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Research Article

ACUTE TOXICITY STUDY OF EXTRACTS OF *EICHHORNIA CRASSIPES* (MART.) SOLMS

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ABSTRACT

Eichhornia crassipes is a water weed and is considered as a threat to the environment and economy. The purpose of the study was to test the acute oral toxicity of the extracts of the plant. Acute toxicity of ethyl acetate, aqueous extracts and methanol fractionate of *Eichhornia crassipes* was evaluated in Swiss mice. The acute toxicity studies were carried out based on OECD guidelines 423 and fixed dosage studies was adopted where the limit dose is 2000mg/kg body weight of test animal. The animals were orally administered a single dose of 100, 250, 500,750, 1000, 2000mg/kg body weight. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of the extract for 14 days. The highest dose administered (2000mg/kg body weight) did not produce mortality or changes in general behaviour of the test animals. These results indicate the safety of the oral administration of ethyl acetate extract, aqueous extract and methanol fractionate of aqueous extract of *Eichhornia crassipes*.

Keywords: Toxicity, *Eichhornia crassipes*, Waterhyacinth, Non-toxic

INTRODUCTION

Eichhornia crassipes (Mart.) Solms (Waterhyacinth) is an aquatic macrophyte, monocotyledon of the family pontederiaceae [1]. Waterhyacinth possesses phytochemicals [2a-3] which are of medicinal importance [4]. The methanol extract of leaves of this plant aids in wound healing process [5] and has tumour inhibition potential [6]. In addition, the extracts of this plant exhibit antimicrobial activity [7].

Plants or drugs must be ensured to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used [8].

The main aim of our study was to evaluate the extracts for their toxic effects before it can be used for applications that are of importance to the public. Hence the ethyl acetate extract, aqueous extract and methanol fractionate of aqueous extract of waterhyacinth were analysed for their acute toxicity profile with reference to behavioural aspects, in Swiss Albino mice. The limit test dose of 2000mg/kg body weight was used following OECD guidelines [9 10].

EXPERIMENTAL

Plant collection

Waterhyacinth is an easily accessible plant and was collected from Singanallur boat house, Coimbatore, Tamilnadu in March, 2010. The plant sample was identified by Dr.G.V.S.Murthy, Scientist F & Head of Office, Botanical Survey of India, Sourthern Regional Centre, Coimbatore- 641 002 with the number BSI/SRC/5/23/2011-12/Tech. The voucher specimen of the plant is kept in the Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women University for future reference. The root portion was cut off and the plant was washed thoroughly to free from debris. The leaves and shoot portion were shade dried for 20 days. The dried plant material was sliced, ground coarsely and stored for further use.

Extraction of the plant

Waterhyacinth (100g) was extracted successively with ethyl acetate (2000mL) and water (1000mL) twice for 6 hours and desolvetised yielding ethyl acetate extract (WHEAE) and aqueous extract (WHAQE). The aqueous extract was fractionated with methanol to yield the methanol fractionate (WHMeF).

Experimental animals

Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423 [11]. The institutional ethical committee of KMCH College of Pharmacy, Coimbatore, Tamilnadu, India approved the protocol for these experiments under number KMCHRET/PhD23/2009-10.

Experiments were performed using healthy young adult female Swiss albino mice, nulliparous, non-pregnant and weighing 25-30 g. Female rats were chosen because of their greater sensitivity to treatment [12].

Assignment of animals

The animals were randomly divided into six groups each containing six mice. They were identified by the markings using a yellow stain. One mouse was unmarked and the others were marked on head, body, tail, head and body, body and tail, to ease the observation.

Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2ºC). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The animals were fed with standard laboratory animal food pellets with water *ad libitum*.

Mode of administration

The test substance was administered in a single dose by gavage using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water).

Administration Dose

Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 100, 250, 500, 750, 1000 and 2000 mg/kg. After the administration of test substance, food for the mice was withheld for 2 h.

Test substance administration volume

The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

Observation period

Animals were observed individually after atleast once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes.

Signs recorded during acute toxicity studies

Direct observation parameters include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then these were maintained for a further 14 days with a daily observation.

Statistical Analysis

Data are presented as a mean **±** SEM (Standard Error of the Mean). Comparisons were made between the treated groups by the use of single way ANalysis Of VAriance (ANOVA). All data were analysed using Sigmastat version 3.1. P< 0.05 was considered as the level statistical significance.

RESULTS

The present study conducted as per the OECD guidelines 423 revealed that the said extracts did not produce any mortality

throughout the study period of 14 days even when the limit dose was maintained at 2000mg/kg body weight. The oral LD_{50} was indeterminable being in excess of 2000mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-toxic.

Table 1 indicates the parameters observed before and after the administration of the test substance for the three extracts of waterhyacinth. The writhing reflux was observed immediately upto 15 min after administration of the test substance at all administered doses for the extracts of waterhyacinth whereas all the other parameters observed were normal even at the highest dosage of 2000mg/kg body weight of the test animal. This clearly indicated that the above extracts of waterhyacinth do not produce oral toxicity. The medium lethal dose (LD_{50}) of the extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect. From the statistical analysis of the dosage administered to the animals, it was found that the values are significant at 5% (Table 2).

Table1: Effect of WHEAE, WHAQE and WHMeF on acute oral toxicity test in mice

S.No	Response	Unmarked		Head		Body		Tail		Head & Tail		Head & Body	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Touch response	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Pain response	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
8	Righting reflux	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
9	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Pinna reflux	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Corneal reflux	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
12	Writhing	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present
13	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
18	Hyper activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 2: Dosage of extracts administered to test animals

Concentration (mg/kg)	WHEAE	WHAQE	WHMeF
100	2.35 ± 0.49^{f}	1.73 ± 0.57^{f}	2.16 ± 0.44^{f}
250	6.25 ± 0.73^{e}	6.12 ± 0.92^{e}	6.75 ± 0.89^{e}
500	13.58 ± 1.49^{d}	12.83 ± 1.45^{d}	12.66 ± 1.01^{d}
750	17.75 ± 1.93^{c}	18.75 ± 1.71^{c}	19.25 ± 1.79^{c}
1000	25.25 ± 2.02^{b}	27.50 ± 2.23^{b}	23.33 ± 1.70^{b}
2000	49.02 ± 3.03^{a}	47.33 ± 2.01^{a}	44.66 ± 1.72^{a}

The values of the dose administered to the animals are expressed as mean ± SD of six samples in each group. A column means followed by a common superscript are not significant at 5% by using Duncan's Multiple Range Test (DMRT).

DISCUSSION

The non-toxic nature of ethyl acetate, aqueous extract and methanol fractionate of waterhyacinth is evident by the absence of mortality of the test animals at oral treatment of 2000mg/ kg body weight. Similar study conducted by Ali et al (2009) [6] showed that the methanolic extract of waterhyacinth at dose of 500mg/kg body weight is non-toxic.

Jayanthi et al. (2011) [2a] demonstrated the presence of phytochemicals like flavonoids, alkaloids, sterols, proteins in the aqueous extract and anthroquinones, phenolics in the ethyl acetate extract of waterhyacinth. Presence of flavonoids, anthroquinones, anthocyanins and carbohydrates in methanol fractionate of waterhyacinth was revealed by Jayanthi and Lalitha (2011) [2b]. These metabolites are generally used in various pharmaceutical and cosmetic preparations [13] which are an indication that these metabolites may be non-toxic. For any therapeutic and cosmetic application, compounds of the plant or its extracts used must be practically non-toxic. Hence to establish the non-toxic nature of waterhyacinth extracts, acute oral toxicity of WHEAE, WHAQE and WHMeF was tested. The non-toxic nature of the ethyl acetate extract, aqueous extract and methanol fractionate of waterhyacinth reveals the non-toxic nature of the foresaid phytochemicals at the tested dosage. Hence, the ethyl acetate extract, aqueous extract and methanol fractionate of waterhyacinth may be exploited for its use in product application like pharmaceuticals/ nutraceuticals/ cosmeceuticals. The oral non-toxic nature of the plant and the use of this plant in dental carries [14] goes hand in hand with a scientific evidence provided by the study.

CONCLUSION

The non-toxic nature of the ethyl acetate extract (WHEAE), aqueous extract (WHAQE) and methanol fractionate (WHMeF) of waterhyacinth is evident from the acute oral toxicity conducted as per OECD guidelines. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the foresaid extracts. Hence waterhyacinth could be safe up to the dose of 2000 mg/kg body weight of the animal. Further studies are warranted for determining chronic toxic symptoms.

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