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

Mustafa Yasar[1] Oguzhan Senogul[2] Neslihan Sirin[3]
Aydan Fulden Agan[3] Nisa Sipahi[3] Kagan Agan[3*]

[1] RTM Clinic, Istanbul, Turkey
[2] Naturin Nutraceuticals, 35477, Izmir, Turkey
[2] Traditional and Complementary Medicine Application and Research Center, Düzce University, 81100, Düzce, Turkey

*Corresponding Author: Kagan AGAN, e-mail: kaganagan@duzce.edu.tr

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

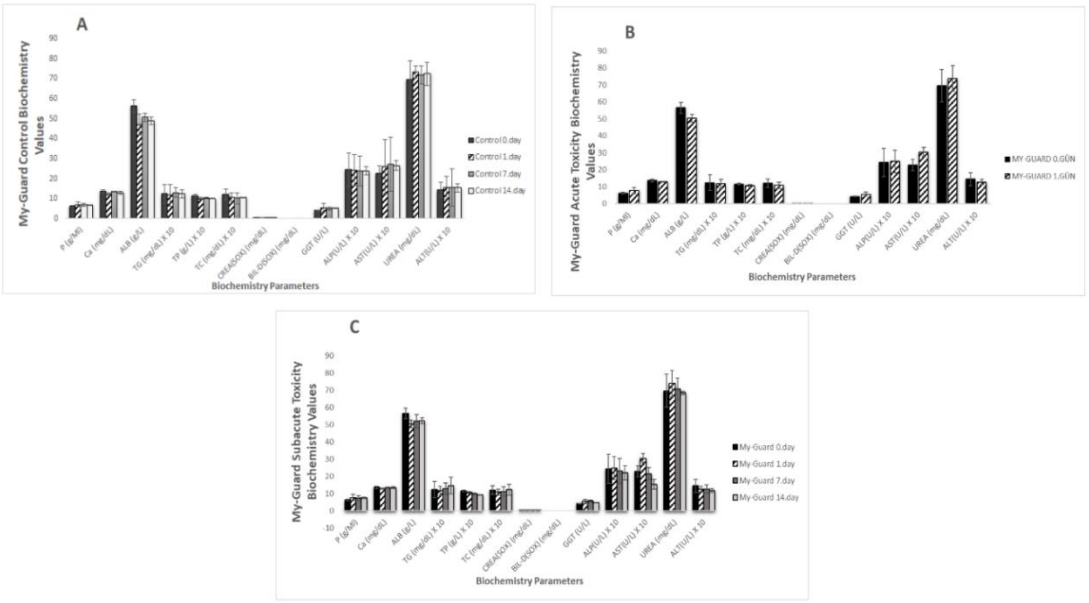
Animals in each experimental group were evaluated routinely during the experiment period by observing breathing, motor activities, convulsions, reflexes, ocular signs, salivation, piloerection, analgesia, muscle tone, gastrointestinal and skin.

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.

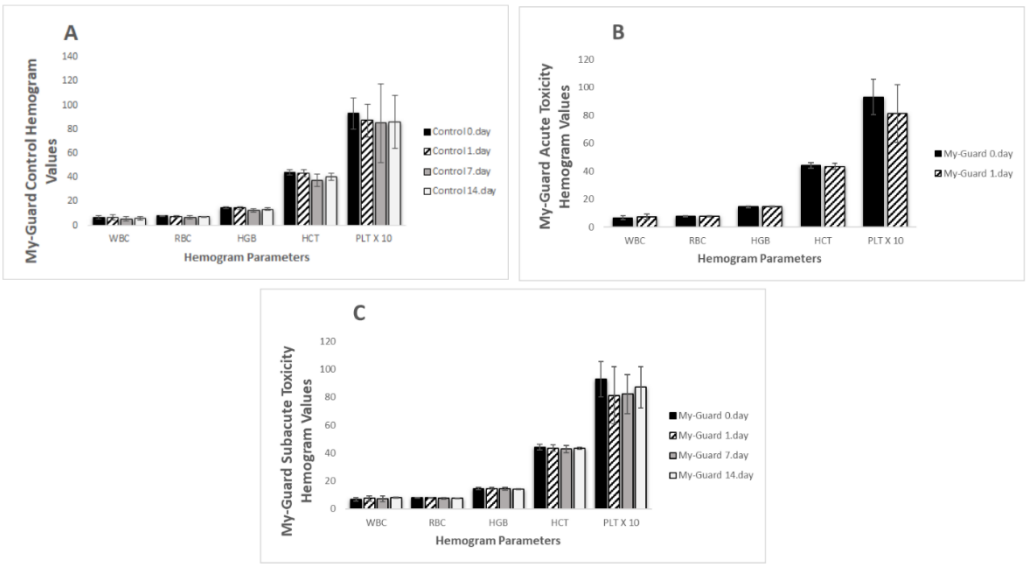
Biochemical and hematological parameters

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.

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