Entomology and Applied Science Letters
Volume 5, Issue 1, Page No: 77-81

Available Online at: www.easletters.com

ISSN No: 2349-2864

Evaluation of the Sub-Acute Toxicity Effects of Amygdalus Eburnea Spach Extract in Mice

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ABSTRACT

To date, the use of medicinal herbs for therapeutic purposes is very common; however, it is required to find the scientific validations for their side effects. This study aimed at identifying the toxicity effects of Amygdalus eburnea Spach extract on hematological and biochemical parameters in mice. Toxicity effects of N. tripedale extract were assessed by evaluating the biochemical and hematological parameters of the treated mice after oral administration of A. eburnea extract at the doses of 250, 500, and 750 mg/kg for 14 days. The results demonstrated that A. eburnea extract at the tested doses did not have considerable toxicity on the biochemical parameters of ALT, AST, ALP, and bilirubin used for the assessment of the liver function, and also Cr and BUN for renal function as well as hematological parameters such as HGB, Hct, WBC, RBC, and PLT counts. But further investigations are compulsory to determine other toxicity features such as genotoxicity, and chronic toxicity.

Keywords: Sub-Acute Toxicity, Mice, Liver, Kidney, Hematology

HOW TO CITE THIS ARTICLE: Mahdi Rezaeifar, Maryam Rezaeifar, Evaluation of the Sub-Acute Toxicity Effects of Amygdalus Eburnea Spach Extract in Mice, Entomol Appl Sci Lett, 2018, 5 (1): 77-81.

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Received: 02/12/2017
Accepted: 05/03/2018

INTRODUCTION

The history of using medicinal plants for treatment is as long as the history of human life. Although chemical and synthetic drugs have been widely used over the past half century, their harmful effects on human life have led to the reoccurrence of the medicinal herbs [1, 2]. Although, using herbs has always been one of the most effective treatments in history, the scientific assessment to discover the side effects of plants in order to achieve therapeutic aims is a very important point [3].

Amygdalus eburnea Spach. is a kind of almond from the Rosaceae family, and native to the regions of Asia Minor and the Middle East but also to other warm and dry regions of the world. This plant that is known among the persians as "Ghosk", in addition to medicinal properties, has non-therapeutic features, such as soil erosion control, and stabilized watersheds [4].

The medicinal attributes of this plant since long time ago, have been much used in Iranian ancient medicine. For example, in southeastern Iran, this plant has been used as a laxative in treatment of constipation, eliminating intestinal worms, and healing the burn wounds. Furthermore, studies based on world-class methods have introduced other therapeutic properties of *A. eburnea* including its role in the treatment of respiratory and digestive illnesses, as well as containing anti-dermatophyte, anti-bacterial, anti-fungal and anti-oxidant characteristics [5-9].

Earlier researches on laboratory animals have confirmed that kidney and liver are the main target organs for drug toxicity. Increase of Serum levels of some important enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST),alkaline phosphatase (ALP) and bilirubin, are events that are followed by liver tissue damage. To evaluate kidney's function, the creatinine blood test (Cr) and blood urea nitrogen (BUN) have been used [10, 11].

Due to the numerous biological effects of *A. eburnea* and the lack of studies done on evaluating the toxicity effects of this plant, this study evaluated toxicity effects of this plant's extract on hematological and biochemical parameters in NMRI mice, for the first time.

MATERIALS AND METHODS

Collection of plant materials

The aerial parts of *A. eburnea* were collected from the rural regions of Baft district in the Southeast of Iran in April 2013. After collection, the identity of the plants was confirmed by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was put at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Iran.

Preparation of the extract

The extraction of 200 g of powdered plant was performed using 80% methanol as solvent using percolation method for 72 h at room temperature according to the method described elsewhere [12-14].

Animals

From the Animal Breeding Stock Facility of Faculty of Medicine, Kerman University of Medical Sciences, (Kerman, Iran), 24 male NMRI mice (6–8 weeks old) were bought .In a colony room with a 12:12-h light/dark cycle at 21 ± 2˚C, the animals were placed and kept in accordance with the standard protocols of experimental animals.

Study design

Randomly, the mice were assigned to four groups (6 mice per each group) as follows:

First group (Control group): For 14 consecutive days, normal saline was given intraperitoneally to the mice.

Second group: The mice in this group received the extract of *A. eburnea* at a dose of 250 mg / kg intraperitoneally for 14 consecutive days.

Third group: The mice in this group received the extract of *A. eburnea* at the dose of 500 mg/kg intraperitoneally for 14 consecutive days.

Forth group: The mice in this group received the extract of *A. eburnea* at the dose of 750 mg/kg intraperitoneally for 14 consecutive days.

Blood collection

By Ketamine-Xylizine, the animals were anesthetized, and then the blood samples from each mouse were collected after opening their hearts. This process was done after two weeks of administration.

Determination of hematological parameters

The acquired total blood was transmitted into tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant for assessing the hematological parameters. In the next step, a number of hematological parameters: such as hemoglobin, hematocrit, white blood cell counts, red blood cell, and platelet counts were evaluated [15, 16].

Determination of biochemical parameters

Some collected blood was put into the tubes without anticoagulant, to procedure the clot. This was done to measure serum biochemical parameters**.** Diagnosis of various clinical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (Cr), blood urea nitrogen (BUN), and bilirubin (direct and total) by Roche diagnostic kits (Mannheim, Germany) and after Separation of serum was done by centrifugation at 5000*g* for 10 min [17, 18].

Statistical analysis

The results of the experiments were analyzed by SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). And $P<0.05$ was considered statistically significant for the differences between test and control groups.

RESULTS

Hematological parameters

The plant extract of *A. eburnea* in doses of 250, 500, and 750 mg/kg were used to evaluate their toxicity effects on hematological parameters in mice. The results of the several doses of the *A. eburnea* extract from the hematological parameters are shown in Table 1. Based on the results, no death of mice was found after administration of *A. eburnea* extract after 14 days. There were no significant differences in hematological parameters after oral administrations of *A. eburnea* at the doses of 250, 500, and 750 mg/kg compared with the control group ($P> 0.05$).

Table 1. The effects of *A. eburnea* extract on hematology parameters on the whole tested blood of the mice (Mean ± SD).

Parameters	*A. eburnea* extract (mg/kg)			Control
	250	500	750	
RBC (×l0^6/µL)	5.7 ± 1.15	8.4 ± 0.33	6.6 ± 0.6	7.8 ± 1.61
HGB (g/dL)	10.1 ± 1.41	12.8 ± 1.07	10.6 ±1.15	11.8 ± 0.67
Hct (%)	37.3 ± 6.5	42.2 ± 3.1	38.3 ± 6.15	41.3 ± 3.01
WBC (×l0^3/µL)	12.8 ± 0.33	13.1 ± 0.43	11.5 ± 0.85	12.3 ± 2.6
PLT (×l0^3/µL)	407.6 ± 26.6	367.6 ± 21.3	398.6 ± 26.6	383.3 ± 30.6

RBC, red blood cell; HGB, hemoglobin; Hct, hematocrit; WBC, white blood cell; PLT, platelet.

Biochemical parameters

The data obtained from the study of the toxicity effects of *A. eburnea* extract at the doses of 250, 500, and 750 mg/kg, on serum biochemical parameters in the mice after 14 days is shown in Table 2. The findings indicated that, despite the increase in these parameters following the increased dosage of the extract, there were no significant differences (p> 0.05) between oral administrations of *A. eburnea* extract at the doses 250, 500, and 750 mg/kg and the control group.

Table 2. Effects of *A. eburnea* extract on biochemical parameters in tested mice.

Parameters	*A. eburnea* extract (mg/kg)			Control
	250	500	750	
AST (U/L)	221.6 ± 16.5	213.3 ± 16.3	233.6 ± 19.3	195.3 ± 21.5
ALT (U/L)	213.2 ± 16.6	183.3 ± 25.6	191.6 ± 11.3	206.3 ± 11.3
ALP (U/L)	183.3 ± 18.6	201.3 ± 20.1	218.3 ± 16.3	221.6 ± 16.5
Cr (mg/dL)	0.37 ± 0.05	0.48 ± 0.08	0.56 ± 0.15	0.4 ± 0.15
BUN (mg/dL)	51.3 ± 6.1	67.1 ± 6.6	73.6 ± 6.3	64.6 ± 7.15
TB (mg/dL)	0.1 ± 0.23	0.09 ± 0.05	0.1 ± 0.05	0.1 ± 0.03

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Cr, creatinine; BUN, Blood urea nitrogen; TB, Total bilirubin; DB, Direct bilirubin.

DISCUSSION

The history of treating diseases with medicinal herbs dates back to the history of human life on the planet. Since man is a part of nature, certainly for every disease, the nature has offered a treatment for it. While only about 10 percent of the plants are used for commercial purposes, about one fifth of them are used for medical aims [1-3].

This study was designed and implemented to evaluate the toxicity of *A. eburnea* extract on biochemical and hematological parameters in mice. Because, laboratory animals like NMRI mice are similar to humans in terms of intestinal and liver metabolism [19]. One of the chief tests for assessing liver function as well as inflammation and injury, such as hepatitis and cirrhosis, used in modern medicine is the evaluation of serum liver enzymes. On the other hand, the decreased kidney function (25%) indicates that serious problems are threatening the health of the body. Therefore, to assess the function of kidney in a variety of conditions, diagnose diseases timely, display people with impaired functions or acute or chronic renal failure, the serum BUN and creatinine levels are usually measured [11].

The research carried out showed that there were no significant differences (p> 0.05) between oral administrations of *A. eburnea* extract at the doses of 250, 500, and 750 mg/kg and control group. These results were achieved while biochemical parameters of ALT, AST, ALP, and bilirubin for the evaluation of the liver function and also Cr and BUN for renal function were increased by increasing the dosage of the extract. In addition, no significant changes were observed in the hematological parameters such as HGB, Hct, WBC, RBC and PLT counts due to the effect of *A. eburnea* extract.

Therefore, according to the toxicity classification, *C. longa* essential oil had no significant toxicity against male NMRI mice [20].

CONCLUSION

With regard to the results of enzymatic and pathological tests, it can be concluded that consuming different dosages of *A. eburnea* extract did not lead to significant toxicity on the liver and kidney organs and also the hematological parameters in NMRI mice for 14 days. More studies are needed to provide data generalization for human community.

Conflict of interest
None.

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