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Investigating the Effect of Acute and Subacute Toxicity of *Ferula macrecolea* (Boiss.) Boiss Essential oil in BALB/c Mice

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ABSTRACT

This research was conducted to evaluate the acute and subacute toxicity of *Ferula macrecolea* essential oil on blood and serum biochemical parameters in BALB/c mice. To determine acute toxicity, four groups of mice (each comprising 6 mice) received a single oral dose of 1.24 ml kg^{-1} of *Ferula macrecolea* essential oil. Subacute toxicity was assessed by examining vital organs such as the liver, kidneys, and blood parameters after administering doses (0.1, 0.2, 0.4, and 0.6 ml kg^{-1}) of the plant essential oil orally for 28 days. The results of acute toxicity studies, where a single dose of 1.24 ml kg^{-1} of plant essential oil was orally administered to the mice, were monitored for 28 days. The median lethal dose (LD_{50}) of this compound was determined to be 1.79 ml kg^{-1}. In the study of acute toxicity, conducted with a dose of 1.24 ml kg^{-1}, no cases of animal death were observed. Additionally, in the study of subacute toxicity with doses of (0.1, 0.2, 0.4, and 0.6 ml kg^{-1}), no toxic effects on vital organs such as the liver and kidneys, as well as blood parameters, were observed. Finally, none of the blood parameters and histological characteristics of the studied organs were altered by the consumption of *Ferula macrecolea* essential oil. Therefore, it exhibits no significant toxicity and can be considered for potential effects on various diseases in future studies.

Keyword: *Ferula macrecolea* (Boiss.) Boiss., Toxicity, Acute toxicity, Subacute toxicity, LD_{50}

INTRODUCTION

In recent years, herbal medicines have secured a special place in the primary healthcare system, particularly in developing countries. A significant portion of the population in most developing countries relies on medicines from traditional healers because it is ingrained in their culture and represents the most accessible and reliable form of healing treatment. The use of herbal medicines is on the rise due to their availability, effectiveness, and social acceptability among patients [1]. These treatments harbor natural healing potentials that combat diseases such as obesity, cardiovascular disorders, arthritis, osteoporosis, diabetes, kidney, and liver diseases. There exists a perception that herbal medicines, being natural derivatives, entail no adverse or toxic side effects compared to synthetic drugs used in conventional medicine [2]. Consequently, it is necessary to conduct toxicity studies on medicinal plants, even though they have been in use for decades, to determine acceptable toxicity levels. Poisoning resulting from the consumption of plants is considered a significant and common issue in clinical toxicology. Such poisonings, especially in children under 5 years old, are prevalent and constitute one of the causes of poisoning in this age group. In terms of prevalence, statistics published in the United States indicate that about 5 to 10 percent of all cases reported to poison control centers in the country involve plant poisonings. Between 1990 and 1998, more than 17,696,714 cases of contact with and consumption of poisonous plants were reported to poison control centers in the United States. In terms of the classification of factors causing plant poisoning by prevalence, it ranks fourth after cleaning substances, pain relievers, and cosmetic-sanitary products. In recent years, owing to the inclination of doctors and people to use plants for medicinal and medical purposes, the prevalence of plant poisoning has increased. This is largely due to people's uninformed and arbitrary use of poisonous plants that they believe have therapeutic value. Many cultures have established the use of medicinal

plants as a reliable and accessible treatment, and a large part of the population, particularly in developing countries, relies on medicinal plants for health and treatment issues. Besides the traditional role of medicinal plants in health systems, many common drugs produced and processed in factories are derived directly or indirectly from medicinal plants [3]. However, it is necessary to conduct pharmacological and toxicological studies on plants and their products to ensure drug safety [4]. The natural origin of a drug does not imply that it is harmless. However, many people believe that using herbal medicines by themselves poses no problems, and they can consume varying amounts of these products without consulting a doctor or pharmacist. The side effects of herbal products, as well as their therapeutic and toxic effects, often manifest over time and during long-term use, rather than immediately after each use. The presence of various chemicals in plants that affect the biological systems of the body can lead to changes in these systems, sometimes resulting in disorders and damage considered as toxicity [5-7]. The effective substances causing damage are referred to as plant toxins, with the most important being alkaloids, organic acids, resins, minerals, and alcohols [8]. Statistics and figures from pharmaceutical information centers in Iran also indicate cases of poisoning with plants and herbal medicines among the population. Given these instances, knowledge of the symptoms, signs, and treatment methods for poisoning caused by plants is essential for medical professionals. Iran boasts a high diversity of plants, and identifying medicinal and poisonous plants and determining the toxic dose of plants is the initial step in preventing poisoning caused by their consumption [9]. The *Ferula* genus, known locally as 'Koma, Barijeh, and Anghozeh,' belongs to the Apiaceae family and is distributed in the Mediterranean region and Central Asia, comprising 133 species [10, 11]. In Iran, a decoction of *Ferula* gum is used for hysteria, black cough, and stomach ulcers. In Brazil, a decoction of dried leaves and stems of *Ferula* is used as a male sexual enhancer. In China, a decoction of *Ferula* is recognized as an anti-worm medicine, and in Egypt, a decoction of dried roots is used as an antispasmodic, anticonvulsant, diuretic, anthelmintic, and analgesic [12]. Some species of *Ferula* are utilized for flavoring food [13], synthesizing green nanoparticles [14], slowing down cancer progression [15], and producing bioactive compounds with antimicrobial properties for making green drugs and pesticides [16-18].

New scientific reports have also confirmed the medicinal effects of *Ferula* species, including antibacterial, antifungal, antioxidant, anti-inflammatory, analgesic, anticonvulsant, antispasmodic, and blood pressure-lowering properties. Different parts of *Ferula* plants are used in the treatment of various diseases such as nervous disorders, inflammation, dysentery, digestive disorders, rheumatism, headache, arthritis, and dizziness. *Ferula macrecolea* (Boiss.) Boiss (Figure 1) is an endemic perennial herbaceous plant of Iran, with very few researches conducted on it thus far. The plant's growth period begins in early spring and lasts until a few days after the onset of summer heat. Then, the immature plants enter a dormant period, remaining in this stage until the next spring; this plant only blooms once in each growing season and is a monocarpic plant [11]. *F. macrecolea* also contains various chemical compounds such as resin, gum essence, free folic acid, sesquiterpene, and elements like iron, strontium, zinc, and copper. Plant poisoning is a significant and common issue in clinical toxicology, making toxicity studies on this endemic medicinal plant necessary. It should be studied for toxicity alongside its medicinal properties and industrial applications. In this study, we investigated the safety assessment of *F. macrecolea* essential oil through acute and subacute toxicity evaluations by oral administration using mice as animal models.

Fig. 1 *Ferula macrecolea* (Boiss.) Boiss

MATERIALS AND METHODS

Plant Preparation

The aerial parts of *F. macrecolea* were collected in spring from the Eslamabad-e-Gharb region in Kermanshah province, Iran (Figure 2). The plant's identification was carried out by botany professors and was cross-referenced with the plant in the Lorestan University of Medical Science Herbarium, assigned the herbarium number (LUMH-0023). The plant materials were dried and stored in the shade for two weeks, then turned into powder before essential oil extraction.

Fig.2 Map of collection (mountain of Eslamabad-e-Gharb region)

Animals and Ethical Consideration

Forty male BALB/c mice (3-4 months old, weighing 30-35 grams) were obtained from the Razi Institute (Karaj, Iran) laboratory animal care and reproduction facility. The animals were housed in a room with a light/dark cycle of 12:12 hours and were maintained at a temperature of 21±2°C. They were tested in accordance with standard protocols for the use of experimental animals. This study received approval from the ethics committee of Lorestan University of Medical Sciences, Khorramabad, Iran, with the ethics number IR.LUMS.REC.1401.193.

Preparation of Plant Essential Oil

To prepare the essential oil from the aerial parts of the plant, 250 g of dried plant powder was added to 1300 ml of water. The essential oil extraction process was conducted using the hydrodistillation method for 2.5 hours, employing a Clevenger-type apparatus. This extraction process took place in the pharmacognosy laboratory of Lorestan Faculty of Pharmacy. The obtained essential oils were separated, dried over anhydrous sodium sulfate, and stored at 5°C in a refrigerator until testing.

Determination of Acute and Sub-acute Oral Toxicity

Acute toxicity was assessed by administering various doses of the plant essential oil (0.1, 0.2, 0.4, and 0.6 ml kg^{-1}) orally to four groups of mice. Mortality was recorded within 24 hours after treatment, and LD_{50} values were calculated using the Probit test in SPSS software [19]. Subacute toxicity was determined by measuring biochemical and hematological parameters in treated mice. Forty mice were randomly divided into five groups, each consisting of eight mice. Group 1 received normal saline orally (gavage). Groups 2 to 4 were orally administered 0.1, 0.2, 0.4, and 0.6 ml $kg^{-1}$of plant essential oil for 14 consecutive days. Group 5 did not receive any medication. After the 14-day period, animals were anesthetized with ketamine injection (100 mg kg^{-1}) and xylazine (10 mg kg^{-1}), and sodium pentobarbital was used as a euthanasia agent. Subsequently, blood samples were taken from the heart, and hematological parameters, including hemoglobin (HGB), hematocrit (Hct), white blood cell count (WBC), red blood cell count (RBC), and platelet count (PLT), were evaluated. To measure biochemical parameters in serum, blood was collected in tubes without anticoagulant, allowed to clot, and serum was separated by centrifugation at 200 g for 20 minutes. Tests for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (Cr), blood urea nitrogen (BUN), and bilirubin (direct and total) were performed using Roche diagnostic kits. Data analysis was conducted using SPSS software [20].

Statistical Analysis

After collecting the data and inputting it into SPSS version 17.0 statistical software, central and dispersion indices were calculated for data analysis. One-way analysis of variance (ANOVA) was performed, and if the results were significant, the Tukey test was employed for pairwise comparisons. In cases where the data did not exhibit normality, the non-parametric Kruskal-Wallis test was used. For post hoc comparisons, the corrected Bonferroni test was applied, and the results were reported at a significance level of 5%.

RESULTS AND DISCUSSIONS

Acute Toxicity

The effects of acute toxicity of the plant essential oil on BALB/c male mice were investigated. The maximum non-lethal dose was found to be 1.24 ml kg^{-1} of body weight. This result is a novel finding that can be compared with previous similar studies. Nevertheless, the results of this study can be analyzed in comparison with the research conducted by Ghorbani and colleagues in 2016, which focused on the toxicity evaluation of the hydroalcoholic extract of the root of *F. gummosa* [21]. In their study, similar to the present one, the subacute toxicity profile (28 days) of orally administered hydroalcoholic extract of *F. gummosa* with doses of 100 and 600 was evaluated in male rats [21]. Consistent with this study, there were no deaths and significant changes in body weight, food and water consumption, organ weight, hematological parameters, serum biochemical characteristics, behavioral changes, and tissue diseases.

Subacute Toxicity on Liver Enzymes

Damage to the entire structure of the liver is assessed through an increase in serum levels of some important enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin. In this research, no mortality was observed in any of the five groups (Table 1). As indicated in Table 1, the serum levels of liver enzymes after the acute oral administration of the essential oil of *F. macrocolea* at doses of 0.1, 0.2, and 0.4 ml kg^{-1} did not show any significant changes during the 14 days, compared to the control group.

Table 1 Subacute toxicity after 28-day oral injection of different doses of *F. macrocolea* essential oil on liver enzymes in BALB/c mice.

Parameters	Dose of essential oil (ml kg^{-1})				Control group
	0.1	0.2	0.4	0.6	
AST (U/L)	87.8±5.7	85.6±8.2	92.3±6.8	105.3±9.1	93.6±7.3
ALT (U/L)	34.3±4.8	31.4±7.2	36.6±5.6	47.6±7.7	38.4±6.6
ALP (U/L)	178.3±14.2	180.2±11.8	187.3±12.3	203.0±15.3	189.4±11.3
TB (mg/dL)	0.40±0.06	0.38±0.14	0.36±0.13	0.48±0.11	0.41±0.11
DB (mg/dL)	0.20±0.06	0.23±0.05	0.21± 0.02	0.27±0.07	0.21±0.015

The liver is a vital organ in the human body responsible for detoxifying foreign compounds, drugs, etc. However, it is susceptible to damage, leading to various liver diseases. Hepatotoxicity and cardiovascular toxicity are the most significant toxicities associated with drugs, prompting the scrutiny of many medications over the last decade. The importance of hepatotoxicity stems from the liver's central role and the extensive biological changes occurring within it. Many substances entering the liver undergo metabolism, potentially forming active metabolites that can overwhelm the body's capacity for detoxification. Complications arising from the detoxification of these metabolites include fatty liver, liver cell death, cholestasis, bile duct injuries, cirrhosis, vascular disorders, and tumors.The detoxification and activation of metabolites are facilitated by microsomal cytochrome P450 enzymes in the liver, which can generate toxic metabolites causing damage to various tissues, including the liver. Liver damage typically involves cell necrosis, increased tissue peroxidation, and decreased tissue glutathione levels. Moreover, the serum levels of biochemical indicators such as SGPT (serum glutamic pyruvate transaminase), SGOT (serum glutamic oxaloacetic transaminase), ALP (alkaline phosphatase), GGT (gamma glutamyl transpeptidase), and bilirubin increase. ALT, AST, and ALP are naturally located inside the

cytosol, and their release into the bloodstream during cell damage makes them valuable indicators for studying liver damage [22].

Subacute Toxicity on Renal Enzymes

As indicated in Table 2, no significant difference ($p > 0.05$) was observed compared to the control group. The kidneys, being the most important excretory organ of the body, play a crucial role in eliminating various exogenous compounds (drugs and poisons) and endogenous compounds (urea, uric acid, and creatine). One of the most common laboratory indicators for assessing kidney function is serum creatinine and BUN. Kidney dysfunction can result in an elevation of blood levels of creatinine, BUN, and many drugs.

Table 2 Subacute toxicity after 28-day oral injection of different doses of *F. macrocolea* essential oil on renal markers in BALB/c mice.

Parameters	Dose of the essential oil (ml kg^{-1})				Control group
	0.1	0.2	0.4	0.6	
Cr (mg/dL)	0.48±0.05	0.52±0.12	0.54±0.16	0.56±0.15	0.49±0.05
BUN (mg/dL)	30.2±6.5	29.3±5.5	30.6±6.5	33.0±7.6	28.6±5.2

Subacute Toxicity on Blood Parameters

Based on the results presented in Table 3, following the oral administration of *F. macrocolea* essential oil at doses of 0.1, 0.2, 0.4, and 0.6 ml kg^{-1} for 28 days, no significant difference ($p > 0.05$) was observed in blood parameters compared to the control group. Blood factors are excellent for toxicity studies due to their rapid predictive power. The bone marrow is the primary site of blood cell production and is considered one of the most important target tissues for toxic compounds. Studies have demonstrated that some compounds isolated from plants can induce changes in blood cells, such as a reduction in RBC and alterations in HCT, Hg, and PLT levels. Due to the risk of severe chronic hematopoietic disorders, it is crucial to screen herbal substances for their hematotoxicity. It is well-documented that changes in serum biochemical parameters directly indicate renal or pancreatic damage.

Table 3 Subacute toxicity after 28 day oral injection of different doses of *F.macrocolea* essential oil on blood parameters in BALB/c mice.

Parameters	Dose of the essential oil (ml/kg)				Control group
	0.1	0.2	0.4	0.6	
RBC ($\times l0^6/\mu L$)	2.3±0.74	2.56±0.63	2.6±0.51	2.77±0.56	2.94±0.71
HGB (g/dL)	10.4±0.54	9.1±0.22	11.2±0.81	10.7±0.47	10.3±0.64
Hct (%)	24.5±4.5	26.5±4.3	30.3±4.5	29.6±5.6	28.7±6.5
WBC ($\times l0^3/\mu L$)	2.2±0.45	2.5±0.25	2.9±0.75	2.6±0.35	2.3±0.55
PLT ($\times l0^3/\mu L$)	134.6±12.5	131.6±14.5	130.3±15.1	127.3±12.2	136.7±13.6

In 2005, Rustaiyan and his colleagues conducted the first phytochemical analysis of the essential oil of the *F. macrocolea* plant. They reported 55 compounds from this plant, with β-pinene (15.9%), α-pinene (10.4%), and β-caryophyllene (8.6%) being the most abundant [23]. A 2021 study by Alyousif et al. specifically evaluated the protoscolicidal effect of *F. macrecolea* species on hydatid cyst protoscoleces. The study revealed that the greatest protoscolicidal property of *F. macrecolea* oil was observed at concentrations of 150 and 300 µl ml^{-1}, resulting in the destruction of 100% of protoscoleces after 30 and 20 minutes of exposure to plant essential oil compounds, respectively. The essential oil of *F. macrecolea* demonstrated strong protoscolicidal effects in vitro and ex vivo [24]. In 2022, Mahmoudvand et al. investigated the chemical composition and anti-parasitic properties of *F. macrecolea* essential oil against Leishmania tropica. The results showed that the oil, especially its chief ingredient terpinolene, exhibited a strong anti-parasitic effect. The main constituents of the essential oil of F. macrecolea were terpinolene (78.72%), n-nonanal (4.47%), and linalool (4.35%) [25]. Naderi et al. conducted a study in 2022, evaluating the antimicrobial effect of *F. macrecolea* extract. The research demonstrated variable effects

against gram-negative and gram-positive bacteria, with varying levels of inhibition. The most and least antibacterial effects were reported on *B. subtilis* and *S. epidermidis* [26]. In the study by Goda et al. in 2015, the acute and subchronic effects of *F. asafoetida* on Sprague Dawley rats were evaluated. No significant changes were observed in weight, biochemical markers, and blood cells. Mild vascular changes were noted in the liver, but no severe toxicity was observed [27]. Fraigui et al. compared the acute and chronic effects of fessoukh (resin gum of *F. communis*) and warfarin in 2001. Fessoukh extract demonstrated moderate toxicity, and its rodenticidal effects were evident in trials on wild rats [28]. Aragno et al. (1988) studied the toxicity of *F. communis* in rats and identified ferulenol and ferprenin as toxic compounds affecting blood coagulation. No liver toxicity was observed with these compounds [29]. Soleimani et al. studied the acute and subchronic toxicity of *F. persica* flower and leaf extract in female rats. The extract showed no significant differences in blood and biochemical factors compared to the control group, and histopathological examinations revealed healthy tissues [31]. Illuri and colleagues evaluated the subacute and acute toxicity of *Ferula asafoetida* and *Silybum marianum* formulations in 2019. The formulations did not cause changes in feed and water intake, body weight, biochemical, and hematological parameters, and asdamarin was considered safe up to a dose of 1000 mg kg^{-1} [32].The results of the current study indicate that the essential oil of *F. macrocolea* does not have a negative effect. Therefore, it is evident that the essential oil, at the tested doses, does not cause liver failure or kidney damage. The essential oil is considered safe and has no harmful effects on the normal functioning of the liver and kidneys. This research places the essential oil of *F. macrocolea* in the category of safe plants for edible use [33]. In the sub-acute examination, no signs of abnormal tissue damage were observed in the animal's vital organs, including the kidney and liver. The essential oil of *F. macrocolea* did not cause any tissue damage due to toxicity in any of the animal's vital organs. Therefore, the essential oil of *F. macrocolea* can be considered safe for therapeutic purposes.

CONCLUSION

This study demonstrates that *F. macrecolea* essential oil is safe and non-toxic. In both acute and subacute toxicity tests, there was no evidence of abnormal tissue damage in vital organs, such as the liver and kidneys, of BALB/c mice. None of the mice's vital organs showed signs of damage due to the essential oil's toxicity. The use of appropriate doses of this essential oil has the potential to reduce ALT and AST levels, suggesting its potential as a dietary supplement for treating liver issues, including fatty liver, various types of liver toxicity, and hepatitis that elevate these enzymes. If additional animal and clinical trials are conducted on various diseases and more precise tests are performed on different laboratory animals using various administration methods, *F. macrecolea* could find applications in therapy systems and dietary supplements [34-37]. Additionally, the active substances in the plant essential oil should be thoroughly examined and biologically validated.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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