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Research Article

Acute Toxicity Study of *Euphorbia helioscopia* L. aqueous Extract in Swiss Albino Mice

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ABSTRACT

Euphorbia helioscopia is an important medicinal herb species in the family Euphorbiaceae. Plants of Euphorbia have been used in the traditional medicine for treatment of cancers, tumors and warts for hundreds. The aim of the present study was to evaluate the *in vivo* toxicity of aqueous extract of *Eeuphorbia helioscopia* in Swiss albino mice. The extract was administered orally at doses of 2000 mg/kg and 5000 mg/kg and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. The findings of this study suggest that the aqueous extract of *Euphorbia helioscopia* did not cause any mortality or signs of toxicity in all treatment groups. Also, there were no differences in body weight, food consumption, organ weights between controls and treated animals, and no change in the biochemical parameters. This suggests that the aqueous extract of *Euphorbia helioscopia* is nontoxic and safe by oral administration in mice.

INTRODUCTION

Herbs are alternative medicines for treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low cost [1]. The public, patients, and consumers are primarily interested in fast access to safe and efficient drugs, as well as in animal welfare. Based on their long-term use by humans one might expect plants used in traditional medicine to have low toxicity. Nonetheless, the latest surveys have indicated that many medicinal plants applied in traditional medicine showed adverse effect [2, 3].

Plant-derived secondary metabolites that are biosynthesized in plants are not only essential for plant survival, but are also extremely important for development, growth and reproduction and plant protection [4-6].

Euphorbia helioscopia L., (Euphorbiacae family), is an annual plant rising 10 to 50 cm high with erected reddish stem, oval alternate leaves and small yellow green flowers.

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It is an indigenous plant of North Africa and most of the Europe and Asia. *Euphorbia helioscopia* has been considered as medicinal plant and was used in folk medicine of various countries around the world [7-10].

Euphorbia helioscopia has been extensively used with a long history to treat diverse health disorders, such as ascites, edema, tuberculosis, dysentery, scabies, lung cancer, cervical carcinoma, and esophageal cancer. It is also believed to have antifungal and antibacterial properties [11]. *Euphorbia* species have yielded numerous diterpenoids and triterpenoids possessing various biological activities with contraverting biological activities, such as tumor promoting and antitumor [12]. *Euphorbia* species have been reported to be toxic and this toxicity is mostly found in the white milky sap called latex, which has been reported to be harmful to humans and livestock [13, 14].

The aim of the present study was to investigate toxic effects of *Euphorbia helioscopia* in albino mice.

MATERIALS AND METHODS

Plant Material

Eeuphorbia was collected in March, from Hidhab region, Wilaya of Sétif in Northeast of Algeria.

Animal Material

Male *Albino Wistar* mice weighing between 35 and 45 g were used for acute toxicity. The animals were obtained from Pasteur Institute (Algiers, Algeria). These animals were kept in the animal house of the faculty of Nature and Life Sciences, University of Sétif, at a temperature of 20°C and a photoperiod cycle of 12 hours light/dark. The animals were housed in plastic cages (3 mice per cage) and had free access to standard commercial diet and tap water.

Preparation of Aqueous Extract

The areal parts were washed in running water, dried, and powdered. 50g of powder was boiled in 500 ml of water for 15 minutes, the resulting was filtered using whatman filter paper and then evaporated in rotary vacuum evaporator.

Acute Toxicity Study

The acute oral toxicity of extract was evaluated using the procedures described by Organization for Economic Co-operation and Development 425 guidelines, the animals were divided into three groups with 3 animals (3 males). The control group was given normal saline. The second and third groups were given a single dose of 2000 mg/kg and 5000 mg/kg of aqueous extract respectively. The animals were fasted (4h) with free access to water prior to administration of single doses of the extract dissolved in distilled water. The general behavior of the mice was continuously monitored after dosing, periodically during the first 24 h (with special attention given during the first 4 hours), and then daily thereafter, for a total of 14 days.

At the end of the treatment, animals were fasted overnight, but allowed access to water and libitum. They were subsequently anesthetized with diethyl ether and blood samples were obtained by retro-orbital puncture and collected in tube containing heparin and centrifuged at 4000 r/min at 4°C for 15 minutes to obtain serum (stored at −20°C until analysis). The organs (kidneys, liver, lungs, heart, and spleen) were weighed.

Body Weight and Food Consumption

The body weight of each mouse was recorded once weekly and the amount of food consumed was measured from the quantity supplied and the amount remaining after 24 hours for 2 weeks of the study period.

Biochemical Analysis

Biochemical analysis was performed using an automatic analyzer (Beckman). Parameters included: aspartate aminotransferase (AST); alanine aminotransferase (ALT).

Organ Weights

After the sacrifice of all animals, the kidneys, liver, heart, lungs, and spleen were carefully removed and weighed individually.

Statistical Analysis

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to assess differences between groups.

RESULTS

Effect of areal parts of *E. helioscopia* was observed on behavior and body weight of the mice. Macroscopic examinations of vital organs and biochemical tests on liver alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of mice were measured in control and treated groups.

Mortality and Signs of Toxicity

Behavioral pattern of mice was observed at various time intervals within 14 days of acute toxicity study period. No signs of toxicity were observed in selected physical parameters of mice and all the mice remained healthy (Table 1).

Table 1: Mortality and signs of toxicity of the *Euphorbia helioscopia* extract administered orally as one single dose in mice.

Dose (g/kg)	Death (D/T)	Adverse effects
0	0/3	Normal
2	0/3	Normal
5	0/3	Normal

Body Weight Changes

For the effect of *E. helioscopia* aqueous extract on mice body weight, There was no body weight loss during the observation period, The weight of the treated mice is stable compared with the weight of the control mice (Table 2), Since changes in body weight have been used as an indicator of adverse effects of drugs and chemicals.

Toxic chemicals decrease the weight of intoxicated body while there is a normal increase in body weight when non-toxic chemicals/drugs are used [15].

Table 2: Effect of *Euphorbia helioscopia* aqueous extract on the body weight in mice

Day	Body Weight (g) of mice		
	control	2000 mg/kg	5000 mg/kg
1st Day	45.62±1.52	35.09±2.35	36.37±4.88
7th Day	39.79±1.62	46.09±1.96	48.69±1.26
14th Day	44.68±2.73	36.28±3.83	35.79±3.44

Values are presented as mean ± SD; N= 3.

Food Consumption

Regarding the consumed food, there is no significant difference between the amount consumed by the control mice and the treated mice, the results are shown in Table 3.

Table 3: Effect of *Eeuphorbia helioscopia* aqueous extract on food consumption in mice

	Food consomption (g)		
	Control	2000 mg/kg	5000 mg/kg
Food	14.00±7.47	19.54±4.05	19.52±1.04

Values are presented as mean ± SD; N= 3.

Biochemical Analysis

While ALT and AST levels in control and treated groups were identical indicating no effect of treatment on this parameter (Table 4).

Table4: Effect of *Eeuphorbia helioscopia* aqueous extract on biochemical parameters of mice.

Parameters	Control	2000 mg/kg	5000 mg/kg
ALT (UI/L)	30.85±2.82	27.93±2.46	44.81±9.93
AST (UI/L)	110.18±30.86	172.83±78.58	209.52±17.46

Values are presented as mean ± SD; N= 3.

Organs Weight

Organ weights values are given in Table 5, and no significant variation among groups was found.

Table 5: Effect of *Eeuphorbia helioscopia* aqueous extract on organ weight in mice

Organ (g)	Control	2000 mg/kg	5000 mg/kg
Liver	1.76±0.17	1.68±0.09	1.47±0.22
Kidneys	0.28±0.00	0.27±0.02	0.27±0.04
Lungs	0.27±0.01	0.21±0.13	0.25±0.15
Herat	0.15±0.03	0.20±0.03	0.21±0.03
Spleen	0.18±0.07	0.21±0.09	0.17±0.02

Values are presented as mean ± SD; N= 3.

On macroscopic examination, no lesion was found in organs of treated groups (liver, heart, kidney, lungs and spleen), the texture was found similar to that of control group organs.

DISCUSSION

Medicinal plants, either as an extract, pure compound or as a derivative, offer unlimited opportunities for the discovery of new drugs. Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. However, there is little information or evidence available concerning the possible toxicity that medicinal plants may cause to the consumers.

The data of the acute and sub-chronic toxicity studies on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in their safety to humans, particularly for use in the development of pharmaceuticals. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects [16].

No abnormality in the general behaviour of the test animals in the short term was observed, Treated animals exhibited normal behaviour as that of control group. No death was observed in any of the group and all the animals lived up to 14 days. The results of our study agreed with the results of the [17] for both methanol extract (L.MT) of the *Euphorbia helioscopia* leaves and plant latex.

The obtained results concerning the effect of *E.helioscopia* L. aqueous extract on mice body weight also suggest that the aqueous extracts of *E. Helioscopia* L. did not affect the increase in body weight as well as food intake, indicative of nontoxic nature of the plant.

In the safety evaluation of the product for therapeutic purpose, it is important to record animal's water and food intake because nutrients are necessary for physiological functions of the body [17].Water and food intake of mice in this study was noted normal in the treated groups. In (2015) [17] found the same observation in his study. This observation stipulates normal processing of lipid, carbohydrate, and protein metabolism inside the animal body [18].

Clinical biochemistry data holds significant role in determining the toxicity induced by drugs. Transaminases ((AST) and (ALT)) are good indicators of liver function and biomarkers to predict the possible toxicity of drugs. Any elevation pertaining to these enzymes indicate their outflow into the blood stream due to damage in liver parenchymal cells [19]**.** Membrane permeability of hepatocytes increases during liver injury that leads to leakage of hepatic enzymes from hepatocytes [20]. Elevated levels of ALT and aspartate aminotransferase are indicative markers of liver injury [21].

From the present study it was seen that there was no significant change in the biochemical parameters (ALT and AST) in the *E. helioscopia* L.extract treated group compared to the normal control group, Butin [21] study, a decrease was found in ALT, ALP and bilirubin levels in latex and LMT treated groups as compared to control. This confirms hepatocurative role of *E. helioscopia* that might be due antioxidant power of this plant. As indicated [21] that methanol extract (L.MT) of the leaves of *E. helioscopia* possessed strong *in vivo* antioxidant activity and can be useful in the treatment of oxidative stress induced diseases.

There were generally no significant differences observed in the organ weights in this study. Hence, the test compounds could be regarded as non-toxic. In 2014 [21] found the same result. The vital organs, heart, liver, kidney, lungs and spleen are metabolically targeted sites for any toxic substance [22]. On macroscopic examination, no lesions on heart, liver, kidney, lungs and spleen were seen when compared with control group organs.

The LD_{50} of this plant was therefore estimated to be more than 5000mg/kg. In(2015) [17] stated in his study that following OECD, the LD_{50} values of latex and LMT were greater than 2000 mg/kg and the plant can be placed in category 5 according to the specification of globally harmonized classification system.

CONCLUSION

In light of these findings, we may conclude that *Euphorbia helioscopia* L. aqueous extract is not toxic in all the doses studied herein and did not produce any toxic signs or evident symptoms at acute *oral* toxicity. *E.* helioscopia extract did not cause any lethality or produce any serum chemical alteration. The preliminary results suggest promising alternatives for exploring therapeutic and pharmaceutical interest in *E.* helioscopia L. extract with a reduction of possible adverse effects.

However, detailed experimental analysis on the sub acute and chronic toxicity is necessary for further support of this extract safety, and clinical trials are needed to be performed before any new phyto therapeutic agent from this plant can be generally recommended for safe use.

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