were performed. After the combined rehabilitation program, significant improvements were observed in upper and lower body strength, lower body flexibility, agility/balance, and endurance parameters. The mean number of repetitions in 30 seconds in the Arm Curl test increased from 14.55±3.63 to 16.68±3.83. The number of repetitions in the Chair Stand test increased from 11.86±2.55 to 14.41±2.95. The results in the Sit and Reach test increased from -2.3 ± 11.11 cm to 2.14 ± 9.19 cm. The time to perform the 8-Foot Up and Go test decreased from 6.63 ± 1.27 seconds to 5.8 ± 0.86 seconds. The results in the 2-Minute Step test increased from 88.27 ± 20.64 to 96.55 ± 16.38 repetitions. This study demonstrated that the functional fitness of elderly individuals with pulmonary disorders improved with speleotherapy combined with pulmonary rehabilitation. [23]

In the study examining the dynamic balance and chest mobility of elderly adults after speleotherapy with pulmonary rehabilitation, endurance, and strength training, 44 elderly adults between the ages of 65 and 77 with chronic respiratory diseases were evaluated. Participants in the outpatient pulmonary rehabilitation program for 3 weeks (5 days a week, 6 hours a day) received a program focused on endurance, strength and breathing exercises, N.A.P. therapy techniques, education, and relaxation. After the application, a significant improvement was observed in the participants' four square-step test results. The average time for the four-square step test decreased significantly from 10.2 ± 1.9 seconds to 9.1 ± 1.7 seconds. The decrease in the four-square-foot test mean in patients with lower respiratory tract disease was 0.8 seconds, while the decline in patients with upper respiratory tract disease was 1.5 seconds. This significant improvement was also observed in chest mobility. The mean increase in thoracic mobility increased significantly from 4.5 ± 5.5 cm to 5.4 ± 2.8 cm. The average increase in chest mobility in this study was found to be 0.9 cm. This study showed that speleotherapy performed together with endurance and strength training increased dynamic balance and chest mobility in elderly adults with chronic respiratory diseases. [24]

In another study examining the effects of N.A.P. treatment combined with standard pulmonary rehabilitation in a salt mine chamber on patients with asthma and chronic upper respiratory tract disease, quality of life and chest mobility were evaluated. The supervised pulmonary rehabilitation program included 54 patients who participated in the study for 3 weeks. In the study group (N=23, 16 women, 7 men), N.A.P. treatment was added to the standard pulmonary rehabilitation program. In contrast, in the control group (N=31, 21 women and 10 men), the standard pulmonary rehabilitation program was applied for 3 weeks. The standard pulmonary rehabilitation program consisted of walking in the salt chamber (20 minutes - twice a day), breathing exercises, fitness exercises including aerobic and flexibility, upper and lower extremity resistance exercises, aerobic exercise (twice a week), health education (30 minutes - once a week), warm-up and cool-down exercises. In the N.A.P. program, which was applied in addition to the standard pulmonary rehabilitation program in the study group, exercises were performed on the ball to shift the nerve structures, regulate the activity of the autonomic system, stimulate breathing and normalize muscle tone. N.A.P. treatment aims to improve postural control and coordination. At the

same time, regulating the activity of the autonomic nervous system was considered one of the goals. The standard pulmonary rehabilitation program has been shown to improve health-related quality of life, the flexibility of the upper body, and chest mobility. The addition of N.A.P. therapy to the standard pulmonary rehabilitation program resulted in a significant improvement in the participants' health-related quality of life. The change in the quality of life 15D score was 0.068 higher in the standard pulmonary rehabilitation + NAP group than in the standard pulmonary rehabilitation group (p=0.022). [25]

In the study investigating the effect of the pulmonary rehabilitation program applied in underground salt chambers on respiratory muscle functions in elderly individuals with chronic respiratory diseases, 44 patients over the age of 65 were included in the 3-week program. The rehabilitation program includes walking, group endurance training under the supervision of a physiotherapist (aerobic or resistance exercises for 30 minutes), breathing exercises, and health-promoting training three times a week (about methods for controlling shortness of breath, behaviors during disease flare-ups, coping with chronic fatigue, sleep hygiene, healthy nutrition and health-promoting behaviors). The study participants were evaluated before and after the 3-week rehabilitation program. After the rehabilitation program, a significant increase of 10.2% in mean maximum inspiratory pressure, 12.3% in maximum expiratory pressure and 28.4% in sniffing nasal inspiratory pressure was observed ($p<0.05$). In patients with lower respiratory tract disease, maximum expiratory pressure increased by 10.7 % and sniffing nasal inspiratory pressure increased by 31.0 %. In patients with upper respiratory tract disease, maximum inspiratory pressure increased by 15.9% and maximum expiratory pressure increased by 14.9%. In the pre- and post-assessment of the pulmonary rehabilitation programme, maximum inspiratory pressure improved significantly by 8.8 cmH_2O, maximum expiratory pressure by 7.1 cmH_2O and sniffing nasal inspiratory pressure by 11.2 cmH_2O. In participants older than 70 years, only the maximum expiratory pressure improved significantly (maximum expiratory pressure increased by 9.3 cmH_2O). It has been reported that speleotherapy combined with pulmonary rehabilitation improves respiratory muscle function in terms of maximum expiratory pressure in elderly individuals with chronic respiratory diseases. [26]

Data on the level of evidence of the reviewed studies are shown in Table 3. The inclusion criteria and source for risk of bias assessment were assessed using the PEDro scale, which includes items such as random allocation, concealed allocation, baseline comparability, blinded cases, blinded therapists, blinded assessors, results for more than 85%, intent-to-treat analysis, intergroup comparisons, mean and variability data, and interventional study design studies ranging from 3 to 7 had a mean score of 4.6. The most common limitations were the lack of random allocation, concealed allocation, blinded cases, and blinded therapist.

Table 3. Risk of Bias (BIAS) (PEDro Scale Score)

Study	2	3	4	5	6	7	8	9	10	11	Total
Freidl et al. (2020)	1	1	1	0	0	0	1	1	1	1	7
Mętel et al. (2020)	0	0	0	0	0	0	1	1	0	1	4
Mętel et al. (2022)	0	0	0	0	0	0	1	1	0	1	4
Mętel et al. (2022)	0	0	1	0	0	0	1	1	1	1	5
Metel et al. (2023)	0	0	0	0	0	0	1	1	0	1	3

DISCUSSION

This study summarizes current studies evaluating the effects of combined salt therapy and exercise on chronic respiratory diseases. It is emphasized that salt therapy and exercise may be more effective for individuals with these diseases to benefit optimally. The findings show that studies using salt therapy and exercise in combination are limited. This may be because natural salt cave environments are not available everywhere and have not been transformed into health tourism. This limitation can be overcome by using artificially designed halotherapy rooms more. The benefits of exercise-oriented rehabilitation programs in these rooms can be further increased.

Halotherapy has been proven effective in chronic respiratory diseases such as COPD, asthma, vasomotor rhinitis, cystic fibrosis, and occupational lung diseases. [27-31] However, studies that have addressed halotherapy sessions and pulmonary rehabilitation together are limited. One study has indicated that halotherapy may have a prophylactic effect on children who frequently get sick. It has been emphasized that it can be used to rehabilitate acute respiratory tract diseases, chronic ear, nose,

and throat diseases, and respiratory and skin diseases seen in children. [32] Another study noted that halotherapy sessions facilitated drainage by reducing secretions in the respiratory tract. It was emphasized that halotherapy applied to a rat COPD model could improve functionality by improving the morphological structure of the lung. In addition, it was shown that immune response could be increased by inhibiting the inflammatory process and oxidative stress. [33]

Clinical studies indicate that salt therapy effectively reduces symptoms and improves functionality in sinusitis, bronchiectasis, chronic bronchitis, mild to moderate asthma, and COPD. [34] It has also been reported that Speleotherapy contributes to the prevention, treatment, and improvement of skin and respiratory disorders with its microclimatic effect. [35] One study investigated the effect of speleotherapy in children with bronchial asthma and found that speleotherapy contributed to the improvement and restoration of impaired bronchial tone. [36] In a study evaluating the effects of cryomassage and sylvinite speleotherapy on patients with asthma, it was emphasized that therapeutic effects could be increased by applying rehabilitative technological applications together. It was stated that anti-inflammatory effects and reduction of bronchial obstruction could be possible in this way. It was emphasized that the physical capacity of the patients could be increased and their psychological well-being could be improved. [37]

In addition to these studies reporting the effectiveness of halotherapy and speleotherapy, studies that include pulmonary rehabilitation programs together with salt therapy applications offer promising approaches. Studies have shown that the therapeutic effect is enhanced when both approaches are considered together. [22-26,38] One study found that pulmonary rehabilitation in COPD patients in an underground salt mine environment was more effective than pulmonary rehabilitation on the surface. This effect was reported to last for 6 months. Exercise tolerance was shown to improve more than pulmonary rehabilitation on the surface. [39] Therefore, considering salt therapy and exercise-based pulmonary rehabilitation programs together in the treatment of chronic respiratory diseases may help to increase the quality of life of patients and reduce the burden on the health ecosystem. In the studies obtained, the fact that the programs applied did not exceed 3 weeks, the number of participants was small, and some studies did not have a control group, which is seen as a limitation of the studies. In this respect, it can be said that randomized controlled studies with a high level of evidence are needed.

Chronic inflammation in chronic respiratory diseases has certain negative effects. Narrowing of small airways, changes in lung parenchyma and pulmonary vasculature, and high levels of pro-inflammatory biomarkers are some of these. The inflammatory process contributes to mortality by increasing the likelihood of muscle loss and cachexia, cardiovascular disease, osteoporosis, and metabolic syndromes in patients. [40,41] Lung inflammation causes systemic inflammation as well. It is assumed that the inflammatory reaction in the lung spills over into the circulation, causing systemic inflammation. [42] In this sense, the anti-inflammatory effect of salt therapy may be considered positive. Increasing this effect may be possible with exercise training. The literature emphasizes that exercise can be a potential therapeutic tool in reducing systemic inflammation with its anti-inflammatory effect. [43] It has been reported that regular exercise protects against diseases associated with chronic systemic inflammation. The acute and long-term effects that occur after exercise are due to the pro-inflammatory and anti-inflammatory cytokines secreted by the body. It has been reported that regular exercise plays a key role in controlling this physiological process. [44]

The effect of halotherapy and speleotherapy on chronic respiratory diseases strengthens the physiological basis that exercise creates in the body. It has been stated that exercise training increases cardiorespiratory fitness, endurance of the muscles required for ambulation, and exercise tolerance. It has been reported that this contributes to an increase in the quality of life-related to health. [45] It has been emphasized that exercise-based pulmonary rehabilitation programs help improve outcomes such as 6-minute walking distance, maximum oxygen consumption, exercise capacity, quality of life, and shortness of breath. [46] It is very important not to underestimate even short-term exercise programs. One study reporting the results of a 4-week program showed significant improvements in quality of life and exercise capacity. [47] Studies reporting positive effects of halotherapy and speleotherapy even after short-term application are important in this respect. It is estimated that longer-term salt therapy and exercise programs combined will increase the benefits. In clinical practice, there is no consensus on creating an optimal exercise prescription for

chronic respiratory diseases. However, current guidelines recommend developing individual exercise prescriptions within the scope of (1) exercise types, (2) frequency, (3) intensity, (4) duration, (5) exercise goals, and (6) precautions. [48] Programs that include endurance training, resistance training, flexibility training, respiratory muscle training, and a combination of these can be implemented as part of pulmonary rehabilitation. Exercise intensity can range from low intensity to high intensity, with exercise training frequency of 20-60 minutes each (lasting at least 8-12 weeks), 3-5 times per week. [48-50] In this sense, it can be said that there is a need for more in-depth studies on the application of personalized exercise programs together with speleotherapy and halotherapy.

In a study written about complementary and alternative medicine for bronchial asthma, it was stated that breathing exercises improve lung function and improve quality of life. Again, in the same study, it was stated that methods such as relaxation, hypnosis, autogenic training, speleotherapy, biofeedback, and psychotherapy contribute to lung function. However, it was emphasized that the obtained effect was not proven to be superior to placebo. It was emphasized that there is a need for randomized controlled studies of superior methodological quality to obtain clearer results. [51] In our study, the lack of randomization in some studies and the absence of a control group draw attention to the weak point of the studies. Conducting research methodologically with a control group and randomized studies may contribute to further clarification of the results shown by these studies. Meta-analysis studies can be conducted for a comprehensive analysis of the effectiveness of speleotherapy and halotherapy combined with exercise. This approach can provide high-quality evidence for determining optimal intervention strategies by evaluating multiple interventions. [52] This can help healthcare professionals make more accurate treatment decisions.

The mean scores of the methodological quality of the studies evaluated with the PEDro scale were found to be moderate. [22-26] Research should include studies with larger samples and higher levels of evidence to more clearly demonstrate how exercise-based pulmonary rehabilitation programs combined with speleotherapy and halotherapies affect clinical outcomes. Treatment strategies that evaluate chronic respiratory diseases separately need to be addressed. These studies can guide clinicians and therapists in terms of creating exercise prescriptions. Individualized exercise programs combined with salt therapy can be beneficial in terms of providing additional evidence. Studies are needed to emphasize the extent to which outcome measures need to be improved to accept the combined effect of salt therapy and exercise as 'successful'.

Our study has some limitations. First, the small number of participants in the studies considered limits the generalizability of the results. Due to the inhomogeneity of individuals and evaluation parameters in the included studies, it is not possible to comprehensively and convincingly discuss the effect of an exercise program based on pulmonary rehabilitation combined with salt therapy (speleotherapy and halotherapy). Secondly, the short-term effects of the studies were discussed. It is unclear whether the benefits obtained will be maintained for a longer period. In this respect, follow-up studies on how much the benefits obtained are maintained will contribute to the literature.

CONCLUSION

According to the evidence, the effectiveness of combined exercise and salt therapy in chronic respiratory diseases is remarkable. Although the number of studies on the effectiveness of salt therapy is increasing, there is a need for high-level evidence studies that combine exercise and salt therapy.

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REVIEW

Aromatherapeutic Remedies to Ease the Work-Related Stress

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Abstract
Stress is defined as a state of worry or mental tension caused by a difficult situation by WHO (World Health Organization). Stress is a natural human response to address challenges and threats in life. Although everyone experiences stress at some level, the way we respond to stress makes a big difference to our well-being. Since individuals spend most of their time at work, managing work-related stress is important for their quality of life. There are numerous approaches for stress management, including synthetic drugs, aromatherapy, and massage applications. Since aromatherapy is notable for its straightforward applicability and reliability, the efficacy of aromatherapeutic applications in managing work-related stress was evaluated.
Keywords: Aromatherapy, Work Stress, Essential Oil, Volatile Oil, Anxiety

INTRODUCTION

There is no doubt that volatile (essential) oils have been one of the most effective healing materials for centuries. The fact that it attracts attention with its pleasant smell has also created the idea that it is the essence of the plant.[1] Aromatherapy is a field that emerged with the use of essential oils for therapeutic purposes. Thanks to their healing power, which is not limited to physical states but also affects mental states, they can be used for various reasons.[2] Aromatherapy can be administered through inhalation of essential oils, topical application with fixed oil, or oral ingestion.[3] Aromatherapy is used to treat multiple conditions, including anxiety, skin, hair, and digestive issues, as well as pain management, wound care, breathing, and sleeping difficulties.[4]

Stress at work is one of the mental illnesses that aromatherapy is frequently used to treat. Employee stress levels rise for various reasons, including role conflicts, workloads, and working environment. Higher stress levels directly impact work productivity.[5] It is evident that stress directly and indirectly negatively affects one's health. The release of neurotransmitters, such as adrenaline and noradrenaline, is a physiological stress response. This results in physiological alterations, including an accelerated heartbeat and respiration. Furthermore, stress stimulates the production of cortisol, a steroid hormone.[6] Cortisol regulates carbohydrate, fat, and protein metabolism and regulates blood pressure and blood sugar. However, higher than normal cortisol levels have also negatively affected cognitive function.[7] In addition, chronic stress can cause many diseases, such as diabetes, obesity, abnormal cholesterol levels, and cardiovascular diseases. For this reason, it is essential to minimize stress levels in working environments where people spend most of their time.

Individuals have employed various approaches to cope with the stress factor. Aromatherapy has also been one solution. *Citrus sinensis* (L.) Osbeck, *Lavandula angustifolia* Mill., *Matricaria recutita* L., *Rosa damascena* Mill. essential oils are frequently used for stress and anxiety states.[8,9] For example, in a study with children, children undergoing dental treatment were treated with sweet orange oil inhalation, resulting in lower cortisol levels than the non-essential oil group[10] In another study of 150 children, although the level of anxiety related to dental treatment decreased in both gender groups, only the decrease in anxiety level of girls was found to be statistically significant.[11] The reduction in cortisol levels shows sweet orange oil's effect on stress. Although there are many clinical studies on stress, the number of clinical studies based on work stress is limited. In this context, aromatherapy applications, which have a place in the literature for work stress, are shown in Table 1. The chemical structures of the effective compounds of the essential oils used are shown in Figure 1.

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Tablo 1. Clinical studies on work stress

Essential oil	Application method	Working group	Results	Reference
Citrus bergamia	Inhalation	Nurses	The work stress score was 46.00 ± 13.93 before the application and 42.08 ± 14.83 after the application ($p<0.05$).	[34]
Rosa damascena	Inhalation	Nurses	The stress score, which was 140.40 ± 32.66 before the application, was measured as 129.93 ± 17.91 after the application ($p<0.05$).	[35]
Lavandula angustifolia	Inhalation	Nurses	It was observed that work stress scores decreased from 88.97 ± 14.95 to 83.44 ± 14.53 in lavender oil application ($p<0.05$).	[36]
L. angustifolia	Inhalation	Nurses	Before the application, the stress value was 2.97 ± 0.99. After applying the change, this value decreased to 2.70 ± 0.92 ($p<0.05$).	[37]
L. angustifolia	Inhalation	Nurses	The stress value, which was 6.1 before the application, was measured as 2.8 after the application ($p<0.05$)	[38]
L. angustifolia	Inhalation	Health personnel working on night shifts	Hemodynamic findings were evaluated after the application. Blood adrenaline value of 0.022±0.02 ng/mL before aromatherapy	[39]

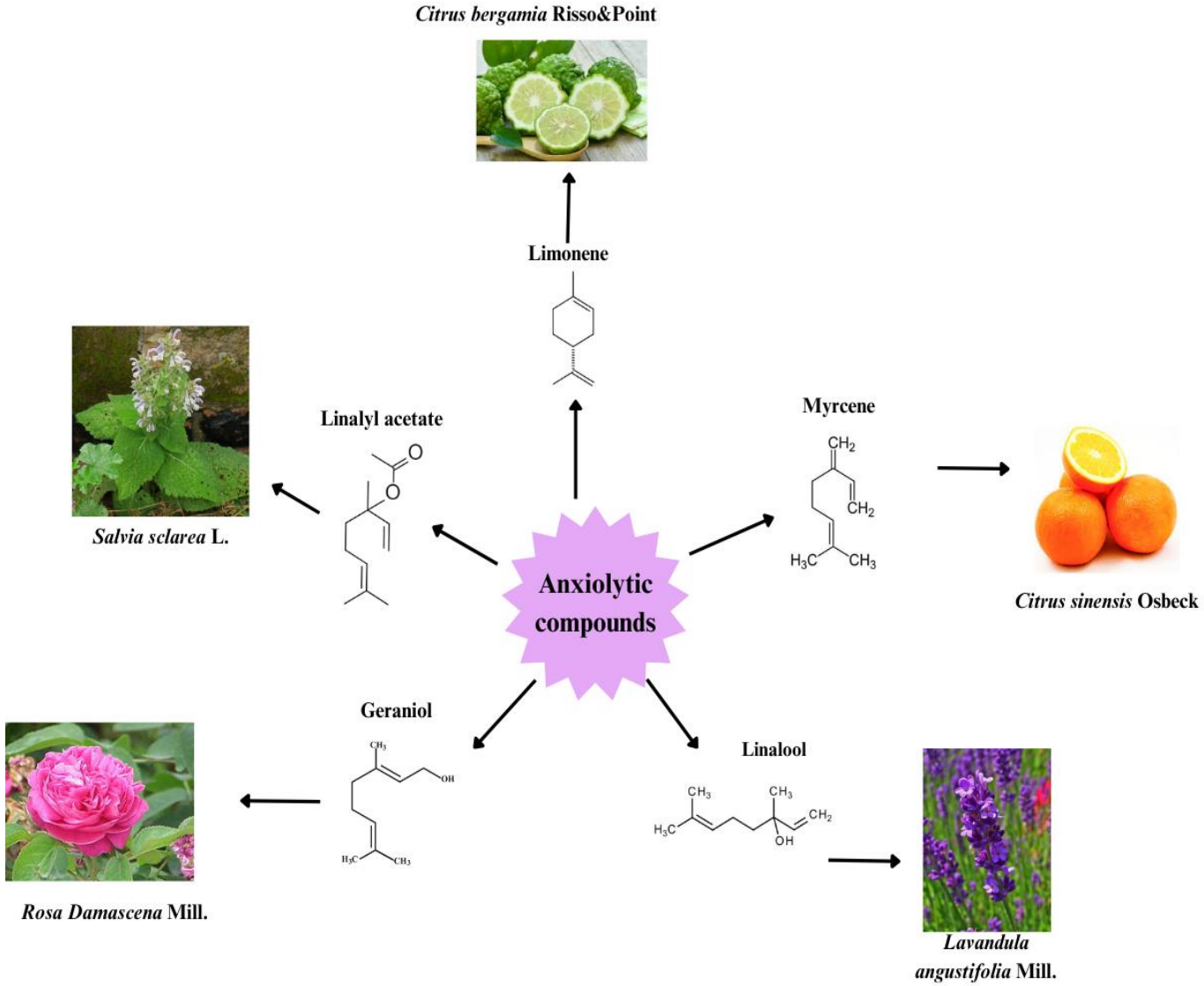

Figure 1. Major chemical compounds of essential oils used in work stress (Illustrated by the author)

1. Essential oils used in work stress

Numerous studies have examined the effects of essential oils on anxiolytic or antidepressant activity. Clinical studies on work stress, a type of stress, have been conducted on certain occupational groups. These studies frequently used essential oils of plants such as *Citrus* species, *Lavandula angustifolia*, *Rosa damascena*, and *Salvia sclarea*.

Citrus bergamia Risso&Poit.

It has been classified within the Rutaceae family. The bergamot essential oil is extracted from the fruit peels mechanically.[12] The main composition of the essential oil is terpenic compounds such as limonene, linalool, *ß*-pinene, linalyl acetate, and γ-terpinene.[13] Especially limonene, linalool, and linalyl acetate compounds are known to have anxiolytic effects due to their impact on the GABAergic system.[14] In an *in vivo* study, the anxiolytic effect of limonene inhalation was investigated using an elevated plus maze model. At the end of the 30 min inhalation time, it was observed that the duration of stay in the open arm increased. [15] This activity of limonene is related to its activity as an adenosine A2A receptor agonist. By this mechanism, it shows anxiolytic activity through GABAergic modulation.[16] This activity of limonene is related to its activity as an adenosine A2A receptor agonist.[17]

Citrus limon (L.) Osbeck

Citrus limon (L.) Osbeck belongs to the Rutaceae family. The essential oil is extracted from the peels using the cold pressing method.[18] The main compounds in the essential oil are limonene, *ß*-pinene, and γ- terpinene.[19] A study has demonstrated that the antidepressant mechanism of action of lemon essential oil is through the GABAergic system, which shows a benzodiazepine-like effect.[20]

Citrus sinensis Osbeck

Citrus sinensis Osbeck has been classified within the Rutaceae family. The sweet orange essential oil is extracted from the fruit peels mechanically.[18] The main composition of the essential oil is terpenic compounds such as limonene, myrcene, *γ*-terpinene, and α-farnesene. This study shows that the anxiolytic effect of sweet orange oil is due to limonene. In an *in vivo* study, the effect of the myrcene compound was investigated in the open-arm test. It was concluded that myrcene had an anxiolytic effect even at a dose of 50 mg/kg, and this effect increased at increasing doses.[21]

Our group conducted a preliminary study to investigate the sweet orange (*Citrus sinensis* by Florame ®) oil effect on 20 female participants, aged between 26 and 51 years, who were full-time employed in a private company. The male participants were excluded from the study due to an inability to demonstrate regular engagement with the prescribed application. The aim was to examine the potential benefits of aromatherapy in reducing work-related stress in employees. The work experience of the participants ranges from 1 to 29 years. 30% of the participants are married, and 70% are single. Additionally, 70% of the participants have obtained a university degree, while 30% have completed high school. The participants were instructed to inhale sweet orange oil twice daily (before noon and afternoon) with a personal diffuser provided by the Florame® for four weeks. Furthermore, sweet almond oil was selected as the placebo as a consequence of the literature review. Stress scores were 13.5 in the placebo group and 12.4 in the sweet orange oil group in the pre-application. At the end of 4 weeks of applications, analyses demonstrated that the stress scores decreased to 9.2 in the placebo group and 4.4 in the sweet orange oil application group. The results of the statistical analysis indicated that the change in the orange oil was statistically ($p < 0.01$) significant (Unpublished results by Altıntaş-Gunduz D. & Koca-Çalışkan U.).

Lavandula angustifolia Mill.

The Lamiaceae family is the taxonomic classification of this species. The lavender essential oil is extracted from the flowers of the plant through steam distillation.[18] The main compounds of the essential oil are terpenic compounds such as linalool, camphor, linalyl acetate, 1,8-cineole, and borneol.[22] In an *in vivo* study, linalool was administered to mice by inhalation for 30 min with an elevated plus maze model. It was observed that the time spent in the open arm and the number of open-arm entries increased.[23] The findings of this study indicate that the anxiolytic effect is attributable to linalool.

Rosa damascena Mill.

The plant belongs to the Rosaceae family. The rosa essential oil is extracted from the petals through steam distillation. The main compounds of the essential oil are terpenic and hydrocarbon compounds, including *ß*-citronellol (48.2%), geraniol (17%), *ß*-phenyl ethyl benzoate (5.4%), phenyl ethyl alcohol (5.1%), and nonadecane (4.3%).[24] It is observed that the geraniol compound may be responsible for the anxiolytic effect. In a study conducted with mice, a forced swimming test was employed. In geraniol applications at different

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doses, no significant activity was observed at a dose of 20 mg/kg, while a significant decrease in immobility time was observed at a dose of 40 mg/kg.[25]

***Salvia sclarea* L.**

The plant is classified in the Lamiaceae family. The essential oil is extracted from the leaves through steam distillation.[18] The main compounds of the essential oil are terpenic compounds such as linalyl acetate (49.1%), linalool (20.6%), (*E*)-caryophyllene (5.1%), *p*-cymene (4.9%), *α*-terpineol (4.9%).[26] The compounds linalyl acetate and linalool are thought to be responsible for the anxiolytic effect. Linalyl acetate compound was administered orally to mice at different doses and tested in the elevated plus maze test. According to the number of entries into the open arms, an effect close to the standard alprazolam compound was observed in the group administered 200 mg/kg linalyl acetate. [27]

2. Effect mechanisms of essential oils in anxiety

GABAergic mechanism

GABA is one of the neurotransmitters that have an inhibitory effect on the central nervous system. GABA reduces the overall activity of the nervous system by slowing down the connection between neurons. When GABA binds to GABA-A receptors, chloride (K^+) channels open, and chloride ions enter the cell, resulting in a hyperpolarisation of the neuron. When GABA binds to GABA-B receptors, potassium (K^+) channels open, and calcium (Ca^{2+}) channels close, leading to a hyperpolarisation of the neuron. In both cases, synaptic transmission is inhibited. Consequently, low GABA levels are associated with increased depression and anxiety.[28] The mechanism is shown in Figure 2.

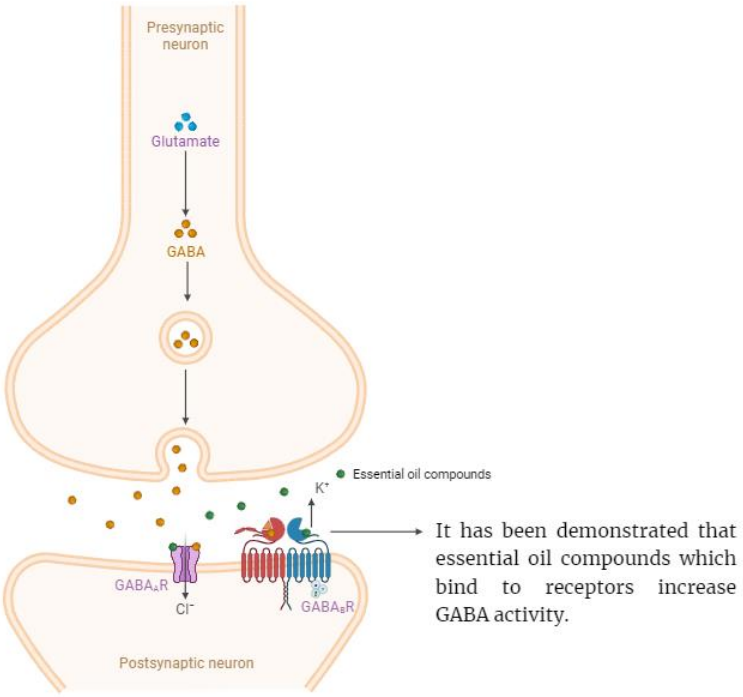

Figure 2. Mechanism of sedative action of GABA (Illustrated by the author)

The anxiolytic effect mechanism of bergamot essential oil was investigated in a study involving rats. The results demonstrated that the mechanism of action of bergamot essential oil is related to GABA receptors.[29] Bergamot essential oil is composed of two primary constituents: limonene and ß-pinene.[30]

Serotonin reuptake inhibition

Serotonin is a neurotransmitter that can control many processes, including mood, cognitive activity, and autonomic function. It is derived from the amino acid tryptophan. Serotonin has an antidepressant effect by increasing the amount of serotonin in the synaptic cleft. For this reason, serotonin reuptake inhibiting (SRI) compounds have antidepressant activity. There are seven main subtypes of receptors (5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6, 5-HT7). The 5-HT1 receptor is responsible for the antidepressant effect.[31] It is important to inhibit the selective serotonin reuptake to achieve an antidepressant effect, as different serotonin receptors have different effects. The mechanism is shown in Figure 3.

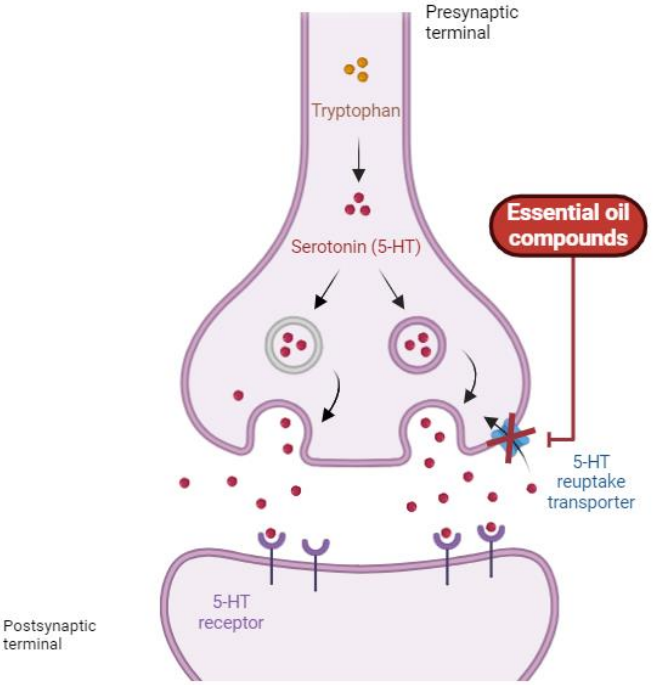

Figure 3. Mechanism of 5-HT reuptake inhibition (Illustrated by the author)

A study has examined the mechanism of action of ylang-ylang essential oil obtained from the Cananga odorata plant. It was observed that the antidepressant effect of this oil was related to serotonin receptor inhibition.[32] The essential oil of ylang-ylang contains linalool, linalool acetate, and α-pinene as the majority of the compounds.[33]

CONCLUSION

Clinical studies have scientifically proven the positive contribution of essential oils to stress levels. Considering that work stress is also evaluated in this context, it shows that aromatherapy applications have a reducing effect on work stress. *In vivo* studies also show that this effect is due to the chemical substances that make up the composition of essential oils. In this context, it can be concluded that the widespread use of aromatherapy applications in workplaces can have a positive effect on work efficiency and human health.

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