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

Mustafa Yasar[1] OguzhanSenogul[2] Ali Gok[3] Kagan Agan[4] Aydan Fulden Agan[4] Ersin Beyazcicek[5*] Sinem Kantarcioglu Coskun[6]

[1] RTM Clinic, Istanbul, Turkey
[2] Naturin Nutraceuticals, 35477, Izmir, Turkey
[3] Experimental Animals Application and Research Center, Düzce University, 81100, Düzce, Turkey
[4] Traditional and Complementary Medicine Application and Research Center, Düzce University, 81100, Düzce, Turkey
[5*] Department of Physiology, Medicine Faculty, Düzce University, 81100, Düzce, Turkey
[6] Department of Pathology, Medicine Faculty, Düzce University, 81100, Düzce, Turkey

*Corresponding Author: Ersin BEYAZÇİÇEK, e-mail: beyazcicek13@gmail.com

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

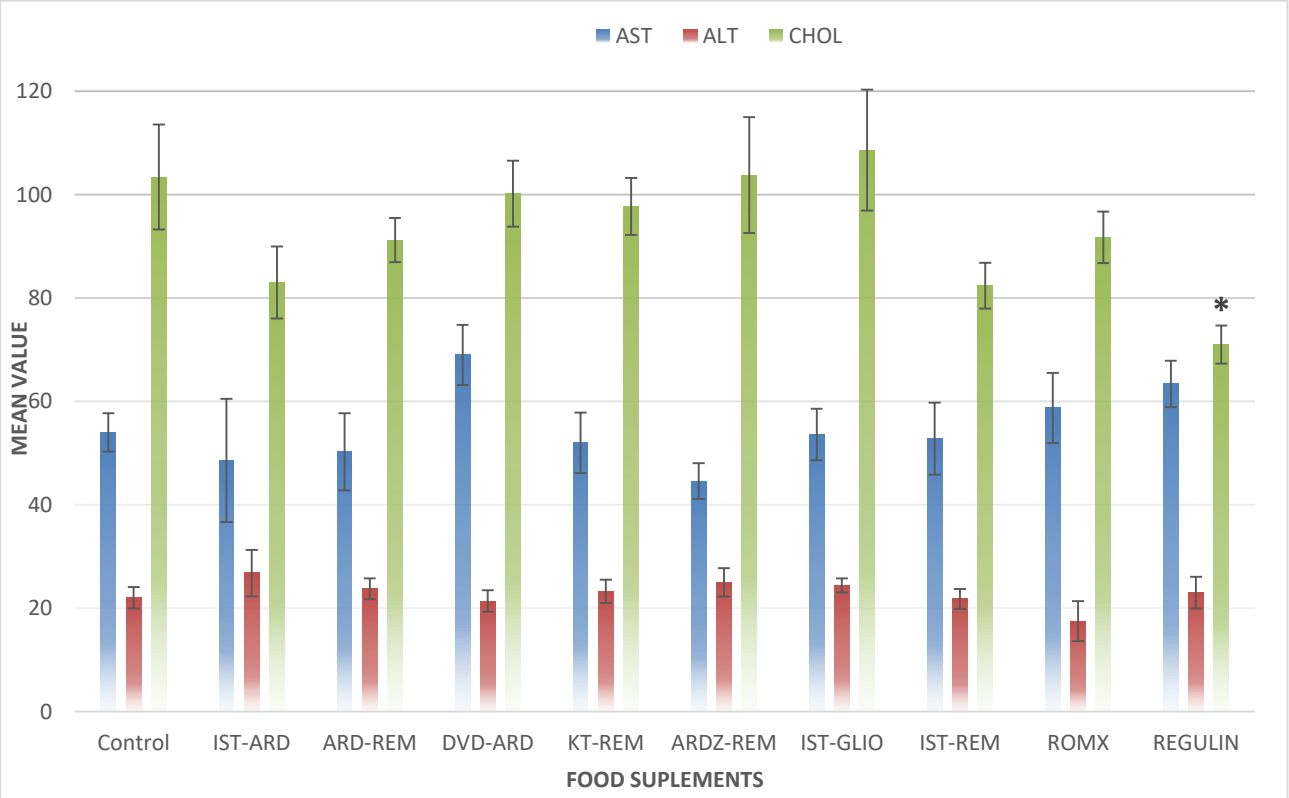
Clinical observation parameters

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

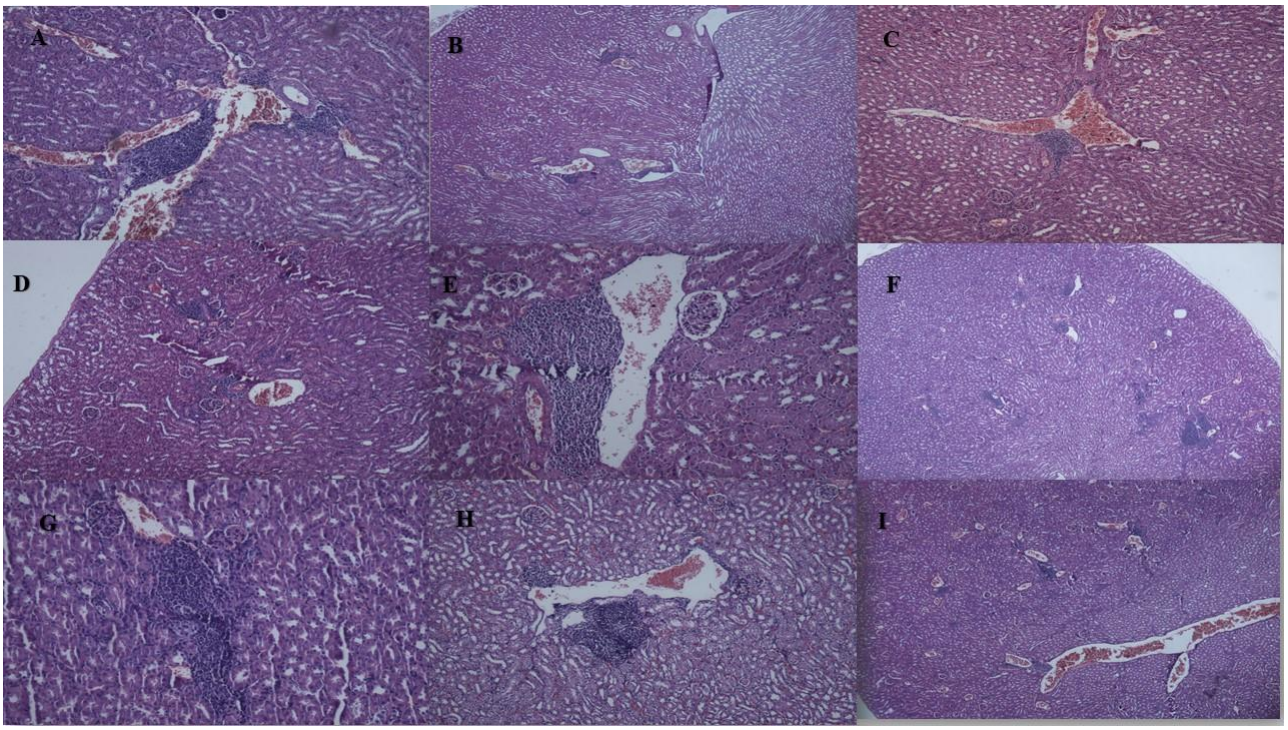


Figure 1. In cortex and corticomedullary junction of the kidneys, perivascular mononuclear inflammatory cells observed in the tubulointerstitial area. (**A:** KBx10, **B:** Control group x4, **C:** Control group x10, **D:** DVD-ARDx10, **E:** KT-REMx20, **F:** ARDZ-REM x4, **G:** IST-GLIOx20, **H:**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

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