INTERNATIONAL JOURNAL OF TRADITIONAL AND COMPLEMENTARY MEDICINE RESEARCH

ULUSLARARASI GELENEKSEL VE TAMAMLAYICI TIP ARAŞTIRMA DERGİSİ

NİSAN / April **2020** **SAYI** / ISSUE: 01 CİLT / VOL: 1 ISSN:

ORGANIZATION, PREPERATION AND CORRESPONDENCE
Duzce University Traditional and Complementary Medicine Application and Research Center, Düzce, TURKEY
Date of Issue: 30.04.2020

• **International Journal of Traditional and Complementary Medicine Research** is an international peer-reviewed journal and is published three times a year. The responsibility of the articles published belongs to the authors.

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Volume:1 **Issue:**1
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

Systemic Investigation of Acute Toxicity of Some Food Supplements on the Market in Turkey

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Abstract

Objective: Along with changing living and working conditions in recent years, changes have occurred in eating habits. People are turning to supplements for many purposes such as supplementing normal nutrition, feeling more energetic, enhancing the immune system by taking vitamins and minerals that they think they do not get enough, and meeting their body's needs when they cannot eat a balanced and healthy diet. Supplements are prepared in various forms using nutrients such as vitamins, minerals, proteins, amino acids, plants, enzymes, fiber and fatty acids. These supplement foods are not intended to treat or prevent any disease. In general, supplement foods; it is not a substitute for natural nutrition, but it is intended to complement its deficiencies. They cannot be recommended and sold instead of medicines. Because of many of these products have natural content, they can be placed on the market without toxicity tests. In the current study, it is planned to observe the effects of acute systemic toxicity of supplements that are used in remember regeneration therapy method (RTM) and sold in the markets with the brands' name of IST-ARD®, ARD-REM®, DVD-ARD®, KT-REM®, ARDZ-REM®, IST-GLIO®, IST-REM®, ROMX®, and REGULIN®.

Material-Method: In the experimental design, there were 10 groups (1 control and 9 application groups) male mice. To observe acute toxicity, clinical observation was performed for 72 hours and the biochemical and histopathological parameters of the animals were evaluated at the end of the application.

Results: According to the results obtained from the study, there were not found significant differences in biochemical and histopathological evaluations between the control and the application groups.

Conclusion: The acute toxicity effect of the food supplements was not determined. May be further studies with bigger numbers of samples investigating the nephrotoxic effects of these products lighten this matter.

Keywords: Acute toxicity, Mice, Supplement foods

INTRODUCTION

Food supplements are known as products used to support substances that are missing the diets of people such as minerals vitamins etc. [1]. In the "Turkish Food Codex Supplementing Food Communiqué"; Supplementary foods are defined as a mixture of vitamins, minerals, proteins, carbohydrates, fibers, fatty acids, amino acids, etc. and vegetable and animal origin substances, bioactive substances that have nutritional or physiological effects concentrates or extracts that are prepared in powdered forms, capsules, tablets, lozenges, liquid ampoules, dropper bottles and whose daily intake dose is determined to reinforce normal nutrition[2]. According to the data of the World Health Organization (WHO), a large part of the world population benefits from traditional medicine in the treatment and prevention of diseases. In this context, food supplements and medicinal plants are increasingly being used as traditional and complementary medicine products. According to WHO, 21,000 plants are suitable for medicines [3]. The widespread use of medicinal

plants and food supplements also leads to an increase in the value of the market.

It is known that taking the nutrients that the body needs completely has positive effects on human health. The use of food supplements is increasing in order to reinforce nutrition in communities. This causes a problem. Adequate safety profiles for food supplements have not yet been fully disclosed. One of the most important problems in food supplements is toxicity. Food supplements produced without toxicity studies can cause health problems. The fourth highest cause of death in the United States is the death caused by side effects [1]. Therefore, it is essential to demonstrate that plants are safe by conducting toxicity studies. The combination of many herbs is likely to cause synergistic or anti-synergistic effects, which may have positive or negative consequences. In a study conducted in 2010, antifungal synergistic effects of ginger and turmeric oils were examined. As a result of the study, it was found that ginger has a higher antifungal effect compared to turmeric, but a mixture of the two shows the highest antifungal effect [4]. It is also stated that turmeric, which has wide usage in the phytotherapy approach, has anticancer properties as epigenetic modulator [5] and has antitumor and antioxidant effects. Mixtures do not always show the synergistic effect. Sometimes they can have anti-synergistic effects. Zhao et al. (2010) study on cell culture technique with adrenal medulla cells, they showed that the *Fructus evodiae* plant inhibits the stimulating effect by *Rhizoma coptidis* [6]. For this reason, it is necessary to identify the plants to be mixed and make safety tests.

In an animal model with an acute systemic toxicity test, it provides information about the potential harmful effects of the food supplement itself, its metabolites, or extracts for less than 24 hours in single or multiple exposure [7,8]. After products metabolize and distribute from the locally exposed area of the orally administered to the distant organ systems, systemic toxicity tests are carried out to determine the general systemic toxicity symptoms, the degree of affection of the organs, and the lethal dose that can be caused by absorption. The dose given at one time and causing the death of half of the population is called LD_{50} (lethal dose). Products with a high LD_{50} value will be safer to use. Today, with the new methods, low animal numbers can be performed in toxicity studies. In these tests, the substance to evaluate the toxicity at once or repeatedly is given to the body within 24 hours. Afterwards, the animals were monitored by observing for 72 hours. This stage constitutes the clinical observation stage. Clinical observations, blood parameters and organs (especially liver and kidneys) were evaluated to observe toxicity [8,9]. It gives us information about the toxic effects and lethal dose of the substance applied mixtures on the body.

The study aim is to research acute toxicity effect of the IST-ARD®, ARD-REM®, DVD-ARD®, KT-REM®, ARDZ-REM®, IST-GLIO®, IST-REM®, ROMX®, and REGULIN®.

MATERIALS AND METHODS

Animals

The animals used in the study were obtained from Düzce University Experimental Animals Application and Research Center. CD1 male mice (2-3-month-old, body weight 35-40 g) which were used for the experiment were kept in the laboratory conditions; 23 ºC room temperature, 60± 5% humidity, and 12:12 light-dark cycle, in optimal values, and with access to food and water ad libitum. The experimental protocol was approved by Düzce University Animal Experiments Local Ethics Committee (Decision Number: 2019/1/1).

Groups, substances and doses

The food supplements used in the study were supplied from Naturin (Natural Products Pharmaceutical and Pharmaceutical Raw Materials Industry Trade Limited Company). The products dissolved in distilled saline were applied as one-time gavage at the doses indicated in Table 1. Control groups were given 1 ml/kg saline again as gavage. Doses for mice were determined by proportioning from products' certain daily usage amounts for humans (Table 1). These products were used for remember regeneration therapy method (RTM)[5].

Table 1. Experimental groups, substances to be applied and doses

Group No	Group	Content	Granted Quantity	Use of Application	Animal Number
1	Control	Salin	1 ml/kg	Oral	8
2	IST-ARD®	*Juniperus communis* extract, *Urtica* sp. seed, *Cirsium arvense* extract, *Peganum harmala* extract, *Nigella sativa* extract, Zingiber officinale extract, *Curcuma longa* extract	63.40 mg/kg	Oral	8
3	ARD-REM®	*Juniperus communis* fruit, *Peganum harmala* seed, *Zingiber officinale* rhizome *Thymus* sp, *Nigella sativa* seed, *Curcuma longa* rhizome, *Foeniculum vulgare* fruit, *Pimpinella anisum* fruit, *Cassia acutifolia* Leaf, *Syzygium aromaticum* flower seed	24.85 mg/kg	Oral	8
4	DVD-ARD®	*Curcuma longa* extrac, *Silybum marianum* extract, *Rosmarinus officinalis* extract, *Juniperus communis* extract, *Fumaria officinalis* extract, *Cichorium intybus* extract	22.30 mg/kg	Oral	8
5	KT-REM®	*Linum usitatissimum* seed, *Urtica* sp. Seed, *Peganum harmala* seed, *Nigella sativa* seed, *Zingiber officinale* rhizome, *Curcuma longa* rhizome	27.40 mg/kg	Oral	8
6	ARDZ-REM®	*Juniperus communis* fruit extract, *Zingiber officinale* rhizome, *Peganum harmala* seed, *Thymus* sp, *Curcuma longa* rhizoma, *Nigella sativa* seed, *Foeniculum vulgare* fruit, *Pimpinella anisum* fruit, *Cassia acutifolia* Leaf, *Syzygium aromaticum* flower	24 mg/kg	Oral	8
7	IST-GLIO®	*Curcuma longa, Curcuma longa* seed, *Peganum harmala, Silybum marianum, Zingiber officinale* extract, *Nigella sativa* seed, *Juniperus communis* fruit, *Thymus sp, Foeniculum vulgare, Pimpinella anisum, Cassia acutifolia, Eugenia caryophyllata*	31.7 mg/kg	Oral	8
8	IST-REM®	*Curcuma longa* extract, *Urtica sp.* Seed, *Silybum marianum* seed, *Peganum harmala* seed, *Nigella sativa* extract, *Zingiber officinale* extract	58.30 mg/kg	Oral	8
9	ROMX®	*Curcuma longa* extract, *Juniperus communis* extract, *Zingiber officinale* extract, *Peganum harmala* extract, *Thymus sp.* Extract, *Nigella sativa* extract, *Foeniculum vulgare* extract, *Pimpinella anisum* extract, *Cassia acutifolia* extract, *Eugenia caryophyllata* extract	18.30 mg/kg	Oral	8
10	REGULIN®	*Curcuma longa* extract, *Silybum marianum* extract, *Rosmarinus officinalis* extrac, *Juniperus communis* extract, *Fumaria officinalis* extract, *Cichorium intybus* extract	16.90 mg/kg	Oral	8

Test procedure

ISO10993-11, ISO10993-2 , and ISO 10993-12 protocols have been used in the planning of acute systemic toxicity tests [8,10,11]. It is based on testing and evaluating 9 food supplements (Table 1) for acute systemic toxicity using the test protocol in vivo experimental animal model followed according to the specified standards. Substances were administered at once within 24 hours, and animals were sacrificed under ketamine/xylazine anesthesia at the end of 72 hours of clinical observation. Blood samples were taken from hearth with cardiac puncture method.

During the experiment and after the experiment, the control group consisting of 8 animals and the application groups consisting of 8 animals were created for each product, and the results were evaluated by looking at the data obtained and the symptoms or findings observed.

Clinical observation parameters

During the study, observations were made at 0 minutes, 30 minutes, 60 minutes, 120 minutes, 240 minutes, 480 minutes, 1440 minutes (1 day), 2880 minutes (2 days), and 4320 minutes (3 days). At the same time, during the study, the video was recorded and evaluated. Dyspnea, apnea, kiss, tachypnea, indeterminate positions and increasing/decreasing tremor, clonic, tonic, tonic-clonic, lacrimation, miosis, mydriasis, bradycardia, tachycardia, arrhythmia, excessive salivation, piloerection, analgesia, hypotonia, hypertonia, diuresis, edema, redness, respiration, motor activity, convulsions, reflexes, salivation, ocular signs, cardiovascular signs, gastrointestinal signs were evaluated.

Biochemical parameters

For the biochemical evaluation, blood samples taken from all groups were collected and waited at +4°C and analyzed with Beckman-Coulter AU5800 Biochemistry Analyzer on the same day in the Düzce University Health Practice and Research Center Biochemistry Laboratory. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzyme levels for liver functions and blood cholesterol (Chol) parameters were examined.

Histopathological evaluation

While taking the samples of the animals in their groups, they were taken in one piece without damaging the organ and kept in a 10% formaldehyde solution. The organs in solution were cassette-tapped and numbered according to macroscopic examination at the Pathology Laboratories of Düzce University Health Practice and Research Center. The process was completed by passing through fixing solutions for a total of 12 hours. After tissue fixation, samples were made into paraffin blocks. 3-4 micrometers thick block sections were taken in the microtome. Sections were stained with hematoxylin and eosin stain. Microscopic examination was performed by a pathologist. Signs of fatty change, spotty necrosis, inflammation, damage for liver tissue; atrial dilatation, inflammation for heart tissue; tubular atrophy, interstitial fibrosis, inflammation, glomerular damage for the renal tissue parameters were evaluated for histopathological evaluation. All animal lesions were scored based on their severity (0 : absent, 1-3 : mild, 3-6 moderate, 6> severe) by pathologist.

Statistical evaluation

The biochemical parameters obtained in our study were analyzed using the one-way ANOVA test using the IBM SPSS Statistics 20 program. The groups that were found statistically significant were determined by post hoc Dunnett's T3 test. $P < 0.05$ was accepted as the statistical significance level. The histopathological assessment obtained in our study were analyzed using the Mann Whitney U test using the IBM SPSS Statistics 20 program. $P < 0.05$ was accepted as the statistical significance level.

RESULTS

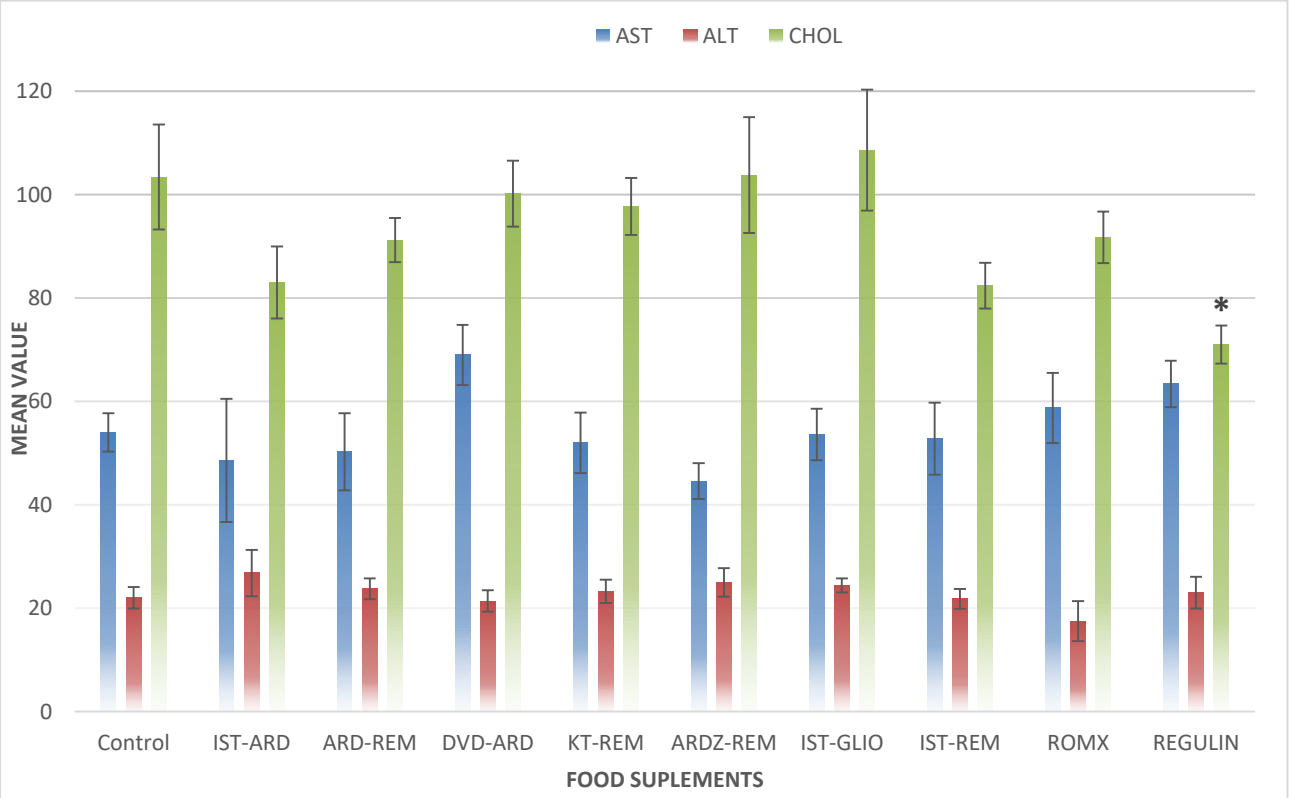
Clinical observation parameters

During the experiment, there was no difference between control group and practice groups in terms of dyspnea, apnea, kiss, tachypnea, increasing / decreasing tremors, clonic, tonic, tonic-clonic, lacrimation, miosis, mydriasis, bradycardia, tachycardia, arrhythmia, excessive saliva, piloerection, analgesia, hypotonia, hypotonia redness, respiration, motor activity, convulsions, reflexes, salivary secretions, ocular signs, cardiovascular signs, gastrointestinal parameters. All animals in the groups were sacrificed under anesthesia after the experiment was completed. There was no loss in the number of animals during the experiment.

Biochemical parameters

When the findings obtained from the study were evaluated, no significant difference was found

between AST and ALT values between the control group and the application groups. In terms of cholesterol values, control group was 103.40 mg/dL, REGULIN® group was calculated as 71.00 mg/dL. This decrease was found statistically significant ($p \leq 0.05$). No statistically significant difference was found in the cholesterol data of other groups compared to the control group cholesterol data. However, it was seen that the cholesterol values of these groups were close to or lower than the control group (Graphic 1, Table 2).



Graphic 1. Biochemistry parameters
* Statistically significant in comparison with the control group ($p \leq 0.05$)

Histopathological evaluation

The data obtained as a result of histopathological evaluation were demonstrated in Tables 3 and 4. According to these results, macroscopically, no deterioration was observed in the natural appearance of the tissues examined. There was no difference between the control and the application groups in terms of the integrity of the organs, shape, and color. Histopathological evaluation of the liver, heart, and kidney tissues of the animals in the experiment was performed. In the comparison of each of the application groups with the control group separately, there was no difference in the parameters of in terms of fatty change, spotty necrosis, inflammation, damage findings for liver tissue; atrial dilatation, inflammation for heart tissue. In the evaluation of the kidney tissues of the groups, especially perivascular tubulointerstitial lymphoplasmacytic cell infiltration in the cortex and corticomedullary junction were observed in the kidney tissues with different proportions of all groups except the ARD-REM® group (Figure 1). In the KT-REM ®, ARDZ-REM®, IST-GLIO®, IST-REM® and control groups, four of five (80%) showed tubulointerstitial nephritis. The rates were 50% for the ROM-X® group and DVD-ARD® group were 20% respectively. The severity of inflammation was inconsistent within the groups. Mostly mild to moderate inflammation was observed but 1 in DVD-ARD®, IST-GLIO®, ROMX® and 2 in ARDZ-REM® and control group had severe tubulointerstitial nephritis respectively.

Table 2. Effects of products on AST, ALT and Chol parameters

BIOCHEMICAL PARAMETERS	GROUPS	MEAN	95% CONFIDENCE INTERVAL FOR MEAN		P VALUE
			Lower Bound	Upper Bound	
AST (IU/L)	Control	54.00±3.71	43.69	64.31	
	IST-ARD	48.60±11.90	15.55	81.65	1.00
	ARD-REM	50.25±7.44	26.56	73.94	1.00
	DVD-ARD	69.00±5.82	52.83	85.17	0.48
	KT-REM	52.00±5.85	33.40	70.60	1.00
	ARDZ-REM	44.60±3.49	34.92	54.28	0.89
	IST-GLIO	53.60±4.97	39.81	67.39	1.00
	IST-REM	52.80±6.97	33.44	72.16	1.00
	ROMX	58.75±6.77	37.19	80.31	1.00
	REGULIN	63.40±4.50	50.90	75.90	0.89
ALT (IU/L)	Control	22.00±2.07	16.24	27.76	
	IST-ARD	26.80±4.50	14.31	39.29	0.75
	ARD-REM	23.75±2.02	17.34	30.16	1.00
	DVD-ARD	21.40±2.09	15.60	27.20	1.00
	KT-REM	23.25±2.25	16.09	30.41	1.00
	ARDZ-REM	25.00±2.77	17.30	32.70	0.97
	IST-GLIO	24.40±1.36	20.61	28.19	0.99
	IST-REM	21.80±1.93	16.43	27.17	1.00
	ROMX	17.50±3.86	5.21	29.79	0.85
	REGULIN	23.00±3.08	14.44	31.56	1.00
CHOL (mg/dL)	Control	103.40±10.16	75.20	131.60	
	IST-ARD	83.00±6.96	63.66	102.34	0.33
	ARD-REM	91.25±4.27	77.66	104.84	0.88
	DVD-ARD	100.20±6.37	82.50	117.90	1.00
	KT-REM	97.75±5.51	80.20	115.30	1.00
	ARDZ-REM	103.80±11.21	72.67	134.93	1.00
	IST-GLIO	108.60±11.70	76.12	141.08	1.00
	IST-REM	82.40±4.45	70.06	94.74	0.30
	ROMX	91.75±4.99	75.87	107.63	0.90
	REGULIN	71.00±3.70	60.72	81.28	0.03*

*p<0.05 in comparison to control

Table 3. Histopathological findings in the liver, heart and kidney of *Mus musculus* exposed to food supplements

Food Supplements	Concentrations	Liver Mean±SE			Heart Mean±SE		Kidney Mean±SE		p Value for TBL
		Fatty change	Spotty necrosis	Inflammation	Atrial dilatation	Inflammation	CJ	TBL	
Control	1 ml/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	0.60±0.40	
ARD-REM	24.85 mg/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	0.00±0.00	0.136
DVD-ARD	22.3 mg/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	0.40±0.40	0.606
KT-REM	27.4 mg/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	1.50±0.28	0.121
ARDZ-REM	24 mg/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	1.40±0.50	0.228
IST-GLIO	31.7 mg/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	0.60±0.24	0.817
IST-REM	58.3 mg/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	1.00±0.31	0.371
ROMX	18.3 mg/kg	0.00±0.00	0.00±0.01	0.00±0.02	0.00±0.03	0.00±0.04	0.00±0.00	1.00±0.54	0.572

TBL: perivascular tubulointerstitial lymphoplasmacytic cell infiltration; CJ : corticomedullary junction

Table 4. Histopathological findings in the liver, heart and kidney of *Mus musculus* exposed to food supplements.

Food Supplements	Concentrations	Liver			Heart		Kidney	
		Fatty change	Spotty necrosis	Inflammation	Atrial dilatation	Inflammation	TBL	CJ
Control	1 ml/kg	0	0	0	0	0	0.60	0
ARD-REM	24.85 mg/kg	0	0	0	0	0	0.00	0
DVD-ARD	22.3 mg/kg	0	0	0	0	0	0.40	0
KT-REM	27.4 mg/kg	0	0	0	0	0	1.50	0
ARDZ-REM	24 mg/kg	0	0	0	0	0	1.40	0
IST-GLIO	31.7 mg/kg	0	0	0	0	0	0.60	0
IST-REM	58.3 mg/kg	0	0	0	0	0	1.00	0
ROMX	18.3 mg/kg	0	0	0	0	0	1.25	0

TBL: perivascular tubulointerstitial lymphoplasmacytic cell infiltration; CJ : corticomedullary junction

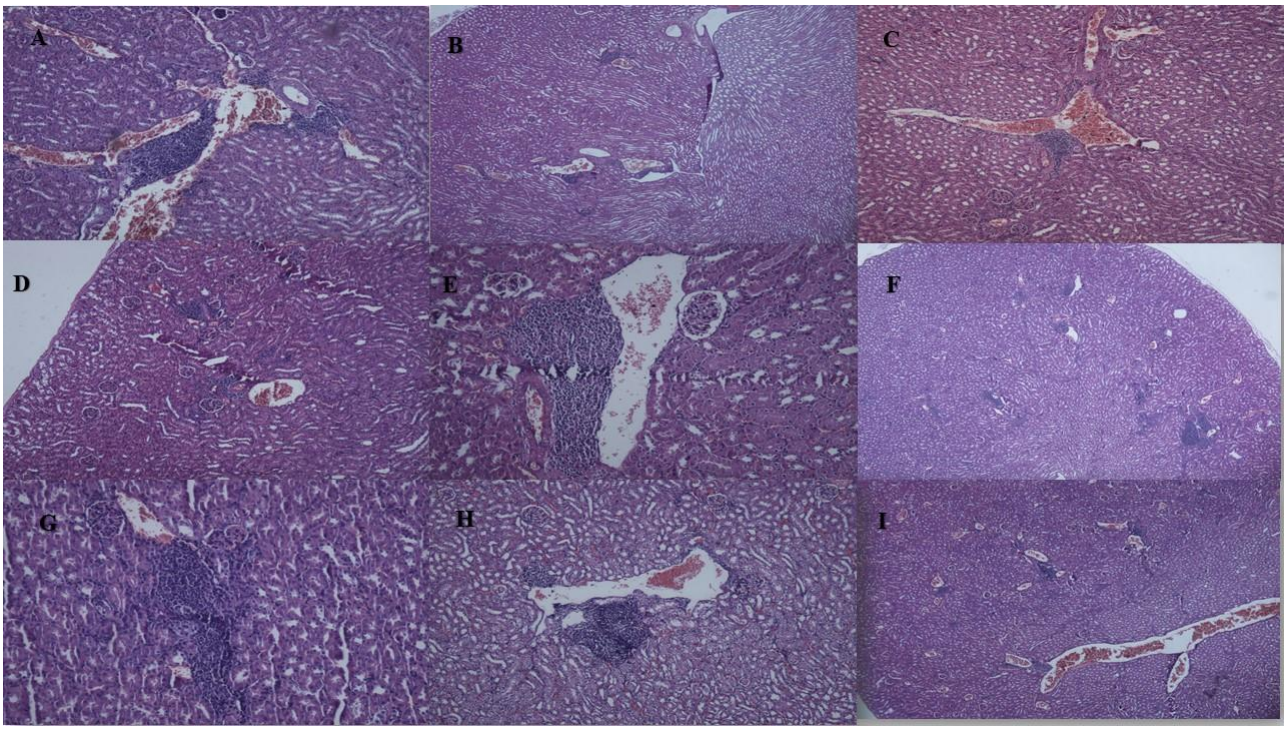


Figure 1. In cortex and corticomedullary junction of the kidneys, perivascular mononuclear inflammatory cells observed in the tubulointerstitial area. (**A:** KBx10, **B:** Control group x4, **C:** Control group x10, **D:** DVD-ARDx10, **E:** KT-REMx20, **F:** ARDZ-REM x4, **G:** IST-GLIOx20, **H:** IST-REM x10, **I:** ROMXx4)

DISCUSSION

Nowadays, it is important to test the final forms of the products such as supplementary foods, which are increasing in use, without being presented for consumption. It is possible that these products, which are thought to be harmless because they consist of natural products, have harmful effects. There are many active ingredients in the content of these products. *Curcuma longa*, *Zingiber officinale*, *Juniperus communis*, *Thymus vulgaris*, *Silybum marianum*, *Urtica dioica*, *Rosmarinus officinalis*, *Nigella sativa* , etc. natural products are created using many plants.

Curcuma longa has an effect that enhances the immune system, reduces or eliminates the risks in diseases such as diabetes and heart diseases that seriously affect human life. It is good for liver diseases [12]. It contributes to the cleaning of toxins in the body. It speeds up the blood circulation and maintains the body temperature. It reduces the risk of getting rheumatological conditions such as arthritis. It is a good antidepressant. It helps to lose weight. In addition, its effects on colorectal cancer have been found effective in a study on rodents [13]. In the study with rats, the LD_{50} value was determined as 1000mg/kg [14].

Zingiber officinale has antioxidant properties owing to the polyphenols it contains [15]. In the study conducted by Zarringhalami et al. (2020), polyphenols have been shown to have cytotoxic effects on osteosarcoma cells [16]. Antiinflammatory, antiapoptotic, antihyperglycemic properties have been found [17]. Studies have shown that orally administered ginger has an antithrombotic effect. In diabetes studies, it has been shown to protect against lipid peroxidation. Besides in the study performed on the transgenic mouse, Alzheimer disease was found to suppress. It significantly reduced symptoms in stomach ulcers [18–21]. The toxic dose in mice was calculated to be 1551 ± 75 mg/kg [22].

Juniperus communis is a potent antioxidant and anti-inflammatory, antidiarrheal, antiseptic, astringent effects [23,24]. It has been demonstrated in studies that it has resisted respiratory disorders,

cough, bronchitis, asthma, and abdominal ailments [23]. Besides it was found that chemotherapeutic drugs reduce the side effects [12]. In the study of mice, the LD_{50} dose was found as 3000mg/kg [25]

Studies on *Thymus vulgaris* have been found to have antimicrobial, antitussive effects. In the LD_{50} study using *Thymus* essential oil, the dose in mice was found to be 4000mg/kg [26].

Silybum marianum contains silymarin and flavonoids. flavonoids are compounds with high antioxidant properties. They also show protective effects for liver cells [27]. The fact that it has been used in fungal poisonings, liver, and bile diseases throughout history supports its hepatoprotective properties. Herbal extracts have been shown to lead to apoptosis in studies on cell culture lung tumor, bladder cancer, and breast cancer cells [28]. In the lethal dose test in rats, the LD_{50} value was found to be 10000 mg/kg [29].

Urtica dioica contains different components such as lignans, polysaccharides, and lectins. There are studies in which these components prevent prostate enlargement. It has anti-inflammatory properties and prevents cell growth. It is known to be used in the treatment of mouth sores, as a spring cure, to stop bleeding, to relieve anemia, to treat hay fever, psoriasis, sciatica, diarrhea, urticaria, rheumatism and prostate [30]. The LD_{50} dose detected in mice was calculated to be 3625 mg/kg [31].

Rosmarinus officinalis has positive effects on the immune system and its antiviral, antibacterial, and antioxidant properties have been demonstrated by studies [32]. In the study with essential oils in rats, the LD_{50} dose was determined to be 5500mg/kg [33].

It is known that *Linum usitatissimum* seed is used in the treatment of colon, breast, prostate cancer types, diarrheal disease and shows positive results [19,33]. It also provided significant reductions in blood cholesterol and LDL levels [33].

There was no difference in clinical observation between the control group and the application groups performed in the experimental process. These results are supported by biochemical and histopathological evaluations. In terms of biochemical parameters, no statistically significant difference was found between the application group and the control group, except for the REGULIN® group. For only cholesterol, the value of the control group is higher than the REGULIN® applied group. In other groups, the level of cholesterol is low or not statistically significant compared to the control group. It is a positive development that the cholesterol is statistically significantly lower as a result of the single-dose administration of the REGULIN group. Both ALT and AST values of the application groups were not statistically different when compared with the values of the control group. This result suggests that the determined doses of the products with acute toxicity studies were below the LD_{50} doses specified in the literature; there is no anti-synergistic effect and no toxic effect among the plants with the mixture.

Acute toxicity effects of the mentioned food supplement products were not found according to the data obtained during the experiment and after the experiment, observed clinical symptoms, biochemical parameter values and gross pathology findings. In addition to the absence of any acute toxicity, these products have positive effects. With detailed histopathological evaluation, none of the animals showed acute toxicity findings in the liver and heart. Tubulointerstitial nephritis was observed in the 23 of 39 samples' kidneys with a variety of severity. But these findings were also seen in the control group commonly so there is not enough data to interpret this particular pathological finding as evidence of toxicity.

According to the obtained results in the study, the acute toxicity effect of the food supplements called IST-ARD®, ARD-REM®, DVD-ARD®, KT-REM®, ARDZ-REM®, IST-GLIO®, IST-REM®, ROMX®, and REGULIN® was not determined. May be further studies with bigger numbers of samples investigating the nephrotoxic effects of these products lighten this matter. It is necessary to plan new studies to identify cellular signaling pathways to elucidate the positive effects of these products.

REFERENCES

1. Atalay D, Selen H, Abant E, et al. Gıda Takviyeleri ve Sağlık Üzerine Etkileri. 2018;4(2):98-111. doi:10.3153/FH18010
2. T.C. Tarım ve Orman Bakanlığı. *Türk Gıda Kodeksi Takviye Edici Gıdalar Tebliği (Tebliğ No:2013/49)*. Türkiye; 2013:4. https://www.resmigazete.gov.tr/eskiler/2013/08/20130816-16.htm.
3. Lange D. Europe's medicinal and aromatic plants: their use, trade and conservation. *Eur Med Aromat plants their use, trade Conserv*. 1998. https://www.cabdirect.org/cabdirect/abstract/19980314129. Accessed October 1, 2018.
4. Sharma M, Sharma R. Synergistic Antifungal Activity of *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger) Essential Oils Against Dermatophyte Infections. *J Essent Oil Bear Plants*. 2011;14(1):38-47. doi:10.1080/0972060X.2011.10643899
5. Yasar M. The remember regeneration therapy method: An overview of new therapy protocol to approach diseases. *J Complement Med Res*. 2019;10(1). doi:10.5455/jcmr.20181229122909
6. Zhao FR, Mao HP, Zhang H, et al. Antagonistic effects of two herbs in Zuojin Wan, a traditional Chinese medicine formula, on catecholamine secretion in bovine adrenal medullary cells. *Phytomedicine*. 2010;17(8-9):659-668. doi:10.1016/j.phymed.2009.10.010
7. Monga M, Sausville EA. Developmental therapeutics program at the NCI: Molecular target and drug discovery process. *Leukemia*. 2002;16(4):520-526. doi:10.1038/sj.leu.2402464
8. Anonymous. ISO 10993-11:2017(en), Biological evaluation of medical devices — Part 11: Tests for systemic toxicity. International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:10993:-11:ed-3:v1:en. Published 2017. Accessed March 30, 2020.
9. Saygı Ş. Deneysel Toksikolojide Toksisite Testleri ve Test Sonuçlarının Önemi. *Gülhane Tıp Derg*. 2003;45(3):291-298. http://cms.galenos.com.tr/Uploads/Article_32654/GMJ-45-291-En.pdf. Accessed March 31, 2020.
10. Anonymous. ISO 10993-2:2006 Biological evaluation of medical devices — Part 2: Animal welfare requirements. International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:10993:-2:ed-2:v1:en. Published 2015. Accessed March 30, 2020.
11. Anonymous. ISO 10993-12:2012 Biological evaluation of medical devices — Part 12: Sample preparation and reference materials. International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:10993:-12:ed-4:v1:en. Published 2012. Accessed March 30, 2020.
12. Kusari S, Lamshöft M, Spiteller M. *Aspergillus fumigatus* Fresenius, an endophytic fungus from *Juniperus communis* L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. *J Appl Microbiol*. 2009;107(3):1019-1030. doi:10.1111/j.1365-2672.2009.04285.x
13. Fernandez A, Edwin Cock I. The Therapeutic Properties of *Juniperus communis* L.: Antioxidant Capacity, Bacterial growth Inhibition, Anticancer Activity and Toxicity. *Pharmacogn J*. 2016;8(3):273-280. doi:10.5530/pj.2016.3.17
14. Pandey G. Active principles and median lethal dose of Curcuma longa Linn. *Artic Int Res J Pharm*. 2014;2(5):239-241.
15. Kazeem MI, Akanji MA, Yakubu MT, Omotayo A, Ashafa T. Protective Effect of Free and Bound Polyphenol Extracts from Ginger (*Zingiber officinale* Roscoe) on the Hepatic Antioxidant and Some Carbohydrate Metabolizing Enzymes of Streptozotocin-Induced Diabetic Rats. *Evidence-Based Complement Altern Med*. 2013;2013. doi:10.1155/2013/935486
16. Zarringhalami R, Hanachi P, Kaya E, Ağan AF, Ağan K, Donmez M. Investigation of total phenolic content of *Tilia dasystyla* and *Polygonatum orientale* desf extracts and their cytotoxic effect on the osteogenic sarcoma (Saos-2) cancer cell line. *Int J Cancer Manag*. 2020;13(2). doi:10.5812/ijcm.94130
17. Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *J Ethnopharmacol*. 1989;27(1-2):129-140. doi:10.1016/0378-8741(89)90085-8
18. Lim GP, Chu T, Yang F, Beech W, Frautschy S a, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neurosci*. 2001;21(21):8370-8377. doi:21/21/8370 [pii]
19. Palla AH, Khan NA, Bashir S, ur-Rehman N, Iqbal J, Gilani A-H. Pharmacological basis for the medicinal use of *Linum usitatissimum* (Flaxseed) in infectious and non-infectious diarrhea. *J Ethnopharmacol*. 2015;160:61-68. doi:10.1016/J.JEP.2014.11.030
20. Sharma RA, Mclelland HR, Hill KA, et al. Pharmacodynamic and Pharmacokinetic Study of Oral Curcuma Extract in Patients with Colorectal Cancer Pharmacodynamic and Pharmacokinetic Study of Oral Curcuma Extract in Patients with Colorectal Cancer 1. 2001;7(July):1894-1900.
21. Jurenka JS, Ascp MT. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern Med Rev*. 2009;14(2):141-153.
22. Ojewole JAO. Analgesic, antiinflammatory and hypoglycaemic effects of ethanol extract of *Zingiber officinale* (roscoe) rhizomes (zingiberaceae) in mice and rats. *Phyther Res*. 2006;20(9):764-772. doi:10.1002/ptr.1952
23. Bais S, Gill NS, Rana N, Shandil S. A Phytopharmacological Review on a Medicinal Plant: *Juniperus communis*. *Int*

Sch Res Not. 2014:1-6. doi:10.1155/2014/634723
24. Manel M, Nouzha H, Rim M, et al. Antibacterial and antioxidant activity of *Juniperus thurifera* L. leaf extracts growing in East of Algeria. *Vet World*. 2018;11(3):373-378. doi:10.14202/vetworld.2018.373-378
25. Bais S, Gill NS, Rana N. Effect of *Juniperus communis* Extract on Reserpine Induced Catalepsy. *Inven Rapid Ethnopharmacol*. 2014;2014(4).
26. Fachini-Queiroz FC, Kummer R, Fernanda Estevão-Silva C, et al. Effects of Thymol and Carvacrol, Constituents of *Thymus vulgaris* L. Essential Oil, on the Inflammatory Response. *Artic ID*. 2012;2012. doi:10.1155/2012/657026
27. Taşdemir B. Ratlarda İndometazinle Oluşturulan gastrik Ülser Üzerine Silimarinin Koruyucu Etkilerinin Araştırılması. 2016. http://adudspace.adu.edu.tr:8080/xmlui/bitstream/handle/11607/2904/BARIŞ TAŞDEMİR.pdf?sequence=1&isAllowed=y. Accessed March 31, 2020.
28. Bayram D. Silibinin İn Vitro Ortamda CCL-228-SW 480 Kolon Kanseri Hücre Hattı Üzerine Apoptotik Etkisinin Araştırılması. *SDÜ Sağlık Bilim Derg*. 2017;8(2):1-1. doi:10.22312/sdusbed.287465
29. Fraschini F, Demartini G, Esposti D. Pharmacology of silymarin. *Clin Drug Investig*. 2002;22(1):51-65. doi:10.2165/00044011-200222010-00007
30. Oktay M. *Kabalcı Şifalı Bitkiler Ansiklopedisi*. Vol 4. 2nd ed. İstanbul: Kabalcı Yayınevi; 2016. https://www.kabalci.com.tr/alternatif-tip-sifali-bitkiler/kabalci-sifali-bitkiler-ansiklopedisi/oktay-mete/kabalci-yayinevi.
31. Joshi BC, Mukhija M, Kalia AN. Pharmacognostical review of *Urtica dioica* L. *Int J Green Pharm*. 2019;8(4):23-30.
32. Malayoğlu HB. Biberiyenin (*Rosmarinus officinalis* L.) Antioksidan Etkisi. *Hayvansal Üretim*. 2010;51(2).
33. Jhala AJ, Hall LM. Flax (*Linum usitatissimum* L.): Current Uses and Future Applications. *Aust J basic Appl Sci*. 2010;4(9):4304-4312.

Volume:1 Issue:1
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

Investigation of Hot Spring Awareness Between Doctors Faculty of Medicine in Düzce University

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Abstract

Objective: Hot spring medicine and hot spring tourism has been increasingly popular in the world and our country for the last 50 years. People in our country have been in search of natural and traditional methods to relieve their discomfort and contribute to their health during their holidays as in the rest of the world. Awareness and knowledge levels of physicians on this subject is important to suggest hot springs individually within the scope of other traditional and complementary medicine and develop internal tourism (health tourism) movements. The aim of this study is the determination of the opinions, knowledge and awareness levels of the physicians working on Düzce University Faculty of Medicine.

Material-Method: The data were collected by survey technique. In the research, a questionnaire form was distributed to all physicians working in the faculty and 72 physicians agreed to fill out the questionnaire. 69 of them were found suitable for evaluation. The survey includes a total of 22 statements apart from demographic questions. The expressions used are five-point Likert scale (I agree-I don't agree). The statements are generally aimed at determining the awareness of physicians about hot spring treatments.

Results: Approximately one-third of the physicians have never been to a hot spring before; almost all of them declared that they do not recommend hot spring treatment to any of their patients. Almost all of them wish to be educated on this subject and consciously recommend and prescribe their patients. It is understood that the majority of physicians lack information about hot spring medicine, use, and awareness.

Conclusion: Almost all physicians have the demand to have information on this subject. There is consensus on increasing scientific research on the subject and ensuring its integration in modern medicine.

Keywords: Hot Spring, Physician, Awareness, Survey

INTRODUCTION

Hot spring has been used in the field of health as a traditional and complementary medicine practice for thousands of years all over the world. As it is accepted in our country, the concept of "Health Tourism" is generally divided into two main areas: Medical Tourism and Hot Spring Tourism[1].

In hot spring tourism, hot spring visits are observed both for protecting and maintaining the health of local tourists in the country and for seeking support for the treatment of their diseases and it is also observed that many people from abroad try to protect and treat their health within natural ways in different cultures[2]. Accordingly, countries have increased their investment and R&D (Research and development) activities. Health and wellness tourism grows 12% annually with changing global demand[3]. Because it has been determined that tourists visiting the countries for health purposes spend money 4 times more than people who visit for sightseeing[4]. Yurdakul and Özgencil (2017), according to the results of the econometric modeling forecasts they developed, an increase in the number of visitors to our country for "health and medical reasons (health tourism)" increased the income from health tourism by 962 dollars on average; the contribution of other alternative tourism types to income is lower[5]. Getting more shares in this type of tourism depends on providing

world-class and quality health services where the number of participants and the income from these countries are increasing[6].

The person who gets the service, regardless of the nation, is a person of all ages and genders who are trying to retain or regain his health. In this regard, this field, which is in direct contact with people and directly related to human health, brings the need for well-equipped healthcare professionals with sufficient knowledge and experience. The subject of the hot spring is not included in the education curriculum of both our allied health personnel and general practitioners or specialists in our country. This is the most important obstacle for our hot spring tourism with 2023 target of 500.000 qualified beds[7].

As a definition or active employee of a certified "hot spring cure physician" is not available in our country instead of existing almost worldwide. This situation stands out as a deficiency in the health examinations of people who try to use the hot springs with traditional methods. The same situation exists in businesses that have to diversify products and earn money in the hot spring sector. Besides, tour operators who will bring tourists to our country for health purposes should also have sufficient knowledge and equipment. It arises as a result of not having hotels and hot springs without certified staff in their catalogs.

It is inevitable to have a certain standard of institutions, buildings and employees to be entrusted in this sector whose basic service target is human. One of the primary reasons for the development of hot spring tourism is that these standards are not known and implemented in our country, which is evident on a world scale. Unfortunately, those who manage the hot spring hotel and facility, the staff serving the facility, those who manage the city or those who serve in the city do not know enough about hot spring medicine and hot spring tourism[8].

The utilization of hot springs is a ritual known in our geography for thousands of years. The first hospital in the world Asklepion for treatment patients with water in Bergama is also the place where two famous physicians such as Hippocrates and Galen grow up and treat patients. The Turkish Baths, which have become a world brand after the Roman baths commonly used in Anatolia, also used hot spring water. It is known that people seeking cleaning and healing have used these baths for centuries[9].

Our hot springs with such an ancient history and tradition are not recommended to patients because they are not known, used, and their mechanism of use and methods of use are not known by general practitioners and specialists working in those regions. This situation results from the fact that our people either cannot benefit from local hot springs or use empirically[10].

Since the operators of the hot springs do not have sufficient knowledge and equipment, in-service training is not possible and a satisfactory answer cannot be given to the questions from the customer. Moreover, there are no application centers or sources for business owners and managers.

Based on all these facts, it is aimed to make a survey among our physicians serving at all levels in the field of hot spring medicine and hot spring tourism and to evaluate the knowledge and awareness of our physicians on the subject.

MATERIALS AND METHODS

In the study, field research methods and survey techniques were used. The target population of the study is composed of physicians from the Faculty of Medicine at Düzce University. The reason for choosing physicians of this faculty is the existence of hot springs in the related city. There are two hot spring sources known in Düzce. Efteni Hot spring, located on the edge of Lake Efteni, has been inactive for several years. However, Derdin hot spring is active and it is still possible for our people too. It is used as a mineral spring cure.

The convenience sampling method was used for data collection. The surveys were applied to physicians in 2019 March. It has been featured to fill in the distributed surveys by 72 willing physicians. At the time of the study 256 physicians work at the faculty. Three surveys due to missing markings have not been evaluated. 69 questionnaires were included in the assessment.

Afterward, it is planned to repeat this survey in ten districts with the contributions of the Ministry of Health, General Directorate of Public Health.The applied questionnaire is as follows (Table).

Table 1. Knowledge scale of physicians for hot springs, treatments, and developments

	Absolutely disagree	Disagree	Agree/ Disagree	Agree	Absolutely agree
I have information about thermo-mineral water	①	②	③	④	⑤
I have information about the effect mechanisms of the hot spring water	①	②	③	④	⑤
I am knowledgeable about the benefits of the thermo-mineral water	①	②	③	④	⑤
I know the contraindications to the use of the thermo-mineral water cure	①	②	③	④	⑤
I have information about the duration of the classic thermo-mineral water cure	①	②	③	④	⑤
I am knowledgeable about regular hot springs use and healthy living connection	①	②	③	④	⑤
I have information about the hot springs in this region	①	②	③	④	⑤
I have information of hot springs legislation	①	②	③	④	⑤
I know the procedure of patient referral to the hot springs	①	②	③	④	⑤
I have information about thermo-mineral water treatment cures (Balneotherapy, thalassotherapy etc.)	①	②	③	④	⑤
I have information about emergencies (thermal crisis etc.) that may occur during the thermo-mineral water treatment	①	②	③	④	⑤
I follow the developments about thermo-mineral water treatment	①	②	③	④	⑤
Combination of traditional and complementary medicine (T&CM) and thermo-mineral water cures is more effective than T&CM treatment alone.	①	②	③	④	⑤
The use of hot springs for therapeutic purposes increases patient satisfaction.	①	②	③	④	⑤
Thermo-mineral water treatment can help fight diseases.	①	②	③	④	⑤
The use of the hot springs provides health and wellness.	①	②	③	④	⑤
Doctors should be more trained in the use of hot springs.	①	②	③	④	⑤
I support the inclusion of T&CM methods such as hot spring use in the medical curriculum.	①	②	③	④	⑤
Integrating the thermo-mineral water treatment into the health system improves patient care.	①	②	③	④	⑤
I support the use of thermo-mineral water cures like medicine treatment.	①	②	③	④	⑤
Research should be conducted on the effectiveness and safety of thermo-mineral water cures.	①	②	③	④	⑤
It is beneficial for patients to build health centers where thermal and other traditional and complementary medicine methods are used together.	①	②	③	④	⑤

The first scale used in the study was created by the researcher to measure how well the physicians know about the hot spring and related treatment methods and developments in this area (Table 1). The scale used in the second part was created by making use of the
scales made before, which measure the perspectives of physicians on traditional medicine and treatment approaches. The scale is based on the perspectives of physicians on using hot springs as a comlementary and traditional treatment method (Table 7).

Statistical analysis

SPSS 22.0 (Statistical Package for the Social Sciences - Statistical Program for the Social Sciences) was used to analyze the survey data obtained from a total of 69 participants. As a result of the evaluation, the descriptive data and attitudes of the participants were determined by frequency analysis.

RESULTS

The survey was prepared to be applied to 256 physicians working at Düzce University Faculty of Medicine. Although it was aimed to reach all physicians, 69 physicians were included in the study.

The expressions other than demographic questions were arranged at a certain interval from “Absolutely Agree” to “Absolutely Disagree” by structuring on a 5-point Likert scale. The data obtained in the study were collected through face

to face questionnaires. The questionnaires were distributed to 72 physicians and 69 of them were evaluated as usable.

The results obtained from the study are shown in the frequency distribution table. The distribution of participants by gender is shown in Table 2. Accordingly, 33 of the participants are women and 35 are men, and 1 person left the gender part blank. Most of the participants are have university and postgraduate degrees in terms of their educational status, and the distribution of these two groups is very close to each other. The distribution of physicians by level of speciality is demonstrated in Table 3. The majority of the participants are between the ages of 30-39 and it is seen that they are in the first five years of working life. In the questionnaire, the physicians were asked whether they had been to the hot spring before. The results are given in Table 4. More than half of them have been to the hot spring before. The percentage of physicians who recommend the hot spring to their patients is shown in Table 5.

Table 2. Descriptive information of the participants

	Number	Percentage
Male	35	50.70
Female	33	47.80
Null	1	1.40
Totaly	69	100.00

Table 3. Distribution of physicians by level of speciality

	Number	Percentage
Practitioner	2	2.90
Asisstant	41	59.40
Specialist	24	34.80
Intern	1	1.40
Null	1	1.40
Totaly	69	100.00

Table 4. Have you been to the hot spring before?

	Number	Percentage
Null	2	2.90
Yes	41	59.40
No	26	37.70
Totaly	69	100.00

Table 5. Did you recommend thermo-mineral water treatment to your patients?

	Number	Percentage
Null	2	2.90
Yes	10	14.50
No	57	82.60
Totaly	69	100.00

Note: While evaluating the tables below, for example, the answer given to "I have information about thermo-mineral water" was found to be 2,3971. This 1 absolutely disagree, 5 strongly agree; it does not correspond to disagree with or disagree. In other words, they do not think that they have information about thermo-mineral waters. Other expressions can also be interpreted in this way. The hot spring existence and awareness survey of physicians is given in Table 6. Also the perspective of physicians to the hot springs is shown in Table 7.

Table 6. Current state of physcians' to existence and awareness of hot springs (average values)

	Number	Average	Standart Deviation
I have information about thermo-mineral water	68	2.3971	1.16080
I have information about the effect mechanisms of hot spring waters	69	2.3623	1.18782
I am knowledgeable about the benefits of the thermo-mineral water	68	2.6471	1.26685
I know the contraindications to the use of the thermo-mineral water cure	68	2.7941	1.29935
I have information about the duration of the classic thermo-mineral water cure	69	2.2029	1.24357
I am knowledgeable about regular hot spring use and healthy living connection	69	2.3768	1.17770
I have information about the hot springs in this region	68	2.3824	1.25816
I have knowledge of the hot spring legislation	69	1.7246	1.02733
I know the procedure of patient referral to the hot springs	69	1.6957	.98972
I have information about thermo-mineral water treatment cures (balneotherapy. thalassotherapy etc.).	69	1.8986	1.20226
I have information about emergencies (thermal crisis etc.) that may occur during the thermo-mineral water treatment	69	2.2464	1.29939
I follow the developments about thermo-mineral water treatment	69	2.0290	1.13722

Table 7. Perspective of physicians on the therapeutic properties of hot springs

	Number	Average	Standart Deviation
The method in which traditional medicine and hot spring types are used is superior to traditional medicine treatment.	68	2.6324	1.07765
The use of hot springs for therapeutic purposes increases patient satisfaction	69	3.4203	1.11679
Thermo-mineral water treatment can help fight diseases	69	3.2899	.97168
Hot springs use provides health and wellness	69	3.3913	.98842
Doctors should be more trained in the use of hot springs	69	3.7971	1.09248
I support the inclusion of CAM methods such as the use of hot springs in the Medical curriculum	69	3.1594	1.09307
Integrating the thermo-mineral water treatment into the health system improves patient care	69	3.3623	.90702
I support the use of thermo-mineral water cures like medicine treatment	69	3.1884	1.11508
Research should be conducted on the effectiveness and safety of thermo-mineral water cures.	69	3.9130	1.10798
It is beneficial for the patients to build health centers where the hot spring and traditional medicine methods are used together.	69	3.5942	1.08897

DISCUSSION

To be able to respond to the increasing health tourism movement in the world and our country and to obtain high income from this type of tourism, a quality health service should be provided. In our country, there is a shortage of qualified, experienced, and certified personnel who are trained in the field of hot spring medicine and hot spring tourism both in public institutions and in the private sector. According to the results of this study and the applied questionnaire, our physicians have a lack of knowledge and awareness on this issue.

The first reason that causes this situation is the absence of hot spring medicine and tourism lessons in the curriculum of medical schools and vocational high school of health. The profession of "cure physician" working in the hot springs of countries where this type of tourism has developed does not exist in our country. In our country, the hot spring is perceived as a place where only physical therapy is carried out, other specialties in the indication of water and preventive medicine are ignored, and the problems are dragged into deadlock by the imposition of certain professional organizations. Besides, physiotherapists who want to be brought to the hot springs for management do not receive hot spring medicine training during their specialization.

CONCLUSION

When this situation is examined in detail, considering our solution suggestions listed below may be beneficial for the development of our country's hot spring medicine and tourism movements:

1) The inventory of the hot springs should be urgently prepared with an existing facility and capacity based classification.

2) A certification training should be organized for physicians who will work in the hot springs, and

the cure physician should be defined professionally.

3) Hot spring medicine and one-month hot spring rotation should be added to the specialist training curricula of all specialist physicians.

4) With the participation of our universities working directly with hot springs, " The Platform of Universities with Hot Spring" should be established and our universities should be supported in training educated personnel, R&D and standardization.

5) All primary care physicians working in the public sector and physicians working in public hospitals should be subjected to in-vocational training related to the hot springs within their duty areas and encouraged healthy and sick individuals to benefit from the hot springs in the population they are responsible for.

6) Scientific researches on hot springs and hot spring tourism should be encouraged and the scientific contributions of our universities should be increased.

The studies to be carried out in line with the given suggestions will contribute to increase the knowledge and experience of physicians and healthcare professionals. It will be ensured that the potential of our country's hot spring and health tourism in this field will have its deserved in the international market with the employment of trained labor force needed in the hot spring facilities.

REFERENCES

1. http://www.saglikturizmi.org.tr/tr/saglik-turizmi/genel bilgi13/08/2019
2. Temizkan SP. Sağlık turizmi. *Ankara: Detay yayıncılık.* 2015 Ekim:1.baskı
3. https://www.businesswire.com/news/home/20180326005490/en/Global-Health-Wellness-Tourism-Report-2018.
4. Barca M, Akdeve İ, Gedik İ. Türkiye sağlık turizm sektörünün analizi ve strateji önerileri. *İşletme araştırmaları dergisi*. 2013;5 (3): 64-92.
5. Yurdakul F, Özgencil G. Türkiye'deki turizm gelirinin belirleyicileri ve alternatif turizm gelirlerinin önemi. *Erzincan Üniversitesi Sosyal Bilimler Enstitüsü Dergisi*. 2017; IV: 173-188.
6. www.saturk.gov.tr/images/pdf/tyst/02.pdf13/08/2019
7. Kültür ve Turizm Bakanlığı Türkiye Turizm Stratejisi (2023). Ankara: 2007.
8. Bahar O, Kozak M. Küreselleşme sürecinde uluslararası turizm ve rekabet edebilirlik. *Ankara: Detay Yayıncılık.*2005.
9. Karagülle MZ, Doğan MB. Kaplıca tıbbı ve Türkiye kaplıca rehberi. *Ankara: Nobel Tıp Kitapevleri.* 2002. Tontus HÖ. Sağlık turizmi tanıtımı ve sağlık hizmetlerinin pazarlanması ilkeleri üzerine değerlendirme. *Disiplinlerarası Akademik Turizm Dergisi*. 2018; 3 (1): 67-88

Volume: 1 Issue: 1
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

Anticancer Effect of Food Supplements on Saos-2 Osteosarcoma Cell

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Abstract

Objective: Nowadays medicinal plants have been considered as complementary medicine in cancer treatment by many researchers. Osteosarcoma, one of the most common malignant tumors, which is more common at a young age; It is one of the most common types of cancer that occur in mesenchymal bone-forming cells and affect the musculoskeletal system. Today instead of cancer drugs due to their numerous side effects that can suppress or prevent cancer treatment, food supplement products that contain plant extracts may be preferred. In this study, the anticancer effects of five different food supplements were investigated on osteosarcoma Saos-2 cancer cell line.

Material-Method: Application doses were as 1000, 2000, 3000, 4000, 5000, 6000 µg/ml and IC50 values were calculated by creating two working groups of 24 and 48 hours.

Results: Extracts of five different herbal product showed cytotoxic effect on Saos-2 cancer cell line due to their mixture of medicinal plants, however REGULIN® showed the most cytotoxic effect among them.

Conclusion: It was determined that REGULIN® used in the remember regeneration therapy method (RTM), had the most anticancer effect in each of the 24 and 48 hour application groups. Cytotoxic effect of these food products on other cancer cell lines and their use in the pharmaceutical and health industries is recommended in future studies.

Keywords: Osteosarcoma, Anticancer, Saos-2, Food Supplement.

INTRODUCTION

Cancer is a serious health problem with a high mortality rate worldwide.[1]. Approximately 300,000 children die every year due to in childhood cancer. Osteosarcoma is primary bone cancer and one of the most common types of bone cancer in childhood and adolescence. Chemotherapy, surgery and postoperative chemotherapy were used as a standard treatment in the treatment of osteosarcoma [2–4]. Many different molecules are currently being investigated in cancer treatments, and the source of such molecules is products with herbal ingredients [5]. Although the mechanism of action of many medicinal plants is still uncertain, herbal products are widely used in traditional medicine as a supplement [6]. The combination of active phytochemicals from more than one plant shows a strong anticancer activity. There are many studies are proving this situation in Chinese medicine that medicinal plants can the improve quality of life in patients with bone cancer [7]. Single-drug approach was only for a period of time, however now the development of multi-drug and conventional pharmaceutical should be approached in the treatment strategy [6].

Food supplements are defined as products used to support substances which are missing in the diets of people such as minerals, vitamins, etc., [8]. Medicinal plants have been increasingly used as traditional and complementary medicine products from east to west. According to WHO, 21.000 plants are suitable for medication [9]. The widespread use of these products also increases the value of the market. It is known that foods have positive effects on human health. Therefore, the use of food supplements is increasing in societies. Urtica contains different components such as lignans, polysaccharides and, lectins. These components decline prostate enlargement. It has anti-inflammatory properties and prevents cancer cell growth. Juniper is a powerful antioxidant with high anti-inflammatory effect [10]. Studies have shown that it has a healing effect for diseases such as

respiratory diseases, cough, bronchitis and, asthma. In addition, chemotherapeutic drugs have been found to reduce the side effects [11]. It has been revealed that it has protective properties for the liver and kidneys. Turmeric has an effect that strengthens the immune system, reduces or eliminates the risks in diseases such as diabetes and heart diseases that seriously affect human life. It is effective for liver diseases [11]. It contributes to the cleaning of toxins in the body. It speeds up the blood circulation and maintains the body temperature. It reduces the risk of getting rheumatological conditions such as arthritis, it is an effective antidepressant, it helps to lose weight. In addition, studies show that it is effective on colorectal cancer in rodents [12]. Ginger has high antioxidant properties. It has been revealed that it has anti-inflammatory, anti-apoptotic, antihyperglycemic properties. Studies have shown that ginger given orally has an anti-thrombolytic effect. In diabetes studies, it has been shown to protect against lipid peroxidation. Also, in the study performed on the transgenic mouse, it was found that it suppresses Alzheimer disease, it has significantly reduced symptoms in stomach ulcers [13–16]. On the anticancer property of rosemary, it has been revealed in the literature that it has healing effects in many cell lines such as colon cancer, breast cancer, prostate cancer, cervical adenocarcinoma and ovarian cancer [17]. The effectiveness of *Peganum harmala*, which has been tested in cells such as leukemia, breast cancer, ovarian cancer and stomach cancer, has been demonstrated by previous studies [18–20]. DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN®, these food supplements with herbal products are used in remember regeneration therapy method (RTM) [21].

The aim of current study is to investigate the anticancer effect of food supplements formed with herbal products with anticancer properties on osteosarcoma.

MATERIALS AND METHODS

Preparation of herbal extracts

Five different herbal product that is being sold in the market in Turkey; DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN® were evaluated in our study (Table 1). Dry extracts of plants were obtained from Naturin Nutraceutical in Turkey. 1 g of dry extract was dissolved in 5 ml of water in a 70 °C water bath for 90 minutes. The extracts were then centrifuged at 2500 rpm for 20 minutes and their supernatant removed. The solution was diluted with DMEM (Dulbecco's Modified Eagle's Medium) culture medium at 1000, 2000, 3000, 4000, 5000, 6000 µg/ml using 0.22 mm filters for sterilization of the solution. Preferred dose ranges of this mixture are preferred over the dose ranges in which these herbal mixtures are known to be effective [22–25].

Table 1. Herbal product contents

Herbal Products	Product Contents
REGULIN®	*Curcuma longa extract* (118 mg), *Silybum marianum extract* (118 mg), *Rosmarinus officinalis extract* (118 mg), *Juniperus communis* extract (118 mg), *Fumaria officinalis extract* (59 mg), *Cichorium intybus extract* (59 mg)
IST-GLIO®	*Curcuma longa* (67 mg), *Curcuma longa seed* (237 mg), *Peganum harmala* (133 mg), *Silybum marianum* (89 mg), *Zingiber officinale* extract (74 mg), *Nigella sativa seed* (68 mg), *Juniperus communis* fruit (44 mg), *Thymus sp* (15 mg), *Foeniculum vulgare* (4 mg), *Pimpinella anisum* (3 mg), *Cassia acutifolia* (3 mg), *Eugenia caryophyllata* (3 mg).
ROMX®	*Curcuma longa* extract (44 mg), *Juniperus communis* extract (158 mg), *Zingiber officinale* extract (127 mg), *Peganum harmala* extract (114 mg), *Thymus sp.* extract (95 mg), *Nigella sativa* extract (38 mg), *Foeniculum vulgare* extract (19 mg), *Pimpinella anisum* extract (15 mg *Cassia acutifolia* extract (15 mg), *Eugenia caryophyllata* extract (15 mg).
IST-REM®	*Curcuma longa* extract (68 mg), *Urtica sp.* seed (272 mg), *Silybum marianum* seed (102 mg), *Peganum harmala* seed (102 mg), *Nigella sativa* extract (68 mg), *Zingiber officinale* extract (68 mg).
DVD-ARD®	*Curcuma longa* extract (118 mg), *Silybum marianum* extract (118 mg), *Rosmarinus officinalis* extract (118 mg), *Juniperus communis* extract (118 mg), *Fumaria officinalis* extract (59 mg), *Cichorium intybus* extract (59 mg).

Cell culture

Experimental study was carried out at Düzce University Experimental Animals Application and Research Center. In the study, Saos-2 cell line was obtained from Bolu Abant İzzet Baysal University. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma) with 10% Fetal Bovine Serum (Sigma) and 1% penicillin + streptomycin (Sigma) broth containing mixture, 37 ˚C in medium containing 5% CO_2 and 95% humidity 25 cm^2 incubated in flasks.

MTT cell viability test

3-(4,5-dimethyltriazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is frequently used as a method for determination of cell viability [26]. Viable cells are easily able to be determined by absorbance of color due to the mitochondria-dependent reaction. MTT reduction property of the cells and MTT assay results obtained in the color density correlates with the number of viable cells. 10 ml MTT (5mg/ml) was added to each well. Cells were incubated at 37 ˚C for 4 hours after treatment with MTT. By removing the supernatant from each well, 100 ml of DMSO was added to the wells and the plate was kept in the incubator for 10 minutes and absorbance was measured at 570 nm by using microplate arrow (Cytation ™ Biotek, USA). Each reading was calculated according to the absorbance x 100/control absorbance formula of the cells. IC 50 values were calculated using the curve by plotting the percentage of extracted different concentrations and cell viability.

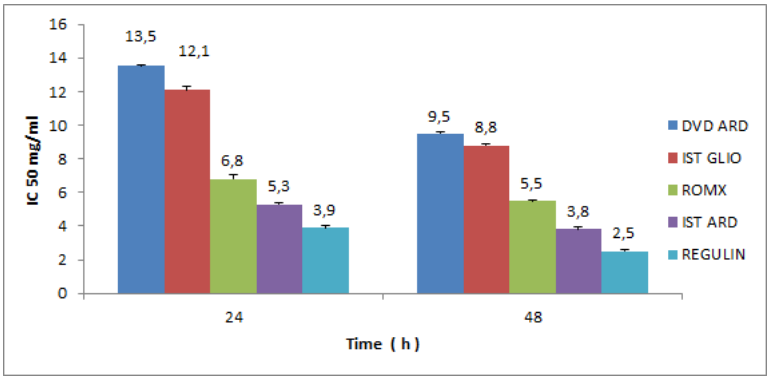
Statistical analysis

Statistical analysis was performed using ANOVA test by SPSS version 24 program and (P value<0.05) was regarded as significant. Experiments were performed in triplicate and expressed as the means ± standard deviation. Experiments were performed in triplicate and expressed as the means ± standard deviation values in each column showed significant differences (P<0.05).

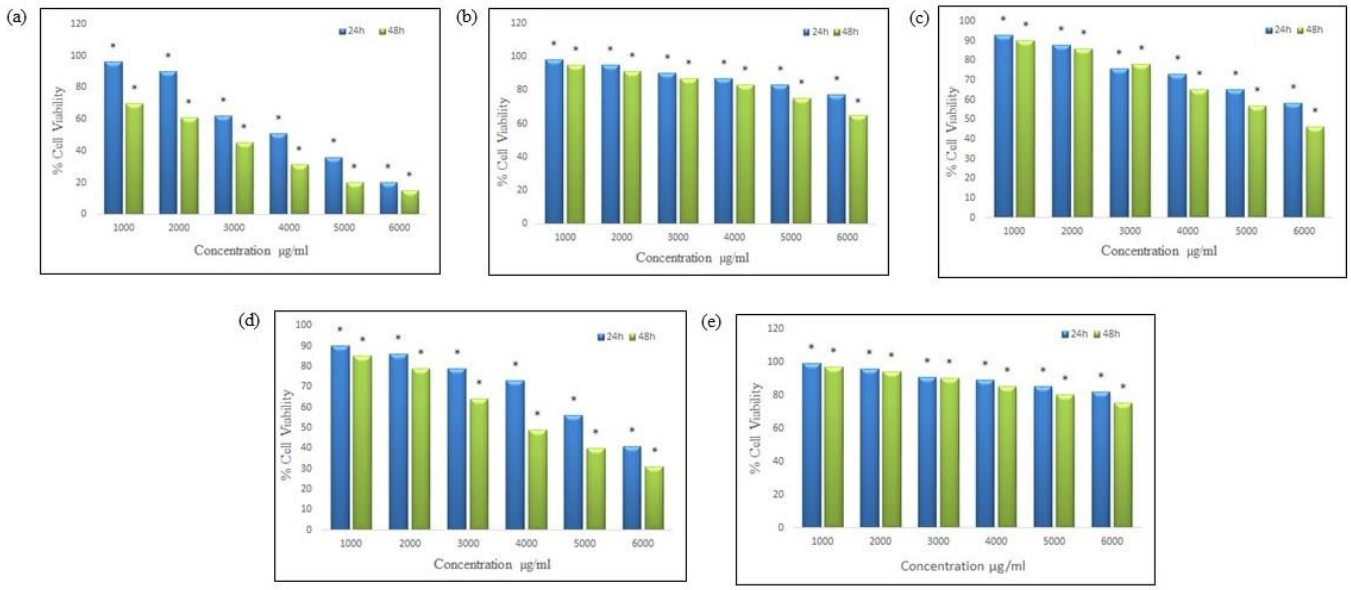
RESULTS

According to the results of graphic 1 the lowest IC_{50} values that shows the most effective extracts belonged to REGULIN® extract at the concentration of 6000 µg/ml, within 48h which was about IC_{50} = 2.5 ± 0.001 mg/ml with 15% cell viability of Saos-2 cells.

The effect of various concentrations of extracts on proliferation of Saos-2 cells within 24 and 48 hours was evaluated by MTT assay. According to the data statistical analysis of Graphic 2, extracts showed a dose-time dependent inhibition of cell viability which means cytotoxic effect of extracts increased with increasing concentrations of extracts also bypassing exposure time of the cells to the extracts. The most cytotoxic effect of extracts belonged REGULIN®>IST ARD®>ROMX®>IST GLIO®>DVD ARD® respectively.



Graphic 1. Mean IC50±SD (mg/ml) values of Saos-2 cell lines treated with different concentrations of DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN® extracts (1-6 mg/ml) for 24 and 48 h.



Graphic 2. Cytotoxic effect of (a) REGULIN®, (b) IST GLIO®, (c) ROMX®, (d) IST ARD®, (e) DVD ARD® (1000-6000 µg/ml) on cell viability of Saos-2 cancer cell line for 24 and 48 h. * In each column indicates significant differences between concentration of 1000-6000 µg/ml ($p<0.05$).

DISCUSSION

Osteosarcoma is a rare but highly malignant bone cancer that occurs in the skeletal system and generally affects children, adolescents and young adults [27]. Current treatments for osteosarcoma include chemotherapy, surgical interventions, or radiotherapy, but these treatments cannot provide adequate survival [28]. In our study, the anticancer effect on osteosarcoma cells was investigated by using food supplements with different herbal ingredients. IC50 values were calculated on Saos-2 cells. Among the five products that we evaluated their effectiveness, REGULIN® was the highest mixture with the IC50 value of 2.5 mg/ml.

There are various plants are that known to have anticancer effects in the content of each of the five different mixtures we used in our study. The anticancer properties of many of these plants have been proven and there is no study comparing the synergistic effects of herbal mixtures available in the market today, but the bioactive compounds of each plant are being investigated on various cancer cells. Plants contain secondary metabolites such as phenol and flavonoids with antioxidant properties, which have strong potential to clear free radicals to prevent diseases such as cancer [27]. There are various extracts that known to have anticancer effects in the content of each of the five different mixtures we used in the study. Anticancer activity of n-hexane turmeric extract from these extracts has been frequently demonstrated. The effect of turmeric on lung cancer was evaluated in the study in which telomerase activity was evaluated and its potential to be converted into drugs [29]. The anticancer effect of turmeric (*Curcuma longa L.*) aqueous extract on sarcoma and breast cancer has been demonstrated [30]. Rosemary (*Rosemarinus officinalis L.*) extract reveals the antitumor potential of prevention, development and drug response in various types of cancer, thereby emphasizing in previous studies that clinical studies should be initiated to confirm the possible use fulness of this extract as a complementary agent for certain cancer patients [31]. The anticancer effect of stinging nettle (*Urtica dioica*) has been investigated in breast cancer cells and it has been revealed that its use with cancer drug paclitaxel may be potential for treatment [32]. Positive results were obtained in prostate cancer, in which the apoptotic effects of nettle herb dichloromethane extract were examined [33]. Juniper (*Juniperus*

phoenicea) activities in human lung, breast, liver cells have been demonstrated [34]. Although the efficacy of more well-known extracts on breast cancer, such as harmal (*Peganum harmala L.*), ginger (*Zingiber officinale*), rosemary (*Rosamarinus officinalis L.*), juniper *(Juniperus communis*), has not been tested, its effectiveness on osteosarcoma has not been investigated [30,35–37]. The anticancer activity of *Caulis spatholobi* ethyl acetate extract, which is frequently used in Chinese medicine with such antioxidant properties, has been demonstrated on human osteosarcoma cell Saos-2 [22]. The anticancer effect has been revealed by targeting molecular mechanisms on osteosarcoma cell [38]. The compound of curcumin, which is an indispensable part of traditional medicine, in turmeric, has been known to have cytotoxic effects in many studies in vitro and in vivo. However, its instability and poor metabolic features limit its clinical application. There are studies investigating the effect of curcumin on Saos-2 [27,39,40]. The anticancer effect of *Tilia dasystyla* and *Polygonatum orientale* extracts on Saos-2 has been demonstrated [25].

In addition to investigating the anticancer effect of different herbal extracts on osteosarcoma, the molecular mechanisms of the synergistic effect of co-administration are planned in our future studies. These components are known to regulate epigenetic mechanisms at the cellular level. So, as mentioned in the literature, the RTM to traditional and complementary medicine treatments with herbal ingredients [21].

CONCLUSION

In this study, results indicate that extracts of five different herbal product including; DVD ARD®, IST GLIO®, ROMX®, IST ARD®, REGULIN® showed cytotoxic effect on Saos-2 cancer cell line due to their mixture of medicinal plants, however REGULIN® showed the most cytotoxic effect among them and the reason of REGULIN® effectiveness requires more detailed investigation in subsequent studies. Cytotoxic effect of these food products on other cancer cell lines and their use in the pharmaceutical and health industries is recommended in future studies.

Additional Information

A part of this study was presented as a poster at the International 2. Traditional and Complementary Medicine Congress, 2019, 24-27 April, Istanbul/Turkey.

REFERENCES

1. Li T, Jiang S, Yang Y. Database selection and heterogeneity?more details, more credibility. *JAMA Oncol*. 2018;4(9):1295. doi:10.1001/jamaoncol.2018.1209
2. Picci P, Mercuri M, Ferrari S, et al. Survival in high-grade osteosarcoma: Improvement over 21 years at a single institution. *Ann Oncol*. 2009;21(6):1366-1373. doi:10.1093/annonc/mdp502
3. Mertens WC, Bramwell V. Osteosarcoma and other tumors of bone. *Curr Opin Oncol*. 1994;6(4):384-390. doi:10.1097/00001622-199407000-00010
4. Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004. *Cancer*. 2009;115(7):1531-1543. doi:10.1002/cncr.24121
5. Winslow LC, David K j. Herbs as Medicines. 2015;158.
6. Ma XH, Zheng CJ, Han LY, et al. Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives. *Drug Discov Today*. 2009;14(11-12):579-588. doi:10.1016/j.drudis.2009.03.012
7. Xiangyong Y, Zhongsheng Y, Wenchao L, et al. External application of traditional Chinese medicine in the treatment of bone cancer pain: a meta-analysis. *Support Care Cancer*. 2016;24(1):11-17. doi:10.1007/s00520-015-2737-2
8. Atalay D, Erge HS. Dietary Supplements and Their Effects on Health. *Food Heal*. 2018;4(2):98-111. doi:10.3153/fh18010
9. Lange D. Europe's medicinal and aromatic plants: their use, trade and conservation. *Eur Med Aromat plants their use, trade Conserv*. 1998. https://www.cabdirect.org/cabdirect/abstract/19980314129. Accessed October 2, 2018.
10. Manel M, Nouzha H, Rim M, et al. Antibacterial and antioxidant activity of Juniperus thurifera L. leaf extracts growing in East of Algeria. *Vet World*. 2018;11(3):373-378. doi:10.14202/vetworld.2018.373-378
11. Kusari S, Lamshöft M, Spiteller M. *Aspergillus fumigatus* Fresenius, an endophytic fungus from *Juniperus communis* L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. *J Appl Microbiol*.

2009;107(3):1019-1030. doi:10.1111/j.1365-2672.2009.04285.x
12. Fernandez A, Journal IC-P, 2016 undefined. The Therapeutic Properties of Juniperus communis L.: Antioxidant Capacity, Bacterial growth Inhibition, Anticancer Activity and Toxicity. *PhcogjCom*. https://sci-hub.tw/http://phcogj.com/article/148.
13. Lim GP, Chu T, Yang F, et al. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci. 2001;21(21):8370-8377. doi:21/21/8370 [pii]
14. Sharma RA, Mclelland HR, Hill KA, et al. Pharmacodynamic and Pharmacokinetic Study of Oral Curcuma Extract in Patients with Colorectal Cancer Pharmacodynamic and Pharmacokinetic Study of Oral Curcuma Extract in Patients with Colorectal Cancer 1. 2001;7(July):1894-1900.
15. Jurenka JS, Ascp MT. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. *Altern Med Rev*. 2009;14(2):141-153. http://www.ncbi.nlm.nih.gov/pubmed/19594223.
16. Palla AH, Khan NA, Bashir S, et al. Pharmacological basis for the medicinal use of Linum usitatissimum (Flaxseed) in infectious and non-infectious diarrhea. J Ethnopharmacol. 2015;160:61-68. doi:10.1016/J.JEP.2014.11.030
17. Moore J, Yousef M, Tsiani E. Anticancer Effects of Rosemary (Rosmarinus officinalis L.) Extract and Rosemary Extract Polyphenols. 2016. doi:10.3390/nu8110731
18. Daoud A, Song J, Xiao F, et al. B-9-3, a novel β-carboline derivative exhibits anti-cancer activity via induction of apoptosis and inhibition of cell migration in vitro. *Eur J Pharmacol*. 2014;724(1):219-230. doi:10.1016/j.ejphar.2013.12.038
19. Gao J, Zhu H, Wan H, et al. Harmine suppresses the proliferation and migration of human ovarian cancer cells through inhibiting ERK/CREB pathway. *Oncol Rep*. 2017;38(5):2927-2934. doi:10.3892/or.2017.5952
20. Hashemi Sheikh Shabani S, Seyed Hasan Tehrani S, Rabiei Z, et al. Peganum harmala L.'s anti-growth effect on a breast cancer cell line. *Biotechnol Reports*. 2015;8:138-143. doi:10.1016/j.btre.2015.08.007
21. Yasar M. The remember regeneration therapy method: An overview of new therapy protocol to approach diseases. *J Complement Med Res*. 2019;10(1):68. doi:10.5455/jcmr.20181229122909
22. Liu B, Liu J, Chen J, et al. A study on anticancer activity of Caulis Spatholobi extract on human osteosarcoma Saos-2 cells. *Afr J Tradit Complement Altern Med*. 2013;10(5):256-260. doi:10.4314/ajtcam.v10i5.6
23. Engel N, Ali I, Adamus A, et al. Antitumor evaluation of two selected Pakistani plant extracts on human bone and breast cancer cell lines. *BMC Complement Altern Med*. 2016;16(1):1-18. doi:10.1186/s12906-016-1215-9
24. Chen X, Gu N, Xue C, et al. Plant flavonoid taxifolin inhibits the growth, migration and invasion of human osteosarcoma cells. *Mol Med Rep*. 2018;17(2):3239-3245. doi:10.3892/mmr.2017.8271
25. Zarringhalami R, Hanachi P, Kaya E, et al. Investigation of total phenolic content of tilia dasystyla and polygonatum orientale desf extracts and their cytotoxic effect on the osteogenic sarcoma (Saos-2) cancer cell line. *Int J Cancer Manag*. 2020;13(2). doi:10.5812/ijcm.94130
26. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1-2):55-63. doi:10.1016/0022-1759(83)90303-4
27. Lima FT, Seba V, Silva G, et al. The curcumin analog CH-5 exerts anticancer effects in human osteosarcoma cells via modulation of transcription factors p53/Sp1. *Int J Mol Sci*. 2018;19(7). doi:10.3390/ijms19071909
28. Zhang Y, Ma R, Cheng S, et al. Marrubenol inhibits osteosarcoma cancer cell growth by inducing autophagic cell death and inhibiting cancer cell migration and invasion. *J BUON*. 2018;23(3):729-734.
29. Mohammad P, Nosratollah Z, Mohammad R, et al. The inhibitory effect of Curcuma longa extract on telomerase activity in A549 lung cancer cell line. *African J Biotechnol*. 2010;9(6):912-919.
30. Ranjbari J, Alibakhshi A, Arezumand R, et al. Effects of Curcuma longa Extract on Telomerase Activity in Lung and Breast Cancer Cells Javad. *Zahedan J Res Med Sci*. 2013;16(10):1-6.
31. Moore J, Yousef M, Tsiani E. *Anticancer Effects of Rosemary (Rosmarinus Officinalis L.) Extract and Rosemary Extract Polyphenols*. Vol 8.; 2016. doi:10.3390/nu8110731
32. Mohammadi A, Mansoori B, Aghapour M, et al. The Urtica dioica extract enhances sensitivity of paclitaxel drug to MDA-MB-468 breast cancer cells. *Biomed Pharmacother*. 2016;83:835-842. doi:10.1016/j.biopha.2016.07.056
33. Mohammadi A, Mansoori B, Aghapour M, et al. Urtica dioica dichloromethane extract induce apoptosis from intrinsic pathway on human prostate cancer cells (PC3). *Cell Mol Biol*. 2016;62(3):78-83. doi:10.14715/cmb/2016.62.3.13
34. Groshi A Al, Evans AR, Ismail FMD, et al. Cytotoxicity of libyan juniperus phoenicea against human cancer cell lines A549, EJ138, Hepg2 and MCF7. *Pharm Sci*. 2018;24(1):3-7. doi:10.15171/PS.2018.02
35. Van Slambrouck S, Daniels AL, Hooten CJ, et al. Effects of crude aqueous medicinal plant extracts on growth and invasion of breast cancer cells. *Oncol Rep*. 2007;17(6):1487-1492. doi:10.3892/or.17.6.1487
36. Vemuri SK, Banala RR, Subbaiah GPV, et al. Anti-cancer potential of a mix of natural extracts of turmeric, ginger and garlic: A cell-based study. *Egypt J Basic Appl Sci*. 2017;4(4):332-344. doi:10.1016/j.ejbas.2017.07.005
37. Hashemi Sheikh Shabani S, Seyed Hasan Tehrani S, Rabiei Z, et al. Peganum harmala L.'s anti-growth effect on a

breast cancer cell line. *Biotechnol Reports*. 2015;8:138-143. doi:10.1016/j.btre.2015.08.007

38. Zhang Y, Xie WP, Zhang YK, et al. Experimental study of inhibitory effects of diallyl trisulfide on the growth of human osteosarcoma saos-2 cells by downregulating expression of glucose-regulated protein 78. *Onco Targets Ther*. 2018;11:271-277. doi:10.2147/OTT.S150933
39. Luo Z, Li D, Luo X, et al. Curcumin may serve an anticancer role in human osteosarcoma cell line U-2 OS by targeting ITPR1. *Oncol Lett*. 2018;15(4):5593-5601. doi:10.3892/ol.2018.8032
40. Walters DK, Muff R, Langsam B, et al. Cytotoxic effects of curcumin on osteosarcoma cell lines. *Invest New Drugs*. 2008;26(4):289-297. doi:10.1007/s10637-007-9099-7

Volume: 1 Issue: 1
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

Effect of My Guard® Food Supplement on Acute and Subacute Toxicity on Rats

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Abstract
Objective: Today, in the field of traditional and complementary medicine, there has been a serious interest in medicinal plants and the treatment methods performed with the use of these plants. The use of natural resources in drug production studies and the promotion of these uses led to the advantage of reaching the drug at a reasonable cost with a rational method. However, these studies should be carried out with objective practices within the framework of scientific ethical rules. Investigating the toxicity dimension in studies related to the subject is also very important for human health to use these products. For this reason, the aim of the study is to determine the toxic symptoms of the My Guard® herbal mixture syrup which is a product used in the remember regeneration therapy method.
Material-Method: In the experimental design, there were 4 groups (1 control and 3 application groups). To observe acute and subacute toxicity, clinical observation, the biochemical, hematological and histopathological parameters of the animals were evaluated at the end of the application times.
Results: According to the results obtained from the study, there were not found significant differences in biochemical, hematological and histopathological evaluations between the control and the application groups.
Conclusion: In our study, the toxic effects of My Guard® product in the dose mentioned in acute and subacute periods were not observed.
Keywords: Traditional and Complementary Medicine, Rat, Supplemental Food Products, Acute Toxicity, Subacute Toxicity

INTRODUCTION

Traditional and medicinal use of plants dates back to the beginning of human history. There is also information that human beings have benefited from plants for nutrition, shelter and healing in the periods before Christ [1,2]. The herbalists think and advocate that the using plant has positive therapeutic effect. This theory is supported by purification of the active ingredients instead of purifying the side effects by neutralizing each other in the plant, instead of using the active substances separately [3].

Our country is home to many plant species due to its existing geographical and ecological features and is considered as the homeland of more than 11 000 plant species. Although medicinal and aromatic plants have an important place among this variety, they are widely used in the pharmaceutical industry and their consumption as a supplement is important. These plants have a serious use in cosmetic applications, pharmaceutical industry and food industries. Phytotherapy, which means therapy with plants, can be applied for therapeutic and protective purposes in disease types of humans and animals. Glycosides and aromatic oils produced by extraction and liquid production from plants have been accepted since ancient times for therapeutic purposes. The type and amount of synthetic compounds used in the health, cosmetics and food industries are increasing every year, threatening the environment and human health, affecting the country's economy negatively [4]. Some types of synthetic drugs bioaccumulate in the environment and cause serious environmental damage [5,6]. Many of these compounds are not

biodegradable and can survive in aquatic environments for a long time [7,8]. Many of these compounds are not biodegradable and can survive in aquatic environments for a long time [9,10]. Regarding all this, in the field of traditional and complementary medicine, there has been a serious interest in medicinal plants and the treatment methods used with these plants. The use of natural resources in drug production studies and the promotion of these uses led to the advantage of reaching the drug at a reasonable cost with a rational method. On the other hand, many complementary and alternative medicine methods are widely available, reasonable and regularly used in many countries. And these therapies such as the remember regeneration therapy method (RTM) are protected by practical evidence of protection and effectiveness [11]. However, these studies should be carried out with objective practices within the framework of scientific ethical rules [2,12]. Investigating the toxicity dimension in studies related to the subject is also very important for human health to use these products.

Toxicity studies are carried out as acute and subacute toxicity tests. Acute toxicity measurement; is carried out in 'finding the dose range' studies that allow the detection of a safe and possible toxic dose range [13]. The most commonly used acute toxicity test is the lethality test. For the acute toxicity test, the dose is determined based on the literature, in vitro results, molecular structure and 24 hours of observation is made to the experimental animals after dosing. The observation is continued for a week to check if there is late toxicity. To determine the subacute toxicity, the chemical is administered to the test animals every day in one or more repeats. For this purpose, the chemical is added to food or drinking water. As experimental animals, preferably rodents (rats, rabbits) may be non-rodent species as well. The test period of some chemicals in humans and rodents is between 1-3 weeks. Three different dose levels are tested on two different animal species [14]. Therefore, xenobiotics are applied daily for 1 week to measure subacute toxicity observations are made and data are recorded. The dosage of xenobiotic to be given is determined according to the dose determined in acute toxicity. In both acute toxicity and subacute toxicity experiments, the organs of the animals that died during the experiment and the tissues of the animals sacrificed at the end of the period are performed for histopathological examination[14].

A detailed investigation of toxicity tests is important in determining the possible effects of herbal products that are widely used and believed to be harmless because they are natural. *Silybum marianum, Rosmarinus officinalis, Zingiber officinale, Fumaria officinalis, Cichorium intybus, Urtica dioica, Achillea millefolium, Thymus vulgaris, Equisetum arvense, Taraxacum officinale, Curcuma longa* and *Juniperus communis* plants are used in combination or separately. The clinical effects and acute toxicity of the products combined with the acute toxicity tests on animals are determined.

Zingiber officinale is a plant that has anti-inflammatory, antipyretic and hypoglycemic potential in addition to its frequent use in digestive disorders, rheumatism, diabetes and edema disorders [15]. *Thymus vulgaris* has antimicrobial, antitussive, expectorant, antispasmodic effects [16]. The use of *Juniperus communis* species in antidiarrheal, anti-inflammatory, astringent, antiseptic and various abdominal disorders [17]. *Lavandula* is a family of *Stoechas Lamiaceae* and is a type of lavender. It was used for the first time in our country in the Ottoman state in the fight against the cholera epidemic, to eliminate the germs causing cholera and to prevent the infection of wounds in the First World War [18].

The aim of the study is to determine the toxic symptoms of the My Guard® herbal mixture syrup which is a product used in the remember regeneration therapy method (RTM), the degree of effectiveness of certain organs such as brain, kidney, liver or lethal dose (lethality) levels.

MATERIALS AND METHODS

Prepration of food supplement

The syrup mixture with herbal material of this study was obtained from Naturin (Natural Products Pharmaceutical and Pharmaceutical Raw Materials Industry Trade Limited Company). Plants included in the plant mixture: *Thymus vulgaris, Zingiber officinale, Juniperus communis, Lavandula stoechas* and *Mentha piperita*. Negative control group was treated with 1 ml saline. 0.06 ml of My Guard® were given to rats by gavage for acute and subacute groups. Doses for the groups to be given food supplements were calculated by proportioning the daily doses of the product delivered to our laboratory according to the weights of the experimental animal.

Experimental animals

The experimental animals used in the study were also obtained from Düzce University Experimental Animals Application and Research Center. The experiment was approved by the "Düzce University Animal Experiments Local Ethics Committee 2020.3.3". In the laboratory, Wistar albino 8 week old, 250-300 g female rats were kept at 20-25˚C room temperature, 55 ± 5% humidity and 12:12 light-dark cycle, with optimal food and water intake free.

The rats were divided into four groups as the control (n=8), the acute toxicity (n=8), the subacute toxicity (7 days) (n=8), and the post-subacute (14 days) (n=8) group. For acute and subacute groups (7 days), 0.06 ml of My Guard® was given to rats by gavage. For subacute groups (14 days), it wasn't given to rats.

Acute and subacute toxicity study

Identifying the target organs affected in acute and subacute toxicity was created preliminary data for subchronic and chronic toxicity tests. Plants in herbal blend syrup are *Zingiber officinale, Thymus vulgaris, Juniperus communis, Lavandula stoechas, Piper nigrum* and *Mentha x piperita* oil.

Animal experiments were carried out using the ISO 10993 toxicity protocol with minor modifications based on recording the weight of the animals prior to application [19]. Animals were sacrificed under ketamine / xylazine anesthesia at the end of the 24-hour clinical observation after product delivery to the acute toxicity experimental group. Blood was drawn from the heart for biochemistry and hemogram parameters. In addition, heart, liver, lung, kidney and spleen organs were fixed and examined histopathologically.

The animals in the sub-acute toxicity experimental group continued to be applied with product gavage for one week (7 days). Clinical observations were made during the experiment period. On the 8th day, blood was taken from the heart to measure its biochemical parameters, sacrificed and tissues fixed in formaldehyde for histopathological examination. Necessary tests were performed for biochemical, hematological and histopathological measurements.

The animals in the group after sub-acute toxicity were continued to be applied with gavage for a week (7 days). Later on, animals were kept under observation for a week (7 days) to see if the effects continued without any product. Clinical observations were made during the experiment period. On the 14th day, blood was taken from the heart for biochemical and hematological parameters, sacrificed and tissues fixed in formaldehyde for histopathological examination. Necessary tests were performed for biochemical, hematological and histopathological measurements.

Clinical observation parameters

Observations were performed to provide acute toxicity data at 0 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours, 8 hours and 24 hours intervals after oral administration of gavage at a time, in proportion to the dose of the mixture given to the test animals. After 1 day, blood was drawn from the acute toxicity test group. Each animal in each experimental group was routinely observed during the experiment in accordance with the criteria of toxicity clinical behavioral symptoms given in Table 1.

Table 1. Observation and evaluation criteria

Clinical Observation	**Observations**	**Systemic Observation**
Respiratory	Dyspnea (abdominal breathing), apnea, eupne, tachypnea	Central nervous system (CNS), circulatory cardiac, respiration
Motor activities	Descending/increasing, Indeterminate positions, tremor	MSS, samatomotor, sensory, autonomous, muscular-nervous systems
The convulsion	Clonic, tonic, tonic-clonic symptoms	CNS, respiration, muscular-nervous, automic
Reflexes	Initial reflex	MSS, Sensory, automic, muscular-nerve
Oculer observation	Lacrimation, miosis, mydriasis	Autonomic nervous system, irritation
Cardiovascular observation	Bradycardia, tachycardia, arrhythmia, vasodilation, vasoconstriction	MSS, autonomous SS, cardiac, circulatory system
Salivation	Quantity	Autonomous SS
The pliorection	Coarse feathers	Autonomous SS
Analgesia	Decreased analgesia	CNS, sensory
Muscle tone	Hypotonia, hypertonia	Autonomous SS
Gastrointestinal	Diüresis	MSS, autonomous SS, kidney, motolite
Skin	Edema, rash	Tissue injury, irritation

Biochemical and hematological parameters

Blood samples were collected intracardiacly without coagulation. About 2 ml of the blood samples were taken to the edited tube for hematology analysis and the other part to biochemistry tubes. The serums were carefully collected by centrifugation (1.500 g, 10 minutes, 4°C) and stored at -20°C until analysis. Biochemistry parameters of Gamma-glutamyl transpeptidase, Total cholesterol, Inorganic phosphorus, Aspartate aminotransferase, Calcium, Albumin, Urea nitrogen, Triglyceride, Total Protein, Creatinine, Alkaline phosphatase, Bilirubin, Alanine aminotransferase was evaluated with Mindray BS-120 device. Hematocrit, red blood cell, hemoglobin, white blood cell, and platelet hemogram parameters were analyzed with the Mindray BC5000Vet device. Biochemical and hematological analyzes were studied in Duzce University Experimental Animals Application and Research Center.

Histopathological evaluation

While taking the samples of the animals in their groups, they were taken in one piece without damaging the organ (liver, heart, kidney, spleen) and kept in a 10% formaldehyde solution. Then the organs removed from solution and were embedded in paraffin blocks. Blocks were numbered for macroscopic examination. 5 micrometers thick block sections were taken in the microtome. Tissues from the alcohol series were stained with Hematoxylin-eosin dye. The preparations were then examined under a Olympus® BX53F microscope.

Statistical evaluation

The biochemical parameters obtained in our study were analyzed using the one-way ANOVA test using the IBM SPSS Statistics 23 program. The groups that were found statistically significant were determined by post hoc Dunnett's T3 test. P <0.05 was accepted as the statistical significance level.

RESULTS

Clinical observation parameters

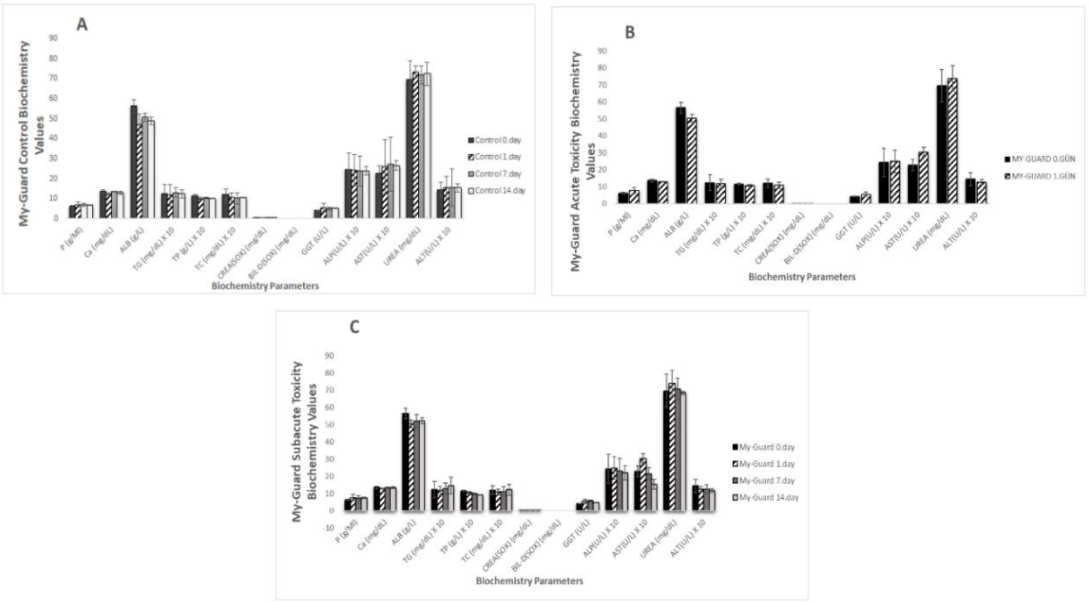
Animals in each experimental group were evaluated routinely during the experiment period by observing breathing, motor activities, convulsions, reflexes, ocular signs, salivation, piloerection, analgesia, muscle tone, gastrointestinal and skin.

The animals in the product group given My Guard® food supplement were found to be similar to the control group compared to the parameters given in

Table 1. There was no statistically significant difference in clinical observation by making observations between groups.

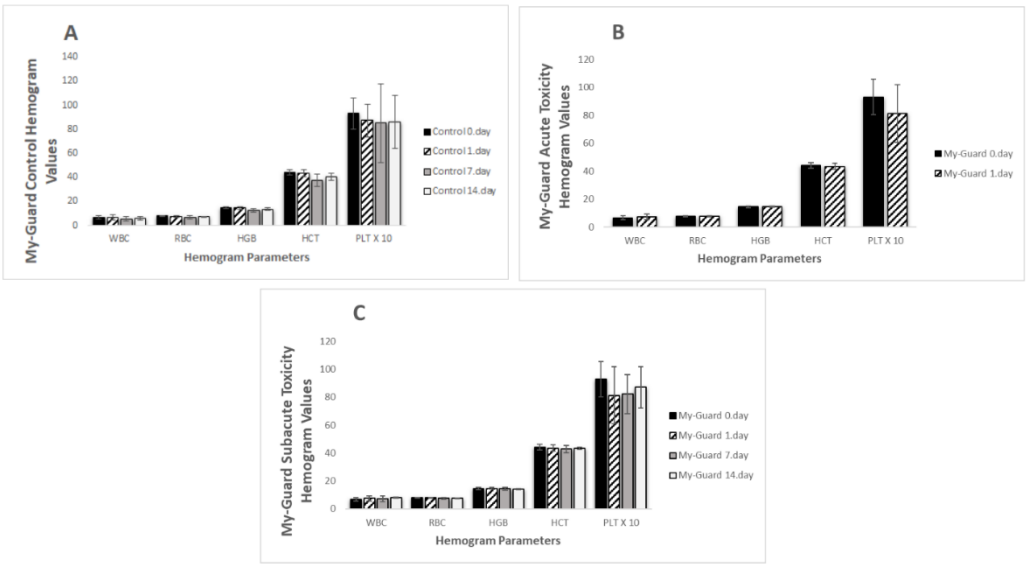
Biochemical and hematological parameters

As seen in Graphic 1, no statistical difference was found between the control and administration groups in terms of biochemical parameters. When the data of biochemical parameters belonging to the acute toxicity and control groups were compared, no statistical significance was found. No statistical significance was found between the subacute toxicity group and the control group.



Graphic 1. A: Control group biochemistry analyze **B:** Acute group biochemistry analyze **C:** Subacute group biochemistry analyze. (The values in the graph are given as mean ± SD.)

As seen in Graphic 2, there was no difference between the control and application groups in terms of hemogram parameters. When comparing the acute toxicity group and 5 different hemogram data on days 0 and 1 of the control group, difference was observed. There was no difference between the subacute toxicity group and the control group at the values of 0, 1, 7 and 14 days.



Graphic 2. A: Control group hemogram analyze **B:** Acute group hemogram analyze **C:** Subacute group hemogram analyze (The values in the graph are given as mean ± SD.)

Histopathological evaluation

For histopathological examination, the histopathology of organs taken by appropriate methods from each animal in each experimental group was generally evaluated.

When the results of lung histopathology of animals in the product group with My Guard® food supplement and animals in the control group were evaluated in terms of interstitial and bronchointerstitial pneumonia, degeneration, hyperemia and necrosis, there was no difference between the groups.

When the results of cardiac histopathology results of animals in the product group given My Guard® food supplement and animals in the control group were evaluated in terms of degeneration, hyperemia, bleeding and necrosis, there was no difference between the groups.

When the liver histopathology results of the animals in the product group given the My Guard® food supplement and the animals in the control group were evaluated in terms of degeneration, hyperemia, bleeding and necrosis, there was no difference between the groups.

When the kidney histopathology results of animals in the product group given My Guard® food supplement and animals in the control group were evaluated in terms of pigmentation, degeneration, hyperemia, bleeding and necrosis, there was no difference between the groups.

When the spleen histopathology results of animals in the product group given My Guard® food supplement and animals in the control group were evaluated in terms of pigmentation, degeneration, hyperemia bleeding and necrosis, there was no difference between the groups.

Histopathologically, no difference was observed when the animals in the My Guard® food supplement group were compared with those in the control group.

DISCUSSION

Some of the side effects of the *Juniperus communis* plant include: overdoses irritate the kidneys[20] and may pose a risk to pregnant women[21]. Akdogan et al. (2012) in a study on juniper plant, rats were applied of various doses of JCL in the treatment of hypercholesterolemia and analyzed their biochemical parameters and histopathological effects on kidney tissue. 35 adult male Wistar albino rats weighing 200-250 g were used. Rats are divided into 5 groups of 7. The control group was fed with a normal pellet feed, the cholesterol (Chol) group was fed with a 2% cholesterol-containing pellet feed, and 50 *Juniperus communis* Lynn (JCL), 100 JCL and 200 JCL groups were 50, 100, 200 in addition to 2% cholesterol feed respectively. It was fed with mg/kg JCL oil. JCL was dissolved in 0.5% Sodium Carboxy Methyl Cellulose (SCMC) and applied with a gavage needle. The experiment ended 30 days later and blood and kidney tissue samples were taken. Complete blood tests and biochemical measurements were made and kidney tissues were analyzed histopathologically. 200 mg/kg JCL administration caused a significant increase in BUN and Crea levels. Chol application significantly increased the TC level, but t administration of JCL in combination with chol prevented this increase. The application of chol led to a significant increase in Ox-LDL levels. However, the administration of 200 mg/kg of JCL with the arm did not result in a significant increase. At the end of the study, JCL oil was found to have an anti-hypercholesterolemic effect. Anemic effect and no significant change was observed in all groups, whereas a significant focal damage was observed in the tubular cast structure [22].

In a toxicity study by Weidner and Sigwart (2000), the teratogenicity of EV.EXT 33, a patented *Zingiber officinale* extract, was investigated in Wistar SPF rats according to the GLP Guidelines. EV.EXT 33 was administered to three groups of 22 pregnant female rats from the 6th to 15th gestation days at concentrations of 100 to 333 and 1000 mg / kg by oral gavage. For comparison, a fourth group of vehicles bought sesame oil. During the treatment, body weight, food and water intake were recorded. Rats were sacrificed on the 21st day of pregnancy and examined for standard reproductive performance parameters. Fetuses were examined

for signs of teratogenic and toxic effects. EV.EXT 33 was well tolerated. No side effects related to death or treatment were observed. Weight gain and food consumption were similar in all groups during pregnancy. Reproductive performance was not affected by treatment with EV.EXT 33. Examination of fetuses for external, visceral and skeletal changes did not show the embryotoxic or teratogenic effects of EV.EXT 33. According to these results, EV.EXT 33 did not cause maternal or developmental toxicity at daily doses up to 1000 mg / kg body weight when administered to pregnant rats during organogenesis [23].

Basch et al. (2009), in order to prevent toxicity in thyme, it was suggested not to exceed oral doses of 10 grams of dried leaves with 0.03% phenol (calculated as thymol) per day. Oregano oil is considered very toxic. Toxicity symptoms include nausea and may include tachypnea and hypotension based on animal studies [24]. LD50 of thyme essential oil is 2.84 g / kg body weight in rats [25]. Oral doses of concentrated thyme extract (0.5-3 g / kg body weight) (4.3-26 g/kg thyme equivalent) reduced locomotor activity and respiratory activity in mice. After 3 months, oral administration of 0.9 g of dried grass as an extract in 95% ethanol led to an increase in liver and testicle weight in mice; 30% of male animals and 10% of female and control animals died.

Yassin et al. (2016) demonstrated the phytochemical screening, anti-inflammatory activities of the extracts from the air part (branches, flowers and leaves) of *Lavandula stoechas* L, anti-inflammatory activities, and sub-acute toxicity of hydro-ethanolic and polyphenols (flavonoids, tannins and mucilage) extracts. Evaluations were made using the Rat Claw Edema method from Carrageenan. Sub-acute toxicity of hydro-ethanolic extract and its fractions was evaluated in vivo after topical application of creams and some biochemical parameters were determined. Phytochemical screening of *L. stoechas* extract revealed the presence of tannins, catechic tannins, flavonoids, sterols, coumarins, quinones, leukocantoses and mucilage compounds. The hydroethanolic extract (5% and 10%) of *L. stoechas* inhibited the carcinogenic-induced inflammation in rats in a dose-dependent manner. At a dose of 10% *L. stoechas* produced a significant inhibition of inflammation at 74 ± 7% compared to 69 ± 10.3% for diclofenac at 1%. Flavonoid and mucilage extracts showed a significant effect in reducing edema (85.1 ± 6.2 and 61.71 ± 7.3%, respectively). No significant change in Relative Organ Weights (ROW) was observed between the body and the control-treated group. In addition, kidney, liver and blood dysfunctions were not recorded in the treated animals compared to control [26].

Thorup et al. (1983) administered doses of peppermint oil to groups of 10 male and 10 female mice at 0, 10, 40 and 100 mg/kg bw/day, respectively, for 28 days. Histopathological changes in cerebellum white matter have been observed especially at dose levels of 40 and 100 mg/kgbw/day and consisted of cyst-like cavities scattered in this white matter. No apparent clinical symptoms were observed due to encephalopathy [27]. In our study, the toxic effects of My Guard® product in the dose mentioned in acute and subacute periods were not observed. Studies have shown the positive effects of these herbs at safe doses below these doses, even though they have toxic effects on the use of high doses. However, new studies are needed to reveal its positive effects and the most effective non-toxic doses.

REFERENCES

1. Baydar SN. *Şifalı Bitkiler Ansiklopedisi: Modern Tıp Alternatif Tıp Ile Elele*. 1st ed. Ankara: Palme Yayıncılık; 2017.
2. Altunbaş M, Türel İ, Yıl Üniversitesi Y, et al. Petroselinum crispum (Maydanoz) Tohumu Uçucu Yağ Özütünün Letal Doz Düzeyleri ve Antienflamatuvar Aktivitesinin Deney Hayvanları Üzerinde Araştırılması* The Lethal Dose Levels of Volatile Oil Extract of Petroselinum crispum (Parsley) Seeds and the Investigation of Antiinflammatory Effect on Laboratory Animals. *YYU Vet Fak Derg*. 2009;20(1):21-25.
3. Fulder S. *The Handbook of Alternative and Complementary Medicine : The Essential Health Companion*. 3rd ed. WILLIAM HEINEMANN; 1997.

4. Yonar T, Kurt A. *Treatability Studies of Hospital Wastewaters with AOPs by Taguchi's Experimental Design*. Vol 19.; 2017.
5. Vergili İ, Kaya Y, Özçelep Z, et al. İlaç Aktif Maddelerinin Sucul Çevrede Bulunuşları, Davranışları ve Etkileri. *Türk Sucul Yaşam Derg*. 2005;4:294-291.
6. Kurt A, Yonar T. Endokrin Bozucu Antibiyotik Bileşiklerinin UV/H2O2 Prosesi ile Taguchi Deneysel Dizaynına Göre Arıtılabilirliği. *Afyon Kocatepe Üniversitesi Fen Ve Mühendislik Bilim Derg*. 2017;17(2):854-860.
7. Arslan-Alaton I, Dogruel S. Pre-treatment of penicillin formulation effluent by advanced oxidation processes. *J Hazard Mater*. 2004;112(1-2):105-113. doi:10.1016/j.jhazmat.2004.04.009
8. Kurt A, Mert BK, Özengin N, et al. Treatment of Antibiotics in Wastewater Using Advanced Oxidation Processes (AOPs). In: *Physico-Chemical Wastewater Treatment and Resource Recovery*. InTech; 2017. doi:10.5772/67538
9. Aslan R. Hekimlikte Alternatif ve Tamamlayıcı Tıbbi Yaklaşımlar Recep ASLAN. *Kocatepe Vet J Kocatepe Vet J*. 2016;9(4):363-371. doi:10.5578/kvj.37336
10. Baytop T. *Türkiye'de Bitkiler Ile Tedavi Geçmişte ve Bugün - Prof. Dr. Turhan Baytop | Kitapyurdu.Com*. 2nd ed. Nobel Tıp Kitabevleri; 1999. https://www.kitapyurdu.com/kitap/turkiyede-bitkiler-ile-tedavi-gecmiste-ve-bugun/37327.html. Accessed May 1, 2020.
11. Yasar M. The remember regeneration therapy method: An overview of new therapy protocol to approach diseases. *J Complement Med Res*. 2019;10(1):68-80. doi:10.5455/jcmr.20181229122909
12. Kayaalp O. *Klinik Farmakolojinin Esasları ve Temel Düzenlemeler[Principles of Clinical Pharmacology and Basic Regulations]*. 5th ed. Pelikan Yayıncılık; 2013.
13. Monga M, Sausville EA. Developmental therapeutics program at the NCI: Molecular target and drug discovery process. *Leukemia*. 2002;16(4):520-526. doi:10.1038/sj.leu.2402464
14. Saygı Ş. Deneysel Toksikolojide Toksisite Testleri ve Test Sonuçlarının Önemi. *Gülhane Tıp Derg*. 2003;45(3):291-298. http://cms.galenos.com.tr/Uploads/Article_32654/GMJ-45-291-En.pdf. Accessed March 31, 2020.
15. Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *J Ethnopharmacol*. 1989;27(1-2):129-140. doi:10.1016/0378-8741(89)90085-8
16. Fachini-Queiroz FC, Kummer R, Fernanda Estevão-Silva C, et al. Effects of Thymol and Carvacrol, Constituents of *Thymus vulgaris* L. Essential Oil, on the Inflammatory Response. *Artic ID*. 2012;2012. doi:10.1155/2012/657026
17. Bais S, Gill NS, Rana N, Shandil S. A Phytopharmacological Review on a Medicinal Plant: Juniperus communis. *Int Sch Res Not*. 2014:1-6. doi:10.1155/2014/634723
18. Kaplan EN, Çaki MN, Paşa S. *Lavandula stoechas* (Karabaş Otu) Bitki Özütünden Kozmetik Ürün Eldesi. *UMUFED Int J West Black Sea Eng Sci*. 2019;1(1):24-37.
19. Fleming T, ed. *PDR for Herbal Medicines.* 2nd ed. New Jersey: Medical Economics Company; 2000.
20. Altundag E, Ozturk M. Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. In: *Procedia - Social and Behavioral Sciences*. Vol 19. Elsevier Ltd; 2011:756-777. doi:10.1016/j.sbspro.2011.05.195
21. Batı Akdeniz Kalkınma Ajansı. *Tibbi Ve Aromatik Bitkiler Sektör Raporu.*; 2012. https://baka.gov.tr/uploads/1357649536TiBBi-VE-AROMATiK-BiTKiLER-SEKTOR-RAPORU-5ARALiK.pdf. Accessed May 1, 2020.
22. Akdogan M, Koyu A, Cirit M, Yildiz K. Anti-hypercholesterolemic activity of *Juniperus communis* Lynn Oil in rats: A biochemical and histopathological investigation. *Biomed Res - An Int J Med Sci*. 2012;23(3):212.
23. Weidner MS, Sigwart K. Investigation of the teratogenic potential of a *Zingiber officinale* extract in the rat. *Reprod Toxicol*. 2000;15(1):75-80. doi:10.1016/S0890-6238(00)00116-7
24. Kagramanov KM. Effect of the essential oils of some thyme growing in Azerbaidzhan on cardiovascular activity and respiration. *Azerbaidzhanskii Meditsinskii Zhurnal*. 1977;54(5):49-51.
25. Skramlik E V. On the toxicity and tolerance of ethereal oils. *Pharmazie*. 1959:435-445.
26. Yassine EZ, Dalila B, Mansouri Latifa E, et al. Phytochemical Screening, Anti-inflammatory Activity and Acute Toxicity of Hydro-ethanolic, Flavonoid, Tannin and Mucilage Extracts of *Lavandula stoechas* L. from Morocco. *Available online www.ijppr.com Int J Pharmacogn Phytochem Res*. 2016;8(1). www.ijppr.com. Accessed May 1, 2020.
27. Thorup I, Würtzen G, Carstensen J, Olsen P. Short term toxicity study in rats dosed with pulegone and menthol. *Toxicol Lett*. 1983;19(3):207-210. doi:10.1016/0378-4274(83)90120-0

Volume: 1 Issue: 1
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

REVIEW

Medicinal Plants Meeting With Mud: Phyto-Peloid

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Abstract

For thousands of years, human beings have used various healing waters, plants, and muds from nature for health and beauty purposes. Nature and return to natural which we observe too often in the world today, has also increased the use of natural products. The use of certain muds for medical purposes is called peloidotherapy.

These subterranean and marine originated materials, which we named as mud, formed by various organic and inorganic substances in geological layers for thousands of years. Today, they are used for health and cosmetic purposes following the characteristics of organic and inorganic substances in their content, which vary according to their geography.

Studies show that; in the cures performed with the addition of peloidotherapy, the number of inflamed joints and the number of painful joints and pain severity decreased significantly ($p < 0.001$), joint function and quality of life significantly increased ($p < 0.05$), and symptoms of rash and itching ($p < 0.05$) decreased as.

As a new approach today; the use of muds obtained by combining medicinal plants widely used in the world for medical and cosmetic purposes is called phyto-peloid treatment. Considering the widespread and various medical mud resources and medicinal plant resources of our country, it is possible to be in the leading position in this regard.

The purpose of our research is to make difference about this value existing in our country, to make determinations and offer solutions on the existing applications and deficiencies in the mud existence and usage areas of our country, by taking into consideration the studies and applications made in the world.

Keywords: Mud, Medicinal Plants, Health, Cosmetics, Antimicrobial

INTRODUCTION

Mud has been used for thousands of years for both treatment and beauty. The beneficial properties of the mud, which is formed by the decay of biological substances, minerals, plant and animal residues for thousands of years, depends on humic substances. These substances such as temperature, content, mechanical which have different effects, are naturally used in the form of mud (peloid) applications. It is a miracle of nature that cleans and repairs the skin, supports the body with the minerals it contains, brings vitality and health, and reduces stress[1]. For this purpose, local and foreign literature and websites have been examined and the data about the usage areas of humic substances in thermal tourism have been evaluated. Peloids are a mixture of inorganic and organic substances for thousands of years as a result of biological and geological effects. There are different applications in the form of whole body, local application or packages for both treatment and cosmetic purposes[2]. The effects of mud treatment are similar to those of treatments with thermo-mineral waters. Depending on the content and physical-chemical differences, muds are used in treatment with their thermal, mechanical and chemical effects. They react in the form of a compliance therapy by stimulating the hypothalamohypophyseal axis[3].

Anatolian lands are the cradle of civilization. When Anatolian history is analyzed, the use of thermo-mineral water, which started in Roman baths in its best-known form, has become widespread in Seljuks and Ottomans and became known worldwide. These waters and muds have been used for health and beauty in these lands for thousands of years[4].

DEFINITION OF PELOID

Peloides are defined as "organic and/or inorganic substances originating from subterranean and sea caused by natural geological and/or biological events". It contains organic materials such as bacteria, algae, diatomas, protozoa, gastropods, as well as healing minerals such as sulfur, iodine, salt, and healing mixtures of inorganic minerals such as clay, quartz, calcite; it is rich in minerals such as magnesium, sodium, calcium, and potassium, which are important in the structure and normal function of our body[5]. Also, cyanobacteria, green algae, and diatoms are gradually colonized and this ensures that peloids have a microbiota. This microbiota generally consists of thermophilic microorganisms[6,7].

Peloids are diversified as follows:

• Peat: High water binding capacity, acid pH peloids. These muds are containing various natural dyes, humic acids and estrogen-like molecules that can be absorbed from the skin.

• Turbas: They are small-grained sediments that settle in still waters. Mineral marshes are formed in environments where thermo-mineral waters originate.

• Marine and Delta Slime: These are inorganic sediments that settle in sea and river deltas.

• Soils: Outside the water communities, they are powdered sediments formed by the crumbling, breaking down and decomposition of rocks or organic matter[8].

The mechanism of action of peloids:

As a thermal feature, there is heat conduction from pellets to particles, molecules to molecules in the form of condensation in the peloid, and convection in water immersion. Peloids mixed with a predetermined amount of thermo-mineralized water or tap water before to use for appropriate density and temperature. Peloid has a high heat holding capacity, therefore it retains heat for a long time. The skin and peloid temperature are equalized by the heat provided by the peloid layer during direct skin contact. Peloid stores heat for a long time and releases it slowly to the area it is applied to. Heat penetrates deep into the body and gradually begins to decrease. For treatment, they are applied to certain body areas in the form of full baths, local wraps, and packs[9].

Peloids are available in different particle sizes in nature that can be made into small particles when necessary. Peloids have high heat capacities, slow transmissions, and can be applied for a longer time at higher temperatures compared to mineral waters. Full, half-peloid baths are applied at 39-40 ° C for 15-20 minutes. In cases where full bath application cannot be performed, the application can be done in the form of sitting and limb baths at a temperature up to 50 ° C. Generally, it is done in total of 15-18 applications in a cure every day or 2-3 days intervals. As a special cure, vaginal and rectal applications are performed at 45-52 ° C in the form of 20 cycles, once a day for 15-20 minutes[10]. After the application period is over, the patient takes a warm shower and cleans the mud on his body. It is appropriate to rest up to 1 hour depending on the patient's condition. This cure application is applied in locomotor system diseases, rheumatic diseases, trauma or post-operative care and rehabilitation, skin diseases, gynecological diseases, and some digestive and urogenital diseases as a result of the examination, depending on the diagnosis and decision of the related physician. Due to its anti-inflammatory effect, it is preferred for infections with different localization of the genital area. Local and general hyperthermia, which occurs with increased blood supply and circulation, helps resorption of the infection. Due to its effect on increasing blood circulation, it stimulates follicles in ovarian failure, increases hormonal activities and regulates its cycle.

Peat, which is also characterized as swamp mud, contains a high rate of estrogen, especially humic acid at acid pH, with high water binding capacity. Another known effect is the stimulating effect on the hypothalamic, pituitary-adrenal system. It is recommended for primary and secondary hypoplasias of the uterus, hypo-hypermenorrhea, amenorrhea, monophasic cycle, corpus luteum insufficiency, and treatment. It is known that especially in the treatment of infertility,

satisfactory results are obtained with local or package applications[3].

The mechanism of action of peloids is not limited only to their thermal effects. There is a microbiota that constitutes its content and this microbiota may have antimicrobial, anti-inflammatory properties for pathogenic species[7]. In this way, it can also be used as a therapeutic agent for lesioned skin. Although the microbiota possessed by peloids is thought to be enriched by active compounds with various metabolic products, the development of microorganisms on peloids has not been studied in detail. Studies for peloids generally focus on their chemical composition and the biological mechanism of their effects[11].

Peloidotherapy is still used in hospitals affiliated to the Ministry of Health. On the website of Konya Training and Research Hospital, section of peloidotherapy indicates that natural mud has pain relieving, antioxidant, cell regenerating and skin tightening effects. Peloidotherapy is actively applied in 4 hospitals of the Ministry of Health, especially in the locomotor system and skin diseases[12].

THE USE OF MUD IN THE FIELD OF HEALTH AND COSMETICS

There are dozens of published studies on the use of mud in the treatment of various diseases in the field of evidence-based medicine worldwide.

In a study conducted by Barassi et al., there are 63 patients with neuromuscular disorders, they showed that kinesiotherapy and mud treatment, which was applied 5 days a week for 2 weeks, was effective in relieving patients' quality of life and physical disability[13].

In a study by Angioni et al., 66 patients with chronic low back pain due to osteoarthritis were discussed. Local mud treatment and thermo-mineralized water bath were applied to these patients for 2 weeks, and only drug treatment was given to the control group. In the patient group who underwent mud therapy, at the beginning of the study, 2nd week and 12th week blood tests were done and the protein levels in the blood were determined.

Accordingly after mud treatment (≥ 2,5 fold), inhibin beta A subunit (INHBA), activin A receptor type 2B (ACVR2B), angiopoietin-1 (ANGPT1), beta-2-microglobulin (B2M), growth differentiation factor 10 (GDF10), C-X-C motif chemokine ligand 5 (CXCL5), fibroblast growth factor 2 (FGF2), fibroblast growth factor 12 (FGF12), oxidized low density lipoprotein receptor 1 (OLR1), matrix metallopeptidase 13 (MMP13) significantly increased. On the other hand (≤0.65 fold), Apolipoprotein C-III (Apoc3), interleukin 23 alpha subunit p19 (IL23A) and syndecan-1 (SDC1) proteins were also found to have a significant decrease. There was a significant clinical improvement in pain score, quality of life, and joint limitation[14].

In a randomized controlled single-blind study by Varzaityte et al., stage III. changes in joint functions after balneotherapy and mud treatment of patients with knee osteoarthritis were investigated. Thermo-mineralized water bath and local mud were applied 10 times for 1 month, and a significant improvement in joint functions, joint pain, and joint stiffness was detected after 1 month[15].

Min KJ et al. investigated the effect of short term thermo-mineralized water and mud treatment on patients with chronic hip pain. As a result, short term application of thermo-mineralized water and mud is significantly beneficial in patients with chronic hip pain. It is an important study in terms of understanding that patients who have short term breaks and who think that long term benefits can be provided in spa treatments are short in their treatments[16].

In a double-blind randomized controlled study by Kiraly et al., 60 patients with knee osteoarthritis were divided into 2 groups. Both groups received 10 times mud treatment from 2 different regions for 2 weeks. In the determination made at the end of the study and after 12 weeks, there was a significant ($p < 0.05$) improvement in pain, joint function, and quality of life in both groups[17].

In the study conducted by Cozzi et al. in Italy, the effects of mud treatment in seronegative spondylarthritis were investigated. In enteropathic spondylarthritis, it has been found that there is a

significant improvement in bowel symptoms without worsening. It is also shown that ankylosing spondylitis is clinically improved with long term especially cutaneous lesions, joint function, psoriatic arthritis[18].

The use of mud, which is used extensively in locomotor system diseases, is also common for the treatment, care and cosmetic purposes of skin diseases. It has numerous benefits for the skin. Some of these are to remove acne and remove blackheads. It also has the feature of removing skin blemishes that occur during pregnancy or from the sun. It softens and revitalizes the skin since it prevents blood collection. It cleans the dead skin and makes the skin smooth by exfoliation. It has been proven to benefit diseases such as psoriasis and eczema. It has the anti-aging effect that combats wrinkles with its antioxidant feature. It makes detox by absorbing the toxins and harmful substances from the body and removing them from the body. It is effective against cellulite, relaxes the body, relieves fatigue, and inflammation[1]. It has been stated that mud application is also actively used in the treatment of seborrheic dermatitis in the article of Borda et al[19].

The physical and chemical response of the skin was investigated in the short-term application of Hamed and his friends with Dead Sea mud on normal people's skin. The moisture of the skin, transepidermal water loss, erythema, and melanin levels and ph values of the skin were checked at 30th and 60th minutes after mud application. Accordingly, it has been observed that mud application increases the moistening of the skin and does not harm barrier resistance, ph and melanin-erythema levels[20].

The efficacy of mud therapy in psoriatic arthritis patients treated with TNF inhibitors (Tumor necrosis factor) has been investigated by Cozzi et al. There are 36 patients treated with TNF inhibitors for the last 6 months were divided into 2 randomized groups of 18. In the first group, in addition to drug treatment, 12 mud packs and 12 thermo-mineralized water treatments were applied, and the other 18 patients continued to receive drug treatment only. In patients, parameters such as sensitive and swollen joints, VAS (Visual Analog Scale) scrub, SF (Short Form)-36 scrub and blood CRP were monitored for 45 days. As a result, significant improvements were detected in PASI ($p < 0.005$), DAS28 ($p < 0.05$), and HAQ ($p < 0.001$) parameters between Day 1 and Day 45 in the mud treatment group. These data demonstrate a marked improvement in joint and skin inflammation and clinical findings in patients treated with mud and thermo-mineral water[21].

Clinical studies conducted by Riyaz et al. reveal the benefits of using mud in psoriasis, atopic dermatitis, vitiligo and many other types of dermatitis[22].

Constantino et al. conducted a study on 30 patients with psoriasis disease. 19 Male and 11 female patients were randomly divided
into 2 groups. Group 1 received mud and thermo-mineralized bath treatment for 12 days, and group 2 received medication only. As a result, it was found that rash and itching symptoms were significantly decreased in the same ratio ($p < 0.05$) in both groups. In this case, the researchers concluded that mud and bath treatment in psoriasis disease has the same value as drug treatment[23].

Carabelli et al. performed thermal mud applications on normal, dry and seborrheic skin and evaluated the results after 14 days of treatment. Accordingly, it was found that cutaneous ph and sebum level improved in seborrheic skins applied with thermal mud[24].

THERAPEUTIC AND COSMETIC USE OF CERTAIN PLANTS

Plants in other words herbs have been used in medicine for thousands of years. Physicians have used many different plants in the treatment of diseases since ancient times. The first accepted medical botanist Dioscorides (AD 40-90) represented more than a hundred medicinal plants in his well-known book De Materia Medica. He also explained the use of these plants and approximately six hundred herbal drugs. We provide some of our important drugs from plants. For instance, one of the most common antipyretic drugs aspirin is a semi-synthetic drug made from

willow bark. Several examples like these have lead to medical practice today to treatment diseases and improve health.

Osteoarthritis is one of the most prevalent chronic joint inflammatory disease in the world. It is mainly affecting significant joint replacements such as the hips, knees, and hands[25] progressing chronic pain, disability, and occasionally swelling in the joints. Research indicates that patients with osteoarthritis prefer complementary alternative medications to conventional drug medications[26]. Various plants have been used for a long time with traditional knowledge for treatments of inflammatory arthritic conditions. In a study reviewed by Dragos et al. shows that topical usage of certain plants such as *Arnica montana*, *Boswellia spp.*, *Symphytum officinalis*, and *Zingiber officinalis* improving the life quality and reducing the pain[27].

Besides joint diseases, herbs are commonly used for skin problems like acne, alopecia, bacterial and fungal infections of skin, chronic venous insufficiency, dermatitis, herpes simplex, herpes zoster, hyperhidrosis, pruritus, psoriasis, wounds and burns[28]. They are also applied for skin care purposes apart from diseases. These herbal remedies are applied topically in different forms, for instance, dried extracts, creams, ointment, solutions and wet-wrap dressings[29]. Herbal products consist of various compounds with different properties in comparison to isolated compounds. Some of these compounds of herbal medicine can act primarily as antimicrobials, others can serve as antioxidants, some may have anti-inflamatory functions and and the rest of them can still maintain healing properties[30].

According to the data from the Ministry of Economy, Turkey; most of the multinational companies operating in the cosmetics industry engage in the production and marketing activities in Turkey. Many foreign investors in the sector produce through license agreements and joint ventures. Depending on economic developments in Turkey, cosmetics and personal care products market has grown an average of 10% each year. It is estimated that the share of natural cosmetics and personal care products in the market is around 5%. This situation is in parallel with the world market. Only 10% of products marketed in Turkey are products originating in Turkey.

Hair care products have the biggest share among the sector products. Shampoos constitute about 59% of hair care products. Shaving products, depilators, bath and shower products, especially hand soaps, lip and eye make-up materials, deodorants and antiperspirant products, perfumes, colognes and baby care products are the main products.

In our country, natural soaps, shampoos, other hair care products and hair dyes, skin care products, body care products and other natural cosmetics have started to be produced in the sector in recent years. Especially natural soap and shampoo production is carried out by many small scale companies across the country. The world-famous laurel and olive oil soaps are produced in large quantities in Turkey.

Some of the companies producing cosmetics also develop products with natural additives. In the cosmetic products category, approximately 170,000 products, 46,000 of which are local, are distributed in the local market. The number of companies in the sector registered in the electronic notification system of the Ministry of Health is 3,250 and 14,000 people are employed in these companies. In addition to large firms in the market, many small and medium sized firms that produce natural, organic and natural additive products also operate[31].

Republic of Turkey Ministry of National Education Ministry of Education Beauty and Hair Care Services Basic Cosmetics 815sbg003 Megep training;

Cosmetics are as old as human history. Since the ages, humans have treated their diseases with plants, water or mud, discovered the benefits of spring waters, burned incense to repel evil spirits, sometimes painted their faces and bodies with earth dyes to impress their gods, sometimes frighten their enemies. As well as men are painted to impress their enemies, women are also painted to decorate. The best example of this in history is the Egyptian

queen Cleopatra. Cleopatra applied not only make-up but also exfoliation applications with Nile sand, entered the beauty baths with milk and applied masks with various herbal mixes, rubbed her body with essences and perfumed after the mud bath. Clay in the form of clay has been used by humans for medicine for thousands of years as it has all the richness of the earth. Today, as it was in the past, it is continued to use the active substances by entering directly into mud baths, and it is continued to be used by taking the valuable substances of the mud or by adding them to oily skin care products and body thinning care products[32].

POSSIBLE USE OF HERBAL MUD AS AN ANTIMICROBIAL/ANTIVIRAL AGENT

Although the microbiota contained by the mud gives it some microbiological active properties, it has not been studied enough. On the other hand, many medicinal plants have antibacterial, antifungal and antiviral actions. *Mentha piperita, Thymus vulgaris, Hydrastis Canadensis, Berberis vulgaris, Urtica dioica, Curcuma longa, Zingiber officinale* are among the commonly used medicinal plants as infusion or tincture[33]. This kind of plants are especially used in the treatment of infectious diseases today. Because in recent years, antimicrobial agent resistance has increased considerably, and besides, causes such as treatment costs, negative side effects of antibiotics/drugs have required low-toxic, easily accessible supportive or therapeutic natural products.*Glycyrrhiza glabra, Cistus creticus,* and *Sambucus nigra* plants stand out due to their antiviral properties. It is sold commercially in forms such as syrup and pastille[33,34].

Medicinal plants can show their antimicrobial effects not only systemically but also locally in topical applications. Various extracts or creams from *Panax ginseng, Calendula officinalis, Arnica montana, Aloe vera, Vitis vinifera seed, Angelica sinensis, Ananas comosus, Symphytum officinale, Glycyrrhiza glabra, Hypericum perforatum, Momordica charantia* plants are used topically. Studies have shown that these medicinal plants have anti-inflammatory, antimicrobial, itch relief, and soothing effects, and proliferative properties on epidermis cells. Accordingly, they have topical use in wound treatment. In some studies, it has been determined that extracts obtained from *Panax ginseng, Calendula officinalis, Arnica montana* plants have anti-inflammatory and antimicrobial properties and have a wound-healing effect in vitro and in vivo testings[35,36,37].

Aloe vera has been shown to improve eczema lesions on animal and human studies and to reduce *Staphlyococcus aureus* colonization, and IgE levels[38, 39, 40]. Theodaris (2017) found in his double-blind randomized controlled study that medicinal plant containing cream significantly reduced psoriasis and eczema lesions[41]. Cream made from *Rheum palmatum* root extract (Chinese rhubarb), *Scutellaria baicalensis* root extract (Chinese skullcap), *Cnidium monnieri* fruit extract (Monnier's snow parsley), *Glycyrrhiza glabra* (licorice) has been shown to reduce atopic dermatitis lesions in open-labelled self-controlled clinical research[42]. It has been shown in vitro that olive oil and propolis have antiviral activity on Herpes viruses that cause shingles and herpes[43]. Lip cream made from propolis extract has been shown to provide significant results for herpes treatment in a double-blind randomized controlled trial[44].

Considering all of these, the use of Traditional and Complementary Medicine applications in dermal treatments is gradually increasing[45, 46]. There are many creams and oil essences on the market used in the treatment of dermatological problems such as psoriasis, eczema, wounds, herpes, and skin inflammation[42,47,40, 48]. Studies are showing that peloids are effective in such diseases[22, 23, 24]. The connection between the positive effect of peloids in other dermatological pathologies with the microbiota in it has not been evaluated much[7]. The microbiota contained by peloids is quite likely to be effective on pathogenic species. Therefore, it is thought that the phyto-pelodies that will occur when mixed with medicinal plants will have a higher antimicrobial effect. On the other hand, it is also possible that the antimicrobial effect of plants will affect the peloid flora and disrupt its natural structure. There is a related data deficiency in the

literature. Accordingly, it is thought that the production of phyto-peloids and their effects are extremely important. But these studies mostly remain at the preclinical level. While in order to have sufficient evidence, it should be supported by clinical research.

CONCLUSION

It is a fact that when an evaluation is made in the light of scientific data, official explanations and practices and market researches, mud has been used in the health and cosmetics industry for many years with pure, processed or additives.

When we look at around 1300 thermo-mineral water sources, hundreds of small lakes and deltas and thousands of years of Anatolian geography that we know exist in our country, we see that the number of muds licensed by the Ministry of Health is still around 10 and there is no inventory of our country. In the scientific community, the number of articles related to mud does not exceed several.

In an environment where the world's demand for natural treatments and natural care products has increased so much, we must recognize our presence in mud and combine mud with natural medicinal plants to create innovations in the field of health and cosmetics. Also, turning this into an investment and earning will undoubtedly be possible by doing more researches in this field. Integrating mud into spa tourism and making mud an indispensable product of every region and every facility requires creating unique products and treatment-care packages with medicinal plants, recognizing mud and medicinal plants and using them correctly. In this regard, determining the potential of our country and activating this potential is necessary for the future.

As mentioned above, it is known that there is a synergistic effect in the treatment mechanisms of herbal products. The compounds they contain are involved in the treatment process in coordination. Accordingly, it can be said that the use of mud with medicinal plants will be more effective than its use alone. Mud treatment should be combined with different medicinal plants on related diseases and the results should be compared with its use unaccompanied.

Mud is a well-expanded health and cosmetic product with its known varieties and materials such as various herbs, essences, oils, fruit extracts etc. added to it. Empirical use of mud, which is a miracle of nature, for thousands of years, has been proven as a result of scientific studies on its contribution to human health and care. Determining the mud sources existing in our country, taking inventory, determining the necessary tests, medical and cosmetic benefits will undoubtedly be possible with the further study and production of our scientists.

We hope that this study will increase awareness of the subject and will enable scientists to conduct more scientific research and produce scientific publications on mud sources and natural plants in their region. The fact that our official institutions and organizations also focus on mud and natural plants while carrying out their duties, to produce joint projects with scientific communities and to support scientific projects, will result in the use of these important values of our country more rationally for the benefit of humanity.

REFERENCES

1. Oyuryüz ZŞ, Gürel A. Humik maddelerin sağlık sektöründe kullanımı ve girişimcilik faaliyetleri. *SAU Fen Edebiyat Dergisi.* 2012; 1.
2. Dönmez A. Balneoterapi yöntemleri. *Balneoloji ve Kaplıca Tıbbı. İstanbul: Nobel Tıp Kitabevleri.* 2002: 57-64.
3. Gürdal H. Peloidler. *Balneoloji ve Kaplıca Tıbbı. İstanbul: Nobel Tıp Kitabevleri.* 2002: 97-112
4. https: //www.saturk.gov.tr.türkiye.
5. Çelik-Karakaya M, Karakaya N. Kaplıca Tedavisinde Kullanılan Termal Çamurların Uygunluğunu Belirleyen Parametreler. *1. Tıbbi Jeoloji Çalıştayı Kitabı.* 2009: 31-43.
6. Quintela A, Terroso D, da Silva, E.F, et al. Certification and quality criteria of peloids used for therapeutic purposes. *Clay Minerals*. 2012; 47(4):441-451
7. Pesciaroli C, Viseras C, Aguzzi C, et al. Study of bacterial community structure and diversity during the maturation process of a therapeutic peloid. *Applied Clay Science*. 2016; 132: 59-67.

8. https://hsgm.saglik.gov.tr/tr/cevresagligi-suguvenligi/su-güvenligi-ve-kaplıcalar-birimi/peloid-nedir.html 17.02.2020
9. Gürdal H. Peloid uygulamaları. *Balneoloji ve Kaplıca Tıbbı. Istanbul: Nobel Tıp Kitabevleri*. 2002: 107-110.
10. Karagülle MZ. Kaplıca tedavisi, balneoterapi ve hidroterapi. *Kaplıca Tıbbı ve Türkiye Kaplıca Rehberi. İstanbul: Nobel Tıp Kitabevleri.* 2011: 1-21
11. Martínez-Villegas N, Muñoz MS, González-Hernández P, et al. Inorganic and organic characterization of Santa Lucía salt mine peloid for quality evaluations. *Environmental Science and Pollution Research.* 2019: 1-15.
12. http://konyaeah.saglik.gov.tr/TR.278802/tibbi-camur-tedavisi.html.
13. Barassi G, Obrero-Gaitan E. Integrated Thermal Rehabilitation: A New Therapeutic Approach for Disabilities. *Adv Exp Med Biol.* 2020; 1251: 29-38
14. Angioni MM, Denotti A, Pinna S. Spa therapy induces clinical improvement and protein changes in patients with chronic back pain. *Reumatismo.* 2019 Oct 24; 71(3):119-131.
15. Varzaityte L, Kubilius R, Rapoliene L. The effect of balneotherapy and peloid therapy on changes in the functional state of patients with knee joint osteoarthritis: a randomized, controlled, single- blind pilot study. *Int J Biometeorol*. 2019 Sep 6. doi: 10.1007/s00484-019-01785-z
16. Min KJ, Choi H, Tae BS, et al. Short-term benefits of balneotherapy for patients with chronic pelvic pain: a pilot study in Korea. *J Obstet Gynaecol.* 2019 Aug 28: 1-6. doi: 10.1080 01443615.2019.1631771.
17. Király M, Kővári E, Hodosi K, et al. The effects of Tiszasüly and Kolop mud pack therapy on knee osteoarthritis: a double-blind, randomized, non-inferiority controlled study. *Int J Biometeorol.* 2019 Aug 3. doi: 10.1007/s00484-019-01764-4
18. Cozzi F, Galozzi P, Ciprian L. Mud-bath treatment of seronegative spondyloarthritis: experience at the Euganean Thermal Area. *Int J Biometeorol.* 2019 Jul 24. doi: 10.1007/s00484-019-01761-7.
19. Topside LJ, Perper M, Keri JE. Treatment of seborrheic dermatitis: a comprehensive review. *J Dermatolog Treat.* 2019 Mar; 30(2):158-169. doi:10.1080/09546634.2018.1473554.
20. Hamed S, Almalty AM. Skin tolerance of three types of dead sea mud on healthy skin: a short-term study. *J Cosmet Sci*. 2018 Jul / Aug; 69 (4): 269-278.
21. Cozzi F, Raffeiner B, Beltrame V. Effects of mud-bath therapy in psoriatic arthritis patients treated with TNF inhibitors. Clinical evaluation and assessment of synovial inflammation by contrast-enhanced ultrasound (CEUS). *Point Bone Spine.* 2015 Mar; 82 (2): 104-8. doi: 10.1016/j.jbspin.2014.11.002.
22. Riyaz N, Arakkal FR. Spa therapy in dermatology. *Indian J Dermatol Venereol Leprol*. 2011 Mar-Apr; 77 (2): 128-34. doi: 10.4103/0378-6323.77450.
23. Costantino M, Lampa E. Psoriasis and mud bath therapy: clinical-experimental study . *Clin Ter*. 2005 Jul-Aug; 156 (4): 145-9.
24. Carabelli A, De Bernardi di Valserra G, De Bernardi di Valserra M. Effect of thermal mud baths on normal, dry and seborrheic skin. *Clin Ter*. 1998 Jul Aug; 149 (4): 271-5.
25. Therkleson T. Topical ginger treatment with a compress or patch for osteoarthritis symptoms. *J Holist Nurs*. 2014;32(3):173–182. doi:10.1177/0898010113512182.
26. Lapane KL, Sands M, Yang S, et al. Use of complementary and alternative medicine among patients with radiographic confirmed knee osteoarthritis. *Osteoarthritis Cartilage*. 2012 Jan;20(1):22-8. doi: 10.1016/j.joca.2011.10.005.
27. Dragos D, Gilca M, Gaman L, et al. Phytomedicine in joint disorders. *Nutrients*. 2017 Jan 16;9(1). pii: E70. doi: 10.3390/nu9010070.
28. Shenefelt PD. Herbal treatment for dermatologic disorders. *Herbal Medicine: Biomolecular and Clinical Aspects*. 2nd edition.
29. Hussain Z, Thu HE, Shuid AN, et al. Phytotherapeutic potential of natural herbal medicines for the treatment of mild-to-severe atopic dermatitis: A review of human clinical studies. *Biomed Pharmacother.* 2017 Sep;93:596-608. doi: 10.1016/j.biopha.2017.06.087.
30. Amparo TR, Seibert JB, De Abreu Vieira PM, et al. Herbal medicines to the treatment of skin and soft tissue infections: advantages of the multi-targets action. *Phytotherapy Research.* 2019;1–10. doi: 10.1002/ptr.6519
31. https://ticaret.gov.tr/data/5b87000813b8761450e18d7b/Kozmetik.pdf 17.02.2020
32. http://megep.meb.gov.tr/mte_program_modul/moduller_pdf/Basic%20Kozmetik.pdf 17.02.2020
33. Bone K, Mills S. Principles and practice of phytotherapy: modern herbal medicine. *2nd edn. Churchill livingstone: Edin-burgh (UK).*
34. Stepień AE, Gorzelany J, Matłok N. et al. The effect of drying methods on the energy consumption, bioactive potential and colour of dried leaves of Pink Rock Rose (Cistus creticus). *J Food Sci Technol*. 2019;56:2386–2394. https://doi.org/10.1007/s13197-019-03656-2

35. Choi, S. Epidermis proliferative effect of thePanax ginseng Ginsenoside Rb 2. *Archives of pharmacal research*, 2002;25(1):71-76.
36. Kim W K, Song SY Oh WK, et al. Wound-healing effect of ginsenoside Rd from leaves of Panax ginseng via cyclic AMP-dependent protein kinase pathway. *European journal of pharmacology*, 2013;702(1-3):285-293.
37. Das T, Debnath J, Nath B, et al. Formulation and evaluation of an herbal cream for wound healing activity. *Int J Pharm Pharm Sci*, 2014;6:693-7.
38. Finberg MJ, Muntingh GL, Van Rensburg CE. A comparison of the leaf gel extracts of Aloe ferox and Aloe vera in the topical treatment of atopic dermatitis in Balb/c mice. *Inflammopharmacology*. 2015;23:337–341.
39. Sirikudta W, Kulthanan K, Varothai S, et al. Moisturizers for patients with atopic dermatitis: an overview. *J Allergy Ther*. 2013;4(4), 1-6.
40. Chew YL, Al-Nema M, Ong VWM. Management and treatment of atopic dermatitis with modern therapies, complementary and alternative medicines: A review. *Oriental Pharmacy and Experimental Medicine*, 2018;18(2):67-76.
41. Theoharides TC, Stewart JM, Tsilioni I. Tolerability and benefit of a tetramethoxyluteolin-containing skin lotion. *International journal of immunopathology and pharmacology*, 2017;30(2):146-151.
42. Bomstein Y, Rozenblat S. Treatment of atopic dermatitis with KAM-3008, a barrier-based, non-steroidal topical cream. *Journal of Dermatological Treatment*. 2015;26(5):426-430. doi: 10.3109/09546634.2015.1021238
43. Altındiş M, Aslan FG, Uzuner H, et al. Zeytin yaprağı ekstresi ve propolisin herpes simpleks virüsü tip 1 üzerine antiviral etkisinin asiklovir ile karşılaştırılması. *Mikrobiyol Bul*. 2020;54(1):79-94.
44. Jautová J, Zelenková H, Drotarová K, et al. Lip creams with propolis special extract GH 2002 0.5% versus aciclovir 5.0% for herpes labialis (vesicular stage). *Wiener Medizinische Wochenschrift.* 2019;169(7-8):193-201.
45. Kalaaji AN, Wahner-Roedler DL, Sood A, et al. Use of complementary and alternative medicine by patients seen at the dermatology department of a tertiary care center. *Complementary therapies in clinical practice.* 2012;18(1):49-53.
46. Murphy EC, Nussbaum D, Prussick R, et al. Reply to:"Response to:'Use of complementary and alternative medicine by patients with psoriasis'". *Journal of the American Academy of Dermatology*, 2019;81(4) e107-e110.
47. Wu J, Li H, Li M. Effects of baicalin cream in two mouse models: 2, 4-dinitrofluorobenzene-induced contact hypersensitivity and mouse tail test for psoriasis. *International journal of clinical and experimental medicine.* 2015;8(2):2128-37
48. Chiang CC, ChengWJ, Lin CY, et al. Kan-Lu-Hsiao-Tu-Tan, a traditional Chinese medicine formula, inhibits human neutrophil activation and ameliorates imiquimod-induced psoriasis-like skin inflammation. *Journal of ethnopharmacology*. 2020;246:1122-46.