

Original Article

Evaluation of Acute Toxicity of Aqueous Extract of Libyan *Globularia alypum* in Albino Mice

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ABSTRACT

Aims. Lorke's method was used to evaluate the acute toxicity effect of aqueous extract from the aerial part of Libyan *Globularia alypum* on albino mice. **Methods.** The study involved intra-peritoneal administration of different doses of the extract to groups of female mice. **Results.** Signs resultant toxicity and possible death of animals were monitored for 14 days to ascertain the median lethal dose (LD50) of the extract. At the end of the two-week study, all the animals in all the dose groups were survived. **Conclusion.** *Globularia alypum* plant can be considered as safe plant, as long as the LD50 is greater than 5000 mg/kg body weight.

Keywords: *Globularia alypum*, Acute toxicity, Lorke's method, LD50.

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INTRODUCTION

Globularia alypum L. is belongs to Globulariaceae family, that found mostly in Mediterranean region and some parts of Europe. In Libya, the family is represented by one genus and two species (*G. alypum* and *G. arabica*) [1]. Many constituents were isolated from *G. alypum*, such as, resins, iridoids, sterols [2]. Flavonoids, phenolic acid, terpenoids [3]. Globularin, cinnamic acids [4]. Saponins, tannins, glycosides, phenylethanoid and coumarins [5]. *G. alypum* is traditionally using in North Africa as folk remedy in the treatment of many illnesses [6]. In Libya, the decoction of leaves and branches are used in traditional medicine as a purgative and as a substitute for Senna. The leaves are also used as a cure for intermittent fever [1].

The ingestion of 800 mg/kg of *G. alypum* ethanolic extract at the first day to sixth day of pregnancy in female rats, did not cause pregnancy failure, but it

significantly reduced the number of viable fetuses, however, the ingestion of 800 mg/kg of this extract for 30 serial days by adult rats had no effect on the occurrence of pregnancy [7]. The orally administered of aqueous extract of *G. alypum* leaves to female and male mice did not produce any death up to 14 following days, even at 10000 mg/kg, however, active spermatogenesis was shown when the animals treated with daily doses, 300 and 600 mg/kg for 30 days [8]. Otherwise orally chronic administration of *G. alypum* butanoic extract (100 mg/kg) exhibited cardioprotective effect [9].

METHODS

Plant Material

The aerial parts of *Globularia alypum* were collected from El-Rojban region of Eljabal Elgarbi, Libya, in March 2016 and a voucher number specimen (No. D 68411) was submitted at herbarium of Botany

Department, Faculty of Science-University of Tripoli, for authentication. The collected plant was allowed to dry for about 15 days at room temperature.

Experimental Animals

Fifteen female albino mice were obtained from the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy-University of Tripoli, Libya. The animals were kept under room temperature and were acclimatized in new environment for period 14 days with free access to food and water, were weights ranged from 30 g to 35 g just before the commencement of the experiment.

Extraction Method

Aqueous extract was prepared in distilled water. Exactly 100 g of the air-dried powder of *G. alypum* was boiled in 500 ml of sterile distilled water contained in a conical flask, for one hour. The extract was there after filtered while hot with filter paper. The filtrate was concentrated in a water bath with its temperature set at 50°C for 2 days. The concentrated extract was finally exposed to air to complete drying [10].

Experimental Design for Acute Toxicity Study

The acute toxicity study was conducted in accord with Lorke's method. It was conducted in two phases using a total of fifteen female mice. In the first phase, nine mice were divided into 3 groups of 3 mice each. Groups I, II and III animals were given 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of the sterile extract respectively, to possibly establish the range of doses producing any toxic effect. In addition, a fourth group of three mice was set up as control group, animals were given only sterile distilled water.

In the second phase, further specific doses (1600, 2900 and 5000 mg/kg body weight) of the sterile extract were administered to three mice (one mice per dose) to further determine the LD50 value. The extract was dissolved in sterile distilled water and finally sterilized by filtration using 0.22 µm Millipore filters, and given via intra-peritoneal route. All animals were

observed frequently on the first day of treatment. The surviving animals were monitored daily for 2 weeks for behavior changes as well as mortality [11].

Statistical Analysis

All data were expressed as means ± standard errors of the mean (SEM), n=3. Using the Statview® version 5.0.1 software package (SAS Institute Inc, Abacus Concept, Inc., Berkeley, CA, USA). A p value of < 0.05 was considered significant.

RESULTS AND DISCUSSION

The acute toxicity effect of *G. alypum* aqueous extract on female albino mice as shown in table (1) shows that no animal died within 24 hours after treatment with extract. The major signs of toxicity included difficulty in breathing, loss of appetite and general weakness, these signs were not seen in all animal groups. Moreover, there was no death among mice in all the groups throughout the two weeks of the experimental. Thus, LD50 being greater than 5000 mg/kg bodyweight. Therefore *G. alypum* aqueous extract is thought to be safe as suggested by Lorke (1983).

Table 1: Acute toxicity effect of aqueous extract of Globularia alypum

Experiment	Doses (mg/kg of bodyweight)	Numbers of dead mice
Phase 1	10	0/3
	100	0/3
	1000	0/3
Control	0	0/3
Phase 2	1600	0/1
	2900	0/1
	5000	0/1

Similar observations were reported that the LD50 value of aqueous extract of Moroccan *G. alypum* leaves in mice, was greater than 14500 mg/kg. Furthermore, there was not any change in behavioral signs [6].

In addition, other previous study, indicated that orally administered of aqueous extract of Tunisian *G. alypum*

leaves, did not induce any mortality in male and female mice even for a dose of 10,000 mg/kg [8].

In contrast to this result, previous work reported that the intra-gastric administration of ethanolic extract of *G. alypum* (From Jordan) at a dose 800 mg/kg to pregnant female rats for 30 serial days, reduced the number of viable fetuses. Therefore, the authors suggested that the ethanol extract of *G. alypum* leaves (800 mg/ml) have some reproductive toxicity in female rats [7].

CONCLUSION

The present work shows that the aqueous extract of Libyan *Globularia alypum*, can be considered safe, as long as the LD50 is greater than 5000 mg/kg bodyweight.

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Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration between authors.

Consent

Authors declare that verbal informed consent was obtained from the participate for publication of this study.

Ethical approval

The study protocol was reviewed and approved by the Ethical Committees of National Authority for Scientific Research (NASR) of Libya in December 2016 by University of Tripoli.

Disclaimer

The article has not been previously presented or published.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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