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Effect of DVD-REG® Food Supplement on Acute and Sub-Acute Toxicity on Rats

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ABSTRACT

The use of herbs that are known to be medically useful in the field of traditional and complementary medicine has been widely used as a mixture in different proportions. Material and temporal restrictions in drug production provide a significant benefit in terms of using supportive herbal ingredients instead of treatment. However, when creating these components, it is necessary to determine the nontoxic dose in many areas, from the origin of plants to the active substance and the dose range that can be used. In this study, acute and subacute toxicity analyzes of DVD-REG® herbal supplements known to be used in Remember Regeneration Therapy Method (RTM) were performed. Clinical observation findings were evaluated according to ISO 10993-11:2017 standards and no changes were made between the control and application groups. Hematological and biochemical blood parameters were evaluated as control group, acute group, subacute group, and post-subacute group and no changes were found between the control and administration groups. There was no pathological finding among the groups in liver, heart, kidney and spleen tissues where histological toxicity was investigated. In this study, which are now used in Turkey food supplement data obtained from the DVD-REG® was researched it will benefit for chronic effects of acute, subacute and post-subacute toxicity tests.

Key words: DVD-REG®, Acute Toxicity, Subacute Toxicity, Traditional and Complementary Medicine**,** Herbal Supplements

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DVD-REG® Takviye Edici Gıda Ürününün Akut Sub-Akut Toksisitesinin Sıçanlar Üzerinde Araştırılması

ÖZET

Geleneksel ve tamamlayıcı tıp alanında faydalı olduğu bilinen bitkilerin karışım olarak kullanılması yaygınlaşmaktadır. İlaç üretimindeki maddi ve zamansal zorluklar, destekleyici bitkisel bileşenlerin kullanma yöneliminde artışa sebep olmuştur. Bununla birlikte, bu bileşenleri oluştururken, bitkilerin kökeninden aktif maddeye ve kullanılabilecek doz aralığına kadar birçok alanda toksik olmayan dozu belirlemek gerekmektedir. Bu çalışmada Remember Regeneration Tedavide (RTM) kullanıldığı bilinen DVD-REG® bitkisel takviyelerinin akut ve subakut toksisite analizleri yapılmıştır. Klinik gözlem bulguları ISO 10993-11:2017 standartlarına göre değerlendirilmiş olup kontrol ve uygulama grupları arasında herhangi bir değişikliğe rastlanılmamıştır. Hematolojik ve biyokimyasal kan parametreleri control grubu., akut grubu., subakut grubu. ve post-subakut grubu. olarak değerlendirilmiş kontrol ve uygulama grupları arasında herhangi bir değişiklik tespit edilmemiştir. Histolojik açıdan toksisitenin araştırıldığı karaciğer, kalp, böbrek ve dalak dokularında gruplar arasında patolojik bir bulguya rastlanılmamıştır. Bu çalışma ile Türkiye'de kullanımakta olan gıda takviyesi DVD-REG®'in akut, subakut ve post-subakut etkileri araştırılmış olup elde edilen veriler kronik toksisite testleri için fayda sağlayacaktır.

Anahtar Kelimeler: DVD-REG®, Akut Toksisite, Subakut Toksisite, Geleneksel ve Tamamlayıcı Tıp, Gıda Takviyesi

INTRODUCTION

Herbal remedies, known as phytochemical or botanical medicines, according to the definition of the World Health Organization (WHO); It consists of herbs, herbal ingredients, preparations from plants and finished herbal products that contain active ingredients of plants or other plant materials or combinations thereof.

According to the reports of the World Health Organization, it is known that approximately 4 billion people in the world apply to herbal medicines without resorting to modern medicine (Phua, Zosel ve Heard, 2009). Although the use of herbal medicines in medicine goes back to ancient times, epigenetic changes, which provide important findings to personalized medicine in understanding the origin of diseases, have been introduced into the literature as a herbal treatment protocol. This treatment method is referred to as Regeneration Recall Therapy Method (RTM). Diseases arise as a reflection of individuals epigenetic changes, but this method (RTM) is a holistic approach that includes a combination of various complementary and traditional medical methods. Since most of these changes, including DNA methylation, chromatin remodeling, histone modifications and non-coding RNA mechanisms, are theoretically reversible, it is suggested that these changes that can cause disease can be reversed with the RTM model (Yasar, 2019). In this treatment method, the interactions of herbal mixtures with each other and their application doses are very important. For this reason, the usage intervals that can be safe and useful should be determined.

There are many different herbal medicines used in traditional and complementary medicine and studies revealing side effects (Drew ve Myers, 1997). On the other hand, there is no study that reveals the synergistic effects of these herbs in herbal medicine. The important thing to know about herbal medicine is the pharmaceutical interactions, but the excessive use of these components and their use with different herbal ingredients can affect the organs that have a role in the detoxification mechanism such as various cardiovascular, hepatic and renal system (Tovar ve Petzel, 2009). The variety of herbal ingredients used today has diversified with the use of these plants. These plants are used in the pharmaceutical industry, household products, drinks, etc. In addition to their use, it is widely used as a food supplement (Chinedu, Arome, ve Ameh, 2013). The excessive use of these herbs or the combination of unconscious combinations can produce toxic effects. These effects can be mild and severe depending on the changes in the active ingredient content of the plant.

Acute toxicity; this is an experimental study to find the safe and possible toxic dose usage range of any drug or herbal ingredient. This test determines the dose range that kills 50% of the experimental animals called LD_{50} (lethal dose). Keeping the LD_{50} to a minimum level will increase the reliability of that product (Shaikh Nusrat ve Maheshwari, 2016). Acute toxicity are negative side effects that occur when such substances are administered within 24 hours or more. These effects occur in living organ pathology, with findings indicating functional disorders such as variability in biochemical parameters (Akhila, Shyamjith, Deepa ve Alwar, 2007).

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, Curcuma longa, Fumaria officinalis, Cichorium intybus* plants are known to be used in combination or separately as a food supplement herbal product. In the study in which the acute and subacute toxicity of asdamarin 250-1000 mg/kg, known as the active ingredient of *Silybum marianum*, was investigated, no biochemical and histopathological toxicity was found (Illuri vd., 2019). Rosemary headache treatment, in the treatment of inflammatory diseases, as well as liver stomach disorders, antiviral, antimicrobial

and anticancer properties are known. No toxic and adverse effects were seen in the test of acute toxicity (2000 mg/kg) in mice (Berhan vd., 2018). Acute administration of *Curcuma longa* was administered to a single dose up to 5g/kg in male and female rats. Sub-chronic administration, 0.1, 0.25 and 0.5g/kg were administered orally daily. There were no negative side effects and toxicity has not been identified. In the *Curcuma longa* samples, were the genotoxicity test was performed, no chromosomal and DNA structure damage was determined (Liju, Jeena ve Kuttan, 2013). *Fumaria indica* is a medicinal plant that has found wide ranging in Indian medicine. In the *Fumaria indica* toxicity study, dental and male mice were administered orally at doses of 1, 2.5 and 5 g/kg, and no toxicity was observed in any of the three doses (Singh ve Kumar, 2011).

MATERIAL and METHODS

1. Animals

The animals used in the study were obtained from Düzce University Experimental Animals Application and Research Center. 8 weeks old and 250-300 g female Wistar Albino rats were kept in the laboratory at 20-25 ºC room temperature, 55 ± 5% humidity and 12:12 light-dark cycle and were fed with standard pellet feed and ad libitum water. Animals were acclimatized for at least one week before using them for the experiments. Principles of laboratory animal care (NIH publication number 85-23, revised in 1985) guidelines were followed. Düzce University Experimental Animals were carried out with the approval of the Local Ethics Committee (2020.3.4). 32 animals were randomly divided into 4 groups. The rats were divided into four groups as the control (n=8) (0 day), the acute toxicity (n=8) (1 days), the subacute toxicity (7days) (n=8), post-subacute groups (14 days) (n=8).

2. Preparation of Study Material

The herbal mixture used as a food supplement was obtained from Naturin Nutraceuticals (Natural Products Pharmaceutical and Pharmaceutical Raw Materials Industry Trade Limited Company). DVD-REG® includes *Silybum marianum, Rosmarinus officinalis, Curcuma longa, Fumaria officinalis, Cichorium intybus* extracts, which are listed in Table 1. 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 animals.

3. Acute, Subacute and Post-Subacute Toxicity Study

In the experiment to be carried out using the ISO 1099311 toxicity protocol with minor modifications, ISO-10993 standards. Animals with acute, subacute and postsubacute toxicity investigated were administered the DVD-REG® food supplement to rats as much as the recommended daily use. The dosage of these products is calculated according to the daily human use dose of DVD-REG®, which is licensed by the Ministry of Agriculture as a food supplement. Dosage amounts were administered at one time via gavage at 10.62 mg/mL according to daily usage rates. The product dissolved in saline was prepared daily fresh stock solution. The control group was given 1 mg/mL saline daily. Weights of rats were recorded before and after application.

Table 1. Herbal Product Content

Herbal Products	The Botanical Part	Purpose and Function	Percentage of Components	Amount in 1 Capsule (mg)
Silybum marianum	Seed	Active Ingredient	24.21%	129.06
Rosmarinus officinalis	Leave	Active Ingredient	14.53%	77.43
Curcuma longa	Rhizome	Active Ingredient	14.53%	77.43
Fumaria officinalis	Aerial Parts	Active Ingredient	14.53%	77.43
Cichorium intybus	Aerial Parts	Active Ingredient	9.69%	51.65

Animals were sacrificed under ketamine-xylazine anesthesia at the end of the 24-hour experimental observation after product delivery to the acute toxicity experimental group. Blood was taken by cardiac puncture method for biochemistry and hematology parameters. Histopathological examination, the heart, liver, lung, kidney and spleen organs were examined.

The daily dose was 10.62 mg/mL in one time. The product in the acute toxicity experimental group continued to be administered gavage for 1 day. Experimental observations were made during the experiment period. At the end of the administration blood was taken from the heart to examine the biochemical parameters and the rats were sacrified. Tissues were taken into formaldehyde for histopathological examination. Then, biochemical, hematological and histopathological examinations were carried out.

For subacute toxicity administration, the animals were administered product by gavage for one week (7 days). Experimental observations were made during the experiment period. On the 7th day, blood was taken from the heart for biochemical and hematological parameters, sacrificed and tissues fixed in formaldehyde for histopathological examination.

For post-subacute toxicity administration, the animals were administered product by gavage for one week (7 days). Between 7-14 days, we stopped administration product. But experimental 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.

4. Experimental Observatory Parameters

Each animal in each experimental groups was routinely observed during the experiment in accordance with the criteria given in Table 2. This standard prepared according to standard ISO 10993-11:2017 with minor modifications (ISO, 2017).

5. Blood Analysis

Biochemical and hematological analyzes were studied in Düzce University Experimental Animals Application and Research Center. After anesthesia with ketamine (85 mg/kg, intraperitoneally, Ketas, Pfizer) to collect blood samples, blood samples were collected cardiac puncture 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. After clotting for 1 hour at room temperature, the serums were carefully collected by centrifugation (1.500 g, 10 minutes, 4 °C) and stored at -20 °C until analysis. After blood sampling, the animals were sacrificed by decapitation. Biochemistry analyzes were studied on Mindray BS-120 device. P (g/mL) (Inorganic phosphorus), Ca (mg/dL) (Calcium), ALB (g/mL) (Albumin), TG (mg/dL) (Triglyceride), TP (g/L) (Total Protein), TC (mg/dL) (Total cholesterol), CRE (mg/dL) (Creatinine), BIL (mg/dL) (Bilirubin), GGT (U/L) (gamma-glutamyl transpeptidase), ALP (U/L) (Alkaline phosphatase), AST (U/L) (Aspartate aminotransferase), UREA (mg/dL) (Urea nitrogen), ALT (U/L) (Alanine aminotransferase) parameters are evaluated.

Hematology has been studied on BC 5000Vet device. HCT (Hematocrit), HGB (hemoglobin), PLT (Platelet), RBC (Red blood cell), WBC (White blood cell) parameters are evaluated.

6. Histopathological Analysis

For histopathological examination, liver, heart, kidney, spleen and lung tissues of each animal in the experimental groups were dissected. When taking samples of animals in the experimental groups, they were taken in one piece without damaging the organ (liver, heart, kidney, spleen, lung) and fixed in 10% formaldehyde solution. The fixed organs were embedded in paraffin blocks. 5 micrometer thick block sections were taken with microtome from tissues embedded in paraffin blocks. Tissues in the alcohol series were stained with Hematoxylin-eosin dye. The prepared preparations were examined under the Olympus® BX53F microscope.

7. 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. $P \leq 0.05$ was accepted as the statistical significance level.

RESULTS

Experimental Observatory Findings

The 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 the DVD-REG® food supplement were found to be similar to the control group compared to the parameters given in Table 2. There was no statistically significant difference in experimental observation by making observations between groups. Also there was no loss in the number of animals during the experiment.

Biochemical Parameters

DVD-REG® product biochemical data belonging to control, acute, subacute and post-subacute groups are given in Figure 1. According to these data, 13 different biochemical parameters were evaluated and no significant difference was seen between the groups.

Table 2. Observation and Evaluation Criteria

Experimental 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	Motor Skill Symptoms (MSS), Samatomotor, Sensory, Autonomous, Muscular-Nervous Systems
The Convulsion	Clonic, Tonic, Tonic-Clonic Symptoms	Central Nervous System (CNS), Respiration, Muscular-Nervous, Automic
Reflexes	Initial Reflex	Motor Skill Symptoms (MSS), Sensory, Automic, Muscular-Nerve
Oculer Observation	Lacrimation, Miosis, Mydriasis	Autonomic Nervous System (ANS), Irritation
Cardiovascular Observation	Bradycardia, Tachycardia, Arrhythmia, Vasodilation, Vasoconstriction	Motor Skill Symptoms (MSS), Autonomous SS, Cardiac, Circulatory System
Salivation	Quantity	Autonomic Nervous System (ANS)
The Pliorection	Coarse Feathers	Autonomic Nervous System (ANS)
Analgesia	Decreased Analgesia	Central Nervous System (CNS), Sensory
Muscle Tone	Hypotonia, Hypertonia	Autonomic Nervous System (ANS)
Gastrointestinal	Diuresis	Motor Skill Symptoms (MSS), Autonomic Nervous System (ANS), Kidney, Motolite
Skin	Edema, Rash	Tissue Injury, Irritation

Table 3. Biochemistry mean and standard error values of groups

Groups / Biochemistry Parameters	Control Mean±SE	Acute Mean±SE	Subacute Mean±SE	Post-Subacute Mean±SE
ALB (g/L)	51,29±1,36	51,23±1,17	47,90±2,64	50,97±2,36
ALP (U/L)	240,16±20,85	264,90±31,44	249,95±43,55	269,90±41,00
ALT (U/L)	142,18±12,92	121,38±10,89	113,93±13,44	134,25±9,85
AST (U/L)	224,19±18,78	250,66±31,48	195,80±17,82	220,85±61,25
Ca (mg/dL)	13,05±0,23	12,68±0,19	13,23±0,37	14,00±0,18
CREA (mg/dL)	0,15±0,02	0,08±0,01	0,12±0,02	0,10±0,02
GGT (U/L)	4,92±0,33	4,55±0,25	5,45±0,36	4,90±0,50
P (g/mL)	6,63±024	6,01±0,46	6,84±0,53	5,81±096
TC (mg/dL)	106,80±6,91	98,34±9,96	85,13±8,18	92,59±11,75
TG (mg/dL)	134,93±7,43	134,83±17,47	161,51±30,96	262,67±38,40
TP (g/L)	104,84±2,19	103,24±1,86	98,20±3,66	98,90±3,10
UREA (mg/dL)	74,67±3,69	73,84±3,10	71,22±2,62	67,96±0,75

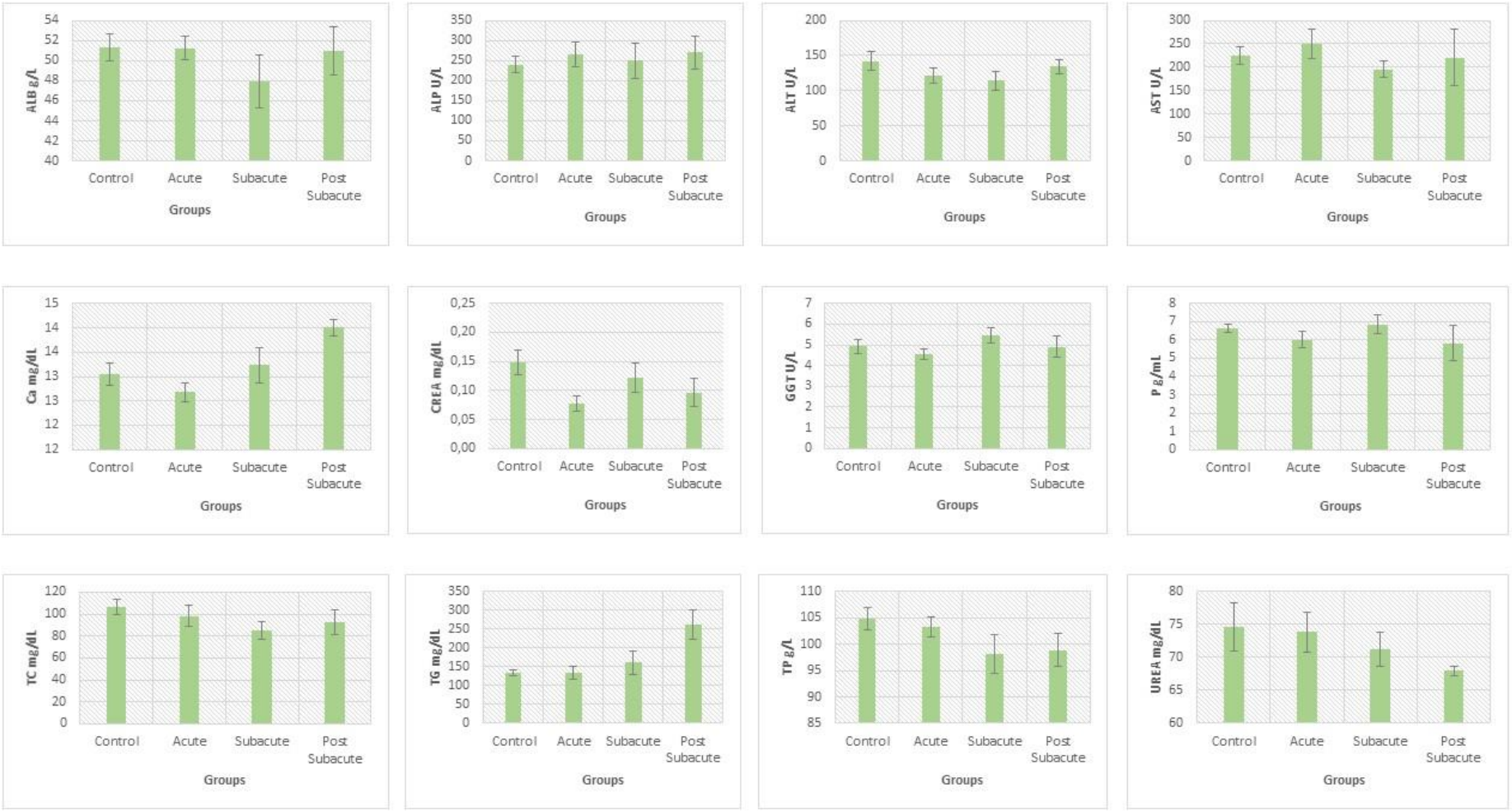

Figure 1. Biochemistry parameters of groups * significant differences with control group ≤ 0.05.

Table 4. Haematological parameters mean and standard error values of groups

Hemogram Parameters	*Groups*	*Mean±SE*	*p Value*
WBC (acute group0^9/L)	**Control**	5,64±0,60	-
	Acute	7,93±0,69	0,11
	Subacute	7,16±0,94	0,45
	Post-Subacute	3,40±0,70	0,30
RBC (10^9/L)	**Control**	7,31±0,22	-
	Acute	7,22±0,16	0,99
	Subacute	7,10±0,23	0,96
	Post-Subacute	6,67±1,28	0,61
HGB (g/dL)	**Control**	13,79±0,40	-
	Acute	13,55±0,41	0,98
	Subacute	13,48±0,34	0,98
	Post-Subacute	12,70±2,31	0,67
HCT %	**Control**	41,66±1,18	-
	Acute	40,35±1,00	0,90
	Subacute	42,16±1,06	1,00
	Post-Subacute	38,13±6,98	0,60
PLT (10^9/L)	**Control**	857,36±45,42	-
	Acute	965,43±17,95	0,74
	Subacute	503,60±248,47	* 0,04
	Post-Subacute	630,50±38,50	0,57

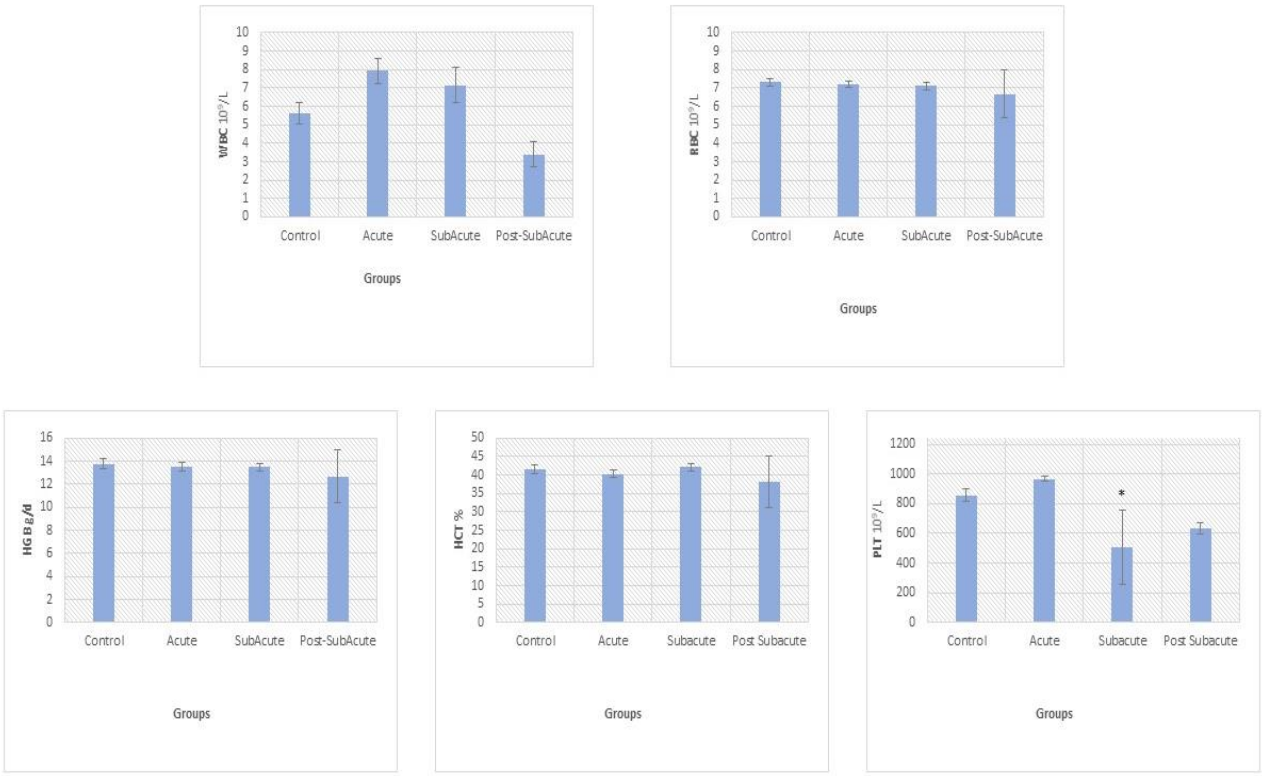

Figure 2. Biochemistry parameters of groups * significant differences with control group ≤ 0.05.

Hematological Parameters

DVD-REG® product hematological data belonging to control, acute, subacute and post-subacute groups are given in Figure 3. According to WBC, RBC, HGB and HTC datas, there was no difference between the groups. In terms of PLT parameter only there is statistically significant between control and the subacute groups. (P= 0.04).

Histopatological Parameters

When removing organs from all experimental groups, they were taken as a single piece without damaging the organ and placed in 10% formaldehyde. Paraffin blocking was performed on the tissues fixed by waiting for 12 hours. Serial sections of 5 micrometer thickness were taken in paraffin blocks. Sections were stained with hematoxylin and eosin stain. Liver tissue preparations examined under the microscope, fat change, spotty necrosis, inflammation, heart tissue preparations, atrial dilatation, inflammation of the heart tissue, kidney tissue tubular atrophy, interstitial fibrosis, inflammation, glomerular damage, lung tissue interstitial and broncho-interstitial pneumonia, degeneration, hyperemia and it was evaluated for necrosis. No pathological finding was found in all preparations examined.

DISCUSSION

Medicinal plants that are used in the world are used to treat diseases, but the components they contain may have side effects for some organisms (Klaassen, 2013). Today, it is a widely accepted complementary treatment method for the use of food supplements containing herbal mixtures. Many people resort to herbal medicines for a number of long-term treatment, such as

diabetes, hypertension, and thyroid disorders (I vd., 2012).

The use of food supplements, and hence the sale, has increased among the public. The majority of food supplements are herbal ingredients. Although these herbal mixtures are often thought to be harmless, it is very important to test their consumption-based state. It is important to test the interaction of active ingredients in these products alone and with each other as toxicology. In this study, acute and subacute toxicity tests of DVD-REG® food supplement, which are used in the markets, were investigated. These evaluations were evaluated by physiologically important experimental observations, blood parameters and histopathological examinations.

Silybum marianum, Rosmarinus officinalis, Curcuma longa, Fumaria officinalis, Cichorium intybus were used in this study that we carried out in order to expand the use of herbal ingredients used in our study and to test the reliability of its use as a food supplement.

It is stated in our study that *Silybum marianum*, which is included in the food supplement, was effective in removing toxic components from the blood. Although there are not many studies evaluating the toxic effect, cancer is prescribed hepatoprotectically for the use of individuals. For example, studies on prostate cancer are known to reduce the level of PSA antigens (Post-White, Ladas ve Kelly, 2007). Many have been in the early experimental trial, but have investigated its effectiveness in treating patients with hepatitis, cirrhosis, or biliary disorders (Angulo vd., 2000; Ferenci vd., 1989; Lucena vd., 2002; Parés vd., 1998; Post-White vd., 2007; Salmi ve Sarna, 1982).

Rosmarinus officinalis is a shrub that can be easily grown in many regions of the world. It has been demonstrated that it is beneficial in preventing diabetes and its complications in diabetic rats with streptozotozine and it can improve lipid metabolism in diabetics (Bakirel, Bakirel, Keleş, Ülgen ve Yardibi, 2008; Ramadan, Khalil, Danial, Alnahdi ve Ayaz, 2013; S. Alnahdi, 2012; Tavafi vd., 2011). It is also known that *Rosmarinus officinalis* has antioxidant, anti-cancer effects (Bakirel vd., 2008; Bourhia vd., 2019; Cattaneo vd., 2015; González-Vallinas, Reglero ve Ramírez De Molina, 2015; Moore, Yousef ve Tsiani, 2016; Stefanovits-bányai vd., 2003). In addition, in a study on rats on the female reproductive system, it reported high abnormal embryo incidence in rats treated with 260 mg/kg rosemary ethanolic extract (Lemonica, Damasceno ve Di-Stasi, 1996).

Curcuma longa, known as turmeric, is a medicinal plant known for its antioxidant, anti-cancer, anti-viral properties. As a result of the studies, histology and cytology of the heart, liver and kidney were evaluated and no toxicity was detected (Tanvir vd., 2017; Tomeh, Hadianamrei ve Zhao, 2019; Zorofchian Moghadamtousi vd., 2014).

Fumaria officinalis is a medicinal plant with proven beneficial effects in many areas from hypertension and heart conditions to gastrointestinal system and liver detoxification (Al-snafi, 2020). It was determined that *Fumaria officinalis* acute and subacute toxicity did not have any toxic effects in the study (Singh ve Kumar, 2011).

Chicory (*Cichorium intybus*) is a plant used as green leafy food. In addition, studies investigating its pharmacological effects have been found to cause hypercholesterolemia, have hepatoprotective effects and inhibit lipid peroxidation (Ahmed, Al-Howiriny, ve Siddiqui, 2003; Atta vd., 2010; Kéry vd., 2001; Kim ve Shin, 1998; Krylova vd., 2006, Rossetto vd., 2005). In the study in which the toxicological effect was investigated, 0.4, 1 and 2.5 g/kg was administered via oral gavage and no toxic effects were detected (Atta vd., 2010).

The herbal mixture we used in our study includes *Silybum marianum, Rosmarinus*

officinalis, Curcuma longa, Fumaria officinalis, Cichorium intybus extracts. Considering the studies investigating the acute and subacute toxicity of these plants, it was found compatible with our data (Atta vd., 2010; Singh ve Kumar, 2011; Tanvir vd., 2017; Tomeh vd., 2019; Zorofchian Moghadamtousi vd., 2014).

Despite the significant decrease in platelet level in the subacute group, no significant change was observed in the postsubacute group. The post-subacute group was administered a single application of the product at the same dose for 7 days with the subacute group. However, before blood and organ samples were taken, animals were observed for 7 days without application of the product in order to evaluate the toxicity that may occur after administration. In addition, when other hematological parameters were evaluated, no significant differences were found between the groups. Since both no significant change was observed in the post-subacute group and other hematological data did not support this finding, it is thought that the decrease in the platelet level seen in the subacute group may occurred temporarily due to the administration.

CONCULISION

In this study, pre-experimental acute and subacute effects of herbal ingredients, which are consumed as DVD-REG® food supplements, on experimental animals were investigated. Through this study, it has been revealed that herbal mixtures can be used when safe dose ranges are investigated in the literature.

REFERENCES

Ahmed, B., Al-Howiriny, T. A. ve Siddiqui, A. B. (2003). Antihepatotoxic activity of seeds of Cichorium intybus. Journal of Ethnopharmacology, 87(2–3), 237–240. https://doi.org/10.1016/s0378-8741(03)00145-4.

Akhila, J. S., Shyamjith, Deepa, ve Alwar, M. C. (2007). Acute toxicity studies and determination of median lethal dose. Current Science, 93(7), 917–920.

Al-snafi, A. E. (2020). Constituents and pharmacology of Fumaria officinalis- A review Constituents and Pharmacology of Fumaria Officinalis - A Review, (January).

Angulo, P., Patel, T., Jorgensen, R. A., Therneau, T. M. ve Lindor, K. D. (2000). Silymarin in the treatment of patients with primary biliary cirrhosis with a suboptimal response to ursodeoxycholic acid. Hepatology, 32(5), 897–900. https://doi.org/10.1053/jhep.2000.18663..

Atta, A. J., Elkoly, T. A., Mouneir, S. M., Kamel, G., Alwabel, N. A. ve Zaher, S. (2010). Hepatoprotective effect of methanol extracts of Zingiber officinale and Cichorium intybus. Indian Journal of Pharmaceutical Sciences, 72(5), 564–570. https://doi.org/10.4103/0250-474X.78521.

Bakirel, T., Bakirel, U., Keleş, O. Ü., Ülgen, S. G. ve Yardibi, H. (2008). In vivo assessment of antidiabetic and antioxidant activities of rosemary (Rosmarinus officinalis) in alloxan-diabetic rabbits. Journal of Ethnopharmacology, 116(1), 64–73. https://doi.org/10.1016/j.jep.2007.10.039.

Berhan, M., Kassahun, D., Ermias, L., Mahlet, A., Tizazu, Z., Gezu, F. ve Awol, M. (2018). Acute skin irritation, acute and sub-acute oral toxicity studies of Rosmarinus officinalis essential oils in mice and rabbit. African Journal of Pharmacy and Pharmacology, 12(26), 389–396. https://doi.org/10.5897/ajpp2018.4957.

Bourhia, M., Laasri, F. E., Aourik, H., Boukhris, A., Ullah, R., Bari, A. ve Gmouh, S. (2019). Antioxidant and Antiproliferative Activities of Bioactive Compounds Contained in Rosmarinus officinalis Used in the Mediterranean Diet. Evidence-Based Complementary and Alternative Medicine, 2019. https://doi.org/10.1155/2019/7623830.

Cattaneo, L., Cicconi, R., Mignogna, G., Giorgi, A., Mattei, M., Graziani, G. ve Marra, M. (2015). Anti-proliferative effect of Rosmarinus officinalis L. extract on human melanoma A375 cells. PLoS ONE, 10(7), 1–18. https://doi.org/10.1371/journal.pone.0132439.

Chinedu, E., Arome, D. ve Ameh, F. S. (2013). A new method for determining acute toxicity in animal models. Toxicology International, 20(3), 224–226. https://doi.org/10.4103/0971-6580.121674.

Drew, A. K. ve Myers, S. P. (1997). Safety issues in herbal medicine: Implications for the health professions. Medical Journal of Australia, 166(10), 538–541. https://doi.org/10.5694/j.1326-5377.1997.tb123246.x.

Ferenci, P., Dragosics, B., Dittrich, H., Frank, H., Benda, L., Lochs, H., Schneider, B. (1989). Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. Journal of Hepatology, 9(1), 105–113. https://doi.org/10.1016/0168-8278(89)90083-4

González-Vallinas, M., Reglero, G. ve Ramírez De Molina, A. (2015). Rosemary (Rosmarinus officinalis L.) Extract as a Potential Complementary Agent in Anticancer Therapy. Nutrition and Cancer, 67(8), 1223–1231. https://doi.org/10.1080/01635581.2015.1082110..

I, Yu, C., Chen, R., Li, J. J., Li, J. J., Drahansky, M., Reading, F. (2012). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. Intech, i(tourism), 13. https://doi.org/10.1016/j.colsurfa.2011.12.014.

ISO. (2017). İnternational Standarts ISO 10993-11. Biological evaluation of medical devices-Part 11. Tests for systemic toxicity., 2017.

Kéry, Á., A. Blazovics, Sz. Fejes, É. Nagy, A. Lugasi, L. Kursinszki, E. Czinner, T. Sz. Kristó, P. Apáti, A. Balázs, ve E. Szőke. (2001). Antioxidant activity of medicinal plants used in phytotherapy. International Journal of Horticultural Science 7(2):28-35.

Kim, M. ve Shin, H. K. (1998). The water-soluble extract of chicory influences serum and liver lipid concentrations, cecal short-chain fatty acid concentrations and fecal lipid excretion in rats. The Journal of Nutrition, 128(10), 1731–1736. https://doi.org/10.1093/jn/128.10.1731.

Klaassen, C. D. (2013). Casarett and Doull's Toxicology and Basic Science of Poisoning. (C. D. Klaassen, Ed.) (Eight Edit). New York: Mc Graw Hill Education.

Krylova, S. G., Efimova, L. A., Vymiatina, Z. K., ve Zueva, E. P. (2006). The effect of cichorium root extract on the morphofunctional state of liver in rats with carbon tetrachloride induced hepatitis model. Eksperimental'naia i klinicheskaia farmakologiia, 69(6), 34–36.

Lemonica, I. P., Damasceno, D. C. ve Di-Stasi, L. C. (1996). Study of the embryotoxic effects of an extract of rosemary (Rosmarinus officinalis L.). Brazilian Journal of Medical and Biological Research, 29(2), 223–227.

Liju, V. B., Jeena, K. ve Kuttan, R. (2013). Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (Curcuma longa L). Food and Chemical Toxicology, 53, 52–61. https://doi.org/10.1016/j.fct.2012.11.027.

Lucena, M. I., Andrade, R. J., de la Cruz, J. P., Rodriguez-Mendizabal, M., Blanco, E. ve Sánchez de la Cuesta, F. (2002). Effects of silymarin MZ-80 on oxidative stress in patients with alcoholic cirrhosis. Results of a randomized, double-blind, placebo-controlled clinical study. International Journal of Clinical Pharmacology and Therapeutics, 40(1), 2–8. https://doi.org/10.5414/cpp40002.

Moore, J., Yousef, M. ve Tsiani, E. (2016). Anticancer Effects of Rosemary (Rosmarinus officinalis L.) Extract and Rosemary Extract Polyphenols. https://doi.org/10.3390/nu8110731.

Parés, A., Planas, R., Torres, M., Caballería, J., Viver, J. M., Acero, D. ve Rodés, J. (1998). Effects of silymarin in alcoholic patients with cirrhosis of the liver: Results of a controlled, double-blind, randomized and multicenter trial. Journal of Hepatology, 28(4), 615–621. https://doi.org/10.1016/S0168-8278(98)80285-7.

Phua, D. H., Zosel, A. ve Heard, K. (2009). Dietary supplements and herbal medicine toxicities-when to anticipate them and how to manage them. International Journal of Emergency Medicine, 2(2), 69–76. https://doi.org/10.1007/s12245-009-0105-z.

Post-White, J., Ladas, E. J. ve Kelly, K. M. (2007). Advances in the use of milk thistle (Silybum marianum). Integrative Cancer Therapies, 6(2), 104–109. https://doi.org/10.1177/1534735407301632.

Ramadan, K. S., Khalil, O. A., Danial, E. N., Alnahdi, H. S. ve Ayaz, N. O. (2013). Hypoglycemic and hepatoprotective activity of Rosmarinus officinalis extract in diabetic rats. Journal of Physiology and Biochemistry, 69(4), 779–783. https://doi.org/10.1007/s13105-013-0253-8

Rossetto, M., Lante, A., Vanzani, P., Spettoli, P., Scarpa, M. ve Rigo, A. (2005). Red chicories as potent scavengers of highly reactive radicals: A study on their phenolic composition and peroxyl radical trapping capacity and efficiency. Journal of Agricultural and Food Chemistry, 53(21), 8169–8175. https://doi.org/10.1021/jf051116n.

S. Alnahdi, H. (2012). Effect of Rosmarinus Officinalis Extract on some Cardiac Enzymes of Streptozotocin-induced Diabetic Rats. International Journal of Health Science, 2(4), 33–37. https://doi.org/10.5923/j.health.20120204.03.

Salmi, H. A. ve Sarna, S. (1982). Effect of silymarin on chemical, functional, and morphological alterations of the liver: A double-blind controlled study. Scandinavian Journal of Gastroenterology, 17(4), 517–521. https://doi.org/10.3109/00365528209182242.

Shaikh Nusrat, K. ve Maheshwari, D. G. (2016). An overview on- toxicity testing methods. International Journal of Pharmacy and Technology, 8(2), 3834–3849.

Singh, G. K. ve Kumar, V. (2011). Acute and sub-chronic toxicity study of standardized extract of Fumaria indica in rodents. Journal of Ethnopharmacology, 134(3), 992–995. https://doi.org/10.1016/j.jep.2011.01.045.

Stefanovits-bányai, É., Tulok, M. H., Hegedûs, A., Renner, C. ve Varga, I. S. (2003). Antioxidant effect of various rosemary (Rosmarinus. Acta Biologica Szegediensis, 47, 111–113.

Tanvir, E. M., Hossen, M. S., Hossain, M. F., Afroz, R., Gan, S. H., Khalil, M. I. ve Karim, N. (2017). Antioxidant properties of popular turmeric (Curcuma longa) varieties from Bangladesh. Journal of Food Quality, 2017. https://doi.org/10.1155/2017/8471785.

Tavafi, M., Ahmadvand, H., Khalatbari, A. ve Tamjidipoor, A. (2011). Rosmarinic acid ameliorates diabetic nephropathy in uninephrectomized diabetic rats. Iranian Journal of Basic Medical Sciences, 14(3), 275–283. https://doi.org/10.22038/ijbms.2011.5006.

Tomeh, M. A., Hadianamrei, R. ve Zhao, X. (2019). A review of curcumin and its derivatives as anticancer agents. International Journal of Molecular Sciences, 20(5). https://doi.org/10.3390/ijms20051033.

Tovar, R. T. ve Petzel, R. M. (2009). Herbal Toxicity. Disease-a-Month, 55(10), 592–641. https://doi.org/10.1016/j.disamonth.2009.05.001.

Zorofchian Moghadamtousi, S., Abdul Kadir, H., Hassandarvish, P., Tajik, H., Abubakar, S. ve Zandi, K. (2014). A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed Research International, 2014. https://doi.org/10.1155/2014/186864.