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Evaluation of Acute and Subacute Toxicity of ISY-CP® Food Herbal Mixture in Rats

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Abstract

Objective: Recently, there is a growing interest in medicinal and aromatic plants also the treatment methods by using these plants in traditional and complementary medicine. On the other hand, possible toxicities of medicinal and aromatic plants has lately begun to be researched and it is extremely important to cognize these possibilities for human consumption. Therefore, a research has been conducted to understand the acute/subacute toxicity characteristics of a plant based herbal mixture, ISY-CP® used in remember regeneration therapy method (RTM) in rats. The product contains a mixture of nettle leaves (*Urtica dioica*), yarrow (*Achillea millefolium*), thyme *(Thymus vulgaris*) and horsetail (*Equisetum arvense*) plants. The aim of this research was to examine the effects of ISY-CP® herbal mixture in term of toxicity.

Material-Method: In the experimental design, 32 female rats were divided into 4 groups (control, acute, subacute and post-subacute). In order to observe acute and subacute toxicity, clinical observations were performed and the biochemical, hematological 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, hematological and histopathological evaluations between the control and the application groups. Only phorphorus data is statistically different between subacute toxicity group and control group.

Conclusion: In our study, the acute and subacute toxic effects of ISY-CP® herbal mixture's doses used in this study were not observed.

Keywords: Traditional and Complementary Medicine, Acute Toxicity, Subacute Toxicity, *Urtica dioica, Achillea millefolium, Thymus vulgaris, Equisetum arvense*

INTRODUCTION

The use of traditional and complementary medicine practices has increased significantly in recent years in the prevention of diseases and sustaining health worldwide. Although the usage rates reported in different sources vary, they have usage ranges between 40% and 90% worldwide; 42% in America; 70% in Canada; 50% in France; 48% in Australia and 90% in Asian and African countries [1]. Many different purposes and treatments have been used in traditional and complementary medicine products. According to Yasar et al. (2019), some plants thought to be modulators in epigenetic mechanisms may exhibit anticancer features with phytotherapy approach [2].

The type and amount of synthetic compounds used in the health, cosmetics and food industries are increasing, threatening the environment and human health, affecting the country's economy negatively every year [3]. Some types of synthetic drugs accumulate biologically in the environment and cause serious environmental damage [4,5]. In this respect, the use of herbal products has increased

worldwide and has highlighted the "safety" issue of these products. Herbal products are considered to be natural; some perceptions that "It does not contain toxins; it does not have undesirable effects; it can be used for a long time and it is safe" is misdirecting. Herbal products are usually in the form of mixture and contain many substances. For this reason, it may have unknown features and effects.

Toxicity may depend on the natural chemical composition of the plant, or it can occur due to possible contamination, adulteration or misidentification of the plant. Dosage and duration of use are extremely important in monitoring toxicity of herbal products. High dosage and long-term use of these products can cause side effects. Also, Toxicity may depend on the active ingredients or dosage in herbal mixture, as well as on user-related factors such as age, genetics, other diseases, and other drugs used. Some plants have their own toxicity at normal therapeutic doses or overdoses [6]. Some substances in the components of some plants, such as ephedra, archtolochic acid, and aconitum, can directly generate toxicity. In some cases, external toxicity can be mentioned [7].

All medicines, cosmetics, pesticides, food additives and chemicals used in industry are evaluated for their toxic potential before they are presented to human use. The effects of these substances on human health are determined by designing toxicity tests by considering the exposure routes and durations. Toxicity tests are also carried out to determine the safe dose values of these substances[8]. One of the most commonly used acute toxicity tests is the 'lethality' test in lots of studies. This test is carried out to determine the toxic symptoms that may occur as a result of interaction with a chemical substance, lethal dose (lethality) value or the degree of influence of certain organs such as brain, kidney, liver. The lethal dose value is considered as an indicator of how safe chemical can be used for human health.

While there are many studies on the benefits of the ingredients of plants, there are a limited number of studies investigating the negative effects of their ingredients. Toxicity studies must be carried out before the products consisting of plants or their ingredients are presented for consumption. Preclinical studies provide detailed information about the effects of toxicity [9]. While any beneficial effects of any substance contained in plants can be seen in some doses, they may show toxic effects or be lethal when combined with other doses or other substances[10].

Toxicity studies are basically divided into 4 parts as acute, subacute, chronic and subchronic [11]. However, there are also special toxicity tests such as immunotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity[12]. These tests provide us with information about the toxicities of the substances contained in the product[13]. The use of products that have been tested for toxicity will make an important contribution in preventing negative effects.

In the current study the acute toxicity and subacute toxicity properties of herbal mixture called ISY-CP®, were investigated.

MATERIALS AND METHODS

Herbal mixture content

ISY-CP® contains a mixture of Nettle Leaf (*Urtica dioica*), Yarrow Perch (*Achillea millefolium*), Thyme (*Thymus vulgaris*) and Horsetail (*Equisetum arvense*) plants. The herbal mixture were supplied from Naturin (Natural Products Pharmaceutical and Pharmaceutical Raw Materials Industry Trade Limited Company). The animals in the acute and sub-acute toxicity groups were administered at 11.8mg/ml ISY-CP® by oral gavage, with the recommended daily use of the ISY-CP herbal mixture (contents is shown in table 1.) adapted to the rats. Herbal mixture was dissolved 1 ml water before gavage. Doses to be given daily were prepared freshly. The control group was given 1 ml water daily.

Table 1. ISY-CP® contents

Herbs	Quantity (1 Capsul)
Achillea millefolium	*184 mg*
Urtica dioica	*92 mg*
Thymus vulgaris	*92 mg*
Equisetum arvense	*92 mg*

Experimental animals

The animals to be used in the study were obtained from Düzce University Experimental Animals Application and Research Center. Wistar Albino 8 weeks old, 250-300 g female rats were used in the laboratory at 20-25 ºC room temperature, 55 ± 5% humidity and 12:12 light-dark cycle, with optimal food and water intake. Animals were provided with commercial food pellets and water *ad libitum*. Experiments were carried out with the approval of the Düzce University Animal Experiments Local Ethics Committee (2020.4.3).

Acute and subacute toxicity study procedure

In the experiment to be carried out using the ISO 10993[11] toxicity protocol with minor modifications, 32 animals were randomly divided into 4 groups.

1 ml of water was given to the control group by gavage for a 7 days. 24 hours after application, blood was taken. At the end of administration, the control group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

The 2nd group was designed as an acute group. They were administered content with once oral gavage. Clinical observations were made. 24 hours after administration, the acute group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

The 3rd group was subacute group. They were administered once time content for 7 days with oral gavage. Clinical observations were made. At the end of 7 days, the subacute group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

The final group was post-subacute group. They were administered once time content for 7 days with oral gavage. Content delivery was stopped after 7 days. Aftewards, Clinical observations were contiuned additional 7 days. At the end of 14 days the post-subacute group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

RESULTS

Clinical observation parameters

In all groups, clinical observation was performed to obtain the data of toxicity at 0. min, 30 min, 60 min, 120 min, 240 min, 480 min and 1440 min during the administration. In this observation, the parameters given in Table 2 were evaluated.

Biochemical and haematological parameters

As seen in Table 3, no statistical difference was found between the control and administration groups in terms of biochemical parameters except 3 parameters.

Table 2. Observation criteria

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

Table 3. Groups' biochemistry mean and standard error values

GROUPS	P MEAN ± SE	CA MEAN ± SE	ALB MEAN ± SE	TG MEAN ± SE	TP MEAN ± SE	TC MEAN ± SE	CREA MEAN ± SE	GGT MEAN ± SE	ALP MEAN ± SE	AST MEAN ± SE	UREA MEAN ± SE	ALT MEAN ± SE
CONTROL	6.71 ± 0.34	13.5 ± 0.09	50.63 ± 0.92	139.92 ± 11.15	101.2 ± 1.71	101.9 ± 12.71	0.11 ± 0.01	4.98 ± 0.27	220.90 ± 33.83	270.90 ± 60.51	71.71 ± 2.01	194.46 ± 46.35
ACUTE	5.95 ± 0.43	12.81 ± 0.2	49.81 ± 1.79	145.65 ± 25.84	104.3 ± 1.78	121.46 ± 17.41	0.11 ± 0.02	4.41 ± 0.19	210.17 ± 16.20	289.66 ± 52.79	78.42 ± 6.28	140.96 ± 13.39
SUBACUTE	10.45 ± 1.93*	12.27 ± 0.72*	46.07 ± 5.60	72.38 ± 22.41	90.4 ± 1.65*	77.61 ± 3.32	0.11 ± 0.07	5.50 ± 0.1	228.33 ± 40.85	365.27 ± 141.86	72.24 ± 4.63	178.27 ± 62.63
POST-SUBACUTE	8.98 ± 0.26	13.31 ± 0.42	50.15 ± 0.45	154.18 ± 20.97	104.7 ± 5.9	98.25 ± 7.13	0.10 ± 0.10	6.10 ± 0.0	161.90 ± 14.80	222.90 ± 41.80	68.61 ± 7.54	108.40 ± 15

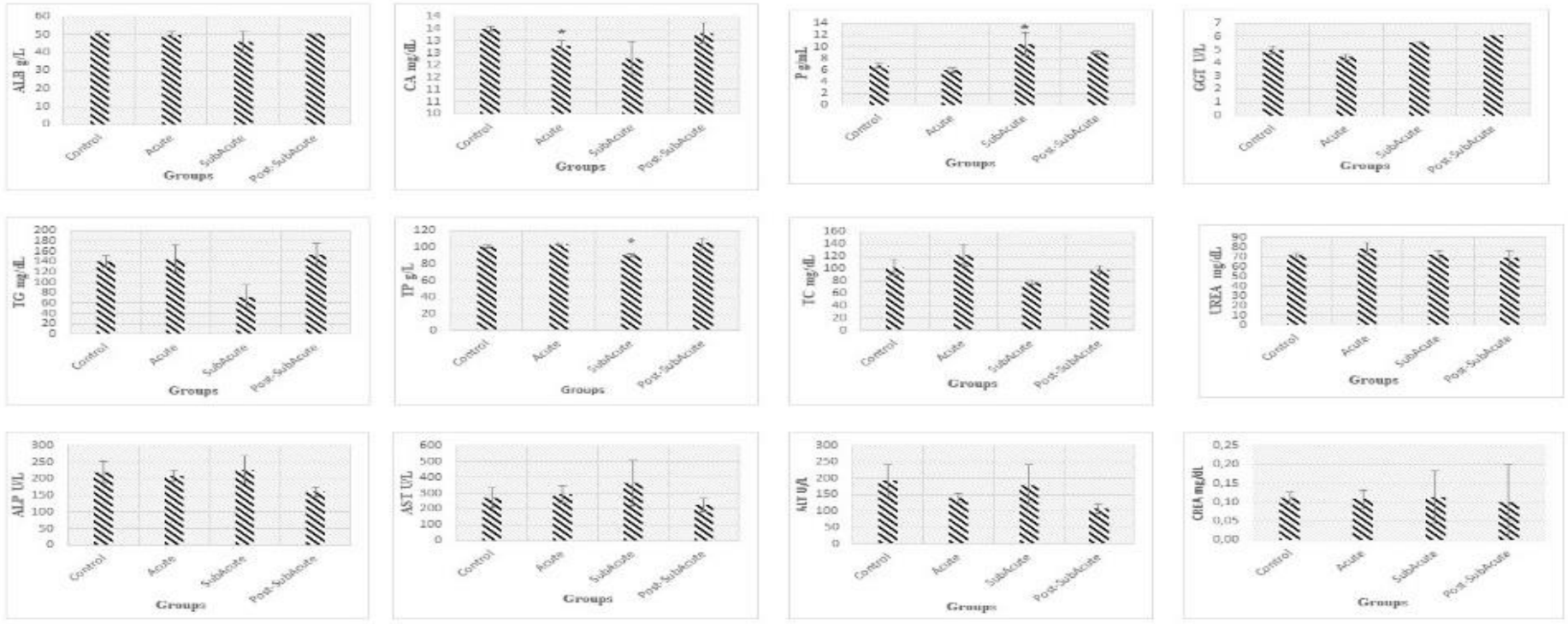

Figure 1. Groups' biochemistry parameters
* significant differences with control group≤0.05

As seen in Table 4, there was no statistically difference between the control and application groups in terms of 5 haematological parameters.

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 the herbal mixture group animals and control group animals given ISY-CP® herbal mixture were evaluated in terms of interstitial and bronchointerstitial pneumonia, degeneration, hyperemia and necrosis, there was no difference between the groups.

For the liver, kidney, spleen histopathology results of the animals in the application group and the control group were evaluated in terms of pigmentation, degeneration, hyperemia, bleeding and necrosis, there was no difference between the groups.

Histopathologically, no comparison was observed between the animals of the herbal mixture group that was given ISY-CP® herbal mixture and the animals of the control group.

Table 4. Groups' haematological parameters mean and standard error values

Groups	WBC Mean ± SE	RBC Mean ± SE	HGB Mean ± SE	HCT Mean ± SE	PLT Mean ± SE
Control	5.64 ± 0.60	7.31 ± 0.22	13.79 ± 0.40	41.66 ± 1.18	721.88 ± 82.24
Acute	8.03 ± 1.12	8.24 ± 0.42	14.93 ± 0.60	45.83 ± 1.86	908.71 ± 68.84
SubAcute	5.64 ± 0.43	6.66 ± 0.21	12.33 ± 0.09	37.7 ± 0.38	966.67 ± 221.40
Post-SubAcute	4.27 ± 0.69	7.09 ± 0.28	13.7 ± 0.70	41.8 ± 2.30	460.5 ± 438.50

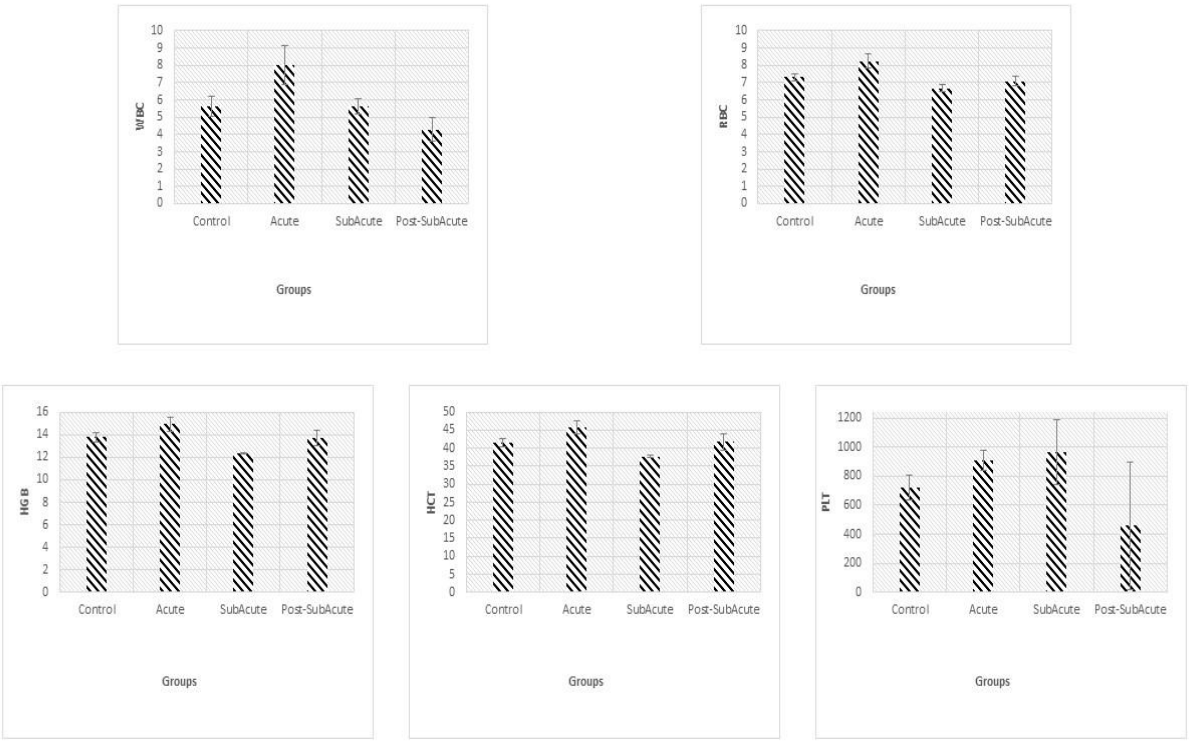

Figure 2. Groups' hemogram parameters

* significant differences with control group≤0.05

DISCUSSIONS

In different studies , it was observed that different target organs were affected by the active substances in herbal mixture. Taylor et al. (2004) observed hepatotoxicity with plants containing senna alkaloids; dermatitis and nephrotoxicity caused by heavy metals such as lead and mercury [14]. So, investigating effects of herbal supplement is important for toxicity.

In a study conducted by Dar et al. (2013) on nettle leaf (*Urtica dioica*), it was found that toxicity tests showed higher safety margins of all solvent extracts with an LC_{50} of 1000 µg/ml on *A. salina* [15]. *Urtica dioica* contains different components such as lignans, polysaccharides, and lectins which prevent prostate enlargement. It prevents cell growth and has anti-inflammatory properties. Nettle leaf has many uses such as stopping bleeding, relieving anemia, sciatica, urticaria, psoriasis, diarrhea, rheumatism, prostate and mouth sores etc.[16]. The LD_{50} dose detected in mice was calculated to be 3625 mg/kg[17].

Yaeesh et al. (2006) were investigated hepatoprotective effects of yarrow (*Achillea millefolium*). D-galactosamine and lipopolysaccharide were administered to mice. All mice were dead. Mortality is 100%. After pre-treatment yarrow crude extract to mice, mortality decreased to 40%. In the light of the obtained data, the extract of yarrow was significantly lower in the alanine aminotranferase (ALT) and aspartate transferase (AST) levels. these results make us think that yarrow may have a liver protective effect[18]. Hasheminia et al. (2011), in a study examining the toxicity properties of the yarrow, LC_{50} and LC_{25} values were found to be 4.19% and 1.69%, respectively[19].

Basch et al. (2009) suggested that not to take oral doses of 10 grams of dried leaves with 0.03% phenol (calculated as thymol) in thyme. Oregano oil might be very toxic. *In vivo* studies revealed that toxicity symptoms may include nausea, tachypnea and hypotension [20]. LD_{50} of thyme essential oil is 2.84 g / kg body weight in rats [21]. Another study by Fakılı (2010) on thyme was found that oregano underground water had neither acute nor chronic toxic effects and was effective on the digestive and cardiovascular system[22].

Equisetum arvense (horsetail) contains abundant calcium and silicon elements[23]. Due to the active ingredients contained in horsetail, it is used in the treatment of ulcers, stopping bleeding, healing wounds, kidney diseases. It also shows antioxidant properties. In addition, there are anticonvulsant, sedative and antioxidant activities in the studies. In addition, there are anticonvulsant, sedative and antioxidant activities [24,25]. In a single-dose toxicity study to determine the LD_{50} value in rats, the toxic dose was found to exceed 5000 mg / kg [26].

Considering the results obtained in our study, post, subacute group ALT, AST and ALP values were not statistically significant, but decreased compared to the control group. These results are Yaeesh et al. (2006) is similar to the results. It is seen that the toxic doses of the plants used are high. The toxic effect of plants was not observed in the ISY-CP® mixture we used in our study.

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 acute, the post-subacute groups with the control group. But subacute phosphorus, calcium and total protein values are statistically lower than control group values. Considering the toxicity parameters (eg AST, ALT, ALP etc.), there is no significant difference between the subacute group and the control group. Therefore, when all the data are evaluated together, it is believed that the ISY-CP® product has no toxic effect at the doses determined in this study. According to the data obtained during the experiment and after the experiment, acute and subacute toxicity effects of the ISY-CP® herbal mixture were not found clinical symptoms observed, biochemical and haematological parameters and pathology findings. Based on our data, there is no toxicity effect. It is necessary to plan new studies to identify ISY-CP® positive effect with physiologically.

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