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Research Article

ACUTE ORAL TOXICITY STUDY OF ETHANOLIC EXTRACT OF *ACTINOSCIRPUS GROSSUS* (L.F.) GOETGH. AND D.A. SIMPSON

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ABSTRACT

Objective: To study the acute oral toxicity of ethanolic extract of *Actinoscirpus grossus* (L.f.) Goetgh. and D.A. Simpson in Wistar albino rats.

Methods: Ethanolic extract of the plant was assessed for single dose acute toxicity by employing Organisation for Economic Co-Operation and Development(OECD) guidelines 425 using Acute Oral Toxicity(AOT) software. The dosed (up or down as per the requirement) rats were observed for 14 days for general appearance, behavior, mortality, and necropsy. A total of 5 healthy female rats of body weight 225±25 g were used.

Results: The test substance did not produce any mortality up to the dose of 2000 mg/kg per oral.

Conclusion: Test substance is without any toxic potential even at the dose of 2000 mg/kg in animals and the Lethal Dose (LD_{50}) value of *A. grossus* (L.f.) Goetgh. and D.A. Simpson was found to be more than 2000 mg/kg body weight.

Keywords: AOT Software, OECD guidelines, Mortality, LD_{50}.

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INTRODUCTION

Actinoscirpus grossus (L.f.) Goetgh. and D.A. Simpson - (*A.grossus*) commonly known as *Kasheruk* in Indian system of Medicine belongs to Cyperaceae family [1]. *A. grossus* (L.f.) Goetgh. and D.A. Simpson has been indicated for different ailments such as antidiarrheal, antiemetic, non-specific antispasmodic, and progesterone like activity such as activity and digestive disorders. The root is slightly sweet in taste and indicated for cooling, laxative, liver disorders, diuretic, used to treat burning sensations, vomiting, and diarrhea and has astringent property [2-4].

Acute oral toxicity study was carried out by administration of single or multiple doses in a period exceeding 24 h, up to a limit of 2000 mg/kg. The objective of the study is to identify a dose causing major adverse effects and estimation of the minimum dose causing lethality, according to regulatory guidelines [5,6].

There are several steps in the drug development procedure one such step is acute toxicity studies. Acute toxicity tests help in getting information about the biological activity of chemical and get insight into its mechanism of action. Toxic profile of plant extract or herbal formulations is important to consider them safe before used as medicines [7,8].

Acute toxicity studies are carried out to fix the dose in animal studies and to estimate the LD_{50} of the herbal product. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity. The test compound should be administered to animals to identify doses causing no adverse effect and doses causing major (life-threatening) toxicity. Acute toxicity studies in animals should ordinarily be conducted using two routes of drug administration [9]. The route intended for human administration and intravenous administration if feasible. When intravenous dosing is proposed in humans, use of this route alone in animal testing is sufficient. Animals should be observed for 14 days after test substance administration. All mortalities, clinical signs, time of onset, duration, and reversibility of toxicity should be recorded. Gross necropsies should be performed on all animals, including those sacrificed moribund, found dead, or terminated at 14 days [10].

METHODS

The plant materials

The authenticated sample of plant tuber powder of *A. grossus* was purchased from Vaidya Hukam Chand Arogyadham Gharaunda, Haryana India. The voucher specimen (No. 495/14101822) was deposited at the Pharmacognosy Laboratory of S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi, for future reference.

Preparation of ethanolic extract of *A. grossus*

A. grossus tuber powder weighing 430 g was taken in 5L round bottom flask. 3.5 L of ethanol was added and allowed to stand for 24 h. Weighed accurately 430 g of the sample in a Round Bottom Flask. 3.5 L of ethanol was added and allowed to stand for 24 h. The contents were filtered, and the extract was concentrated by distillation and solvent was removed by evaporation on a water bath. It was completely dried under vacuum. The percentage of dried extract with reference to the sample taken was recorded.

Experimental animals

Female Wistar albino rats weighing 225±25 g were procured from animal house attached to the Research Centre NITTE University. The animals for the current study were approved by an Institutional Animal Ethical Committee of NITTE University approval (Reg. no.115/1999/CPCSEA). The selected animals were kept under acclimatization for 7 days before dosing. The animals were marked with a saturated picric acid solution in water for proper identification. Rats were housed in

each cage of polypropylene with stainless steel top grill. The dry husk was used as bedding material and was changed every morning. The animal was exposed to 12 h light and 12 h dark cycle with the relative humidity 50–70% and the ambient temperature 22±03°C, standard commercial rat feed was provided throughout the study period except on the previous night of dosing, i.e. (overnight), fasting before dosing.

Experimental design

The *A. grossus* ethanol extract (AGEE) was made a suspension with 0.5% of gum acacia and dosed 1 ml/100 g body weight with the help of gastric gavage attached with syringe (Fig. 1). Three dose levels 175 mg/kg, 550 mg/kg, and 2000 mg/kg, were selected according to the OECD guidelines 425 using AOT software (Table 1). The animals were observed every hour after drug administration for 4 consecutive hours followed by 24 and 48 h for its morbidity and mortality. The following cage side observations were done without disturbing the animal attention and at the end of every hour, the animal was individually exposed to open arena for recording the behavioral changes such as increased or decreased motor activity, convulsions, straub's reaction (Fig. 2), muscle spasm, catatonia, spasticity, opisthotonus, hyperesthesia, muscle relaxation, anesthesia, arching and rolling, lacrimation, salivation, diarrhea, writhing, mode of respiration, changes in skin color, central nervous system depression - hypo activity, passivity, relaxation, ataxia, and narcosis.

Animals were observed at 1, 2, 3, 4, 24 h, and 48 h after dosing and thereafter daily once for mortality during the entire period of the study (i.e., 14 days) [11,12].

RESULTS

Physical and behavioral examination

There were no physical and behavioral changes - (except mild decrease in motor activity, irritability, and Straub's reaction (Fig. 2) and ataxia seen in 3 rats in the group 2000 mg/kg in all the treated animals on day 1 at ½, 1, 2, 3, and 4 h intervals after dosing and thereafter once daily for 14 consecutive days. Thus, the data obtained from the study on single dose administration of AGEE oral administration up to 14 days of observation period does not result in any physical and behavioral changes (Table 2).

Mortality

All the animals belonging to the treated group survived throughout the 14 days observation period after dosing.

DISCUSSION

A. grossus is a principal weed of four South East Asian Countries it occurs in swampy and inundated places such as pools, ditches, and marshes. There are many families of phytochemicals, and they help the human body in a variety of ways. Limited studies on traditional medicinal plants hinder utilization of plant-based medicine; therefore, to develop safe, natural plant products, preliminary study is necessary [13]. It is important to check its safety profile in animals before starting any trial. Acute oral toxicity is usually carried out in small groups of animal's gives information about the toxic profile of the plant extract. In any pharmaceutical preparation, acute oral toxicity studies are necessary to use in humans [14-16]. In the present study, the acute oral toxicity of ethanolic extract of *A. grossus* (L.f.) Goetgh. and D.A. Simpson was carried out, and no significant toxic changes were observed for any of the 3 dose levels taken in the study, and hence, the LD_{50} was found to be more than 2000 mg/kg.

CONCLUSION

Ethanolic extract of tuber of *A. grossus* (L.f.) Goetgh. and D.A. Simpson did not produce any mortality up to the dose of 2000 mg/kg per oral which is evident from the acute oral toxicity study conducted as per the OECD guidelines. At the dose level studied, the drug also did not

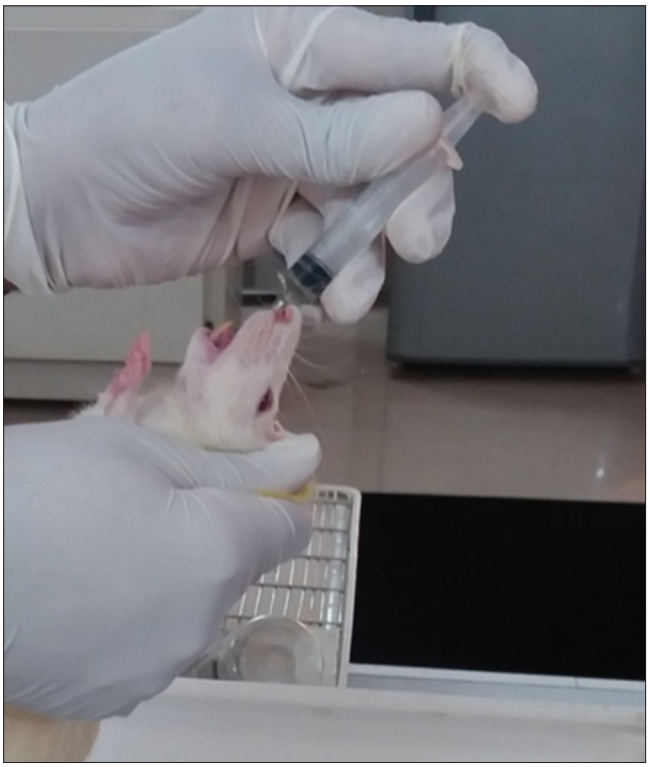

Fig. 1: Oral dosing of *Actinoscirpus grossus* ethanolic extract using gastric gavage attached to syringe

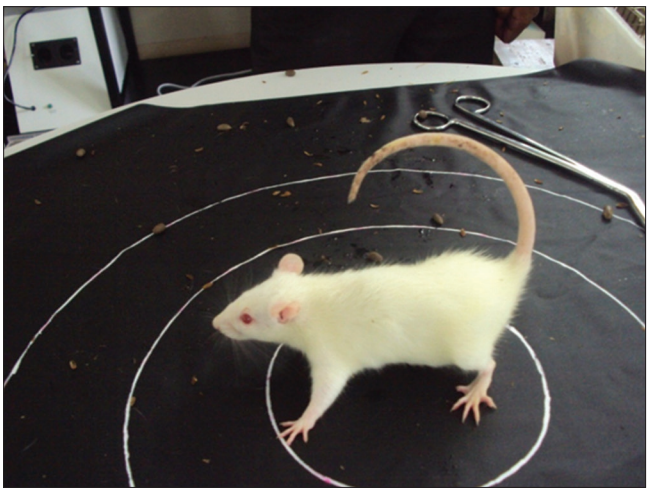

Fig. 2: Straub's reaction seen with rats given with the ethanolic extract of *Actinoscirpus grossus* 2000 mg/kg

Table 1: Animal grouping and dose calculation

Sl. No.	Identification of animals	Desired dose (according to AOT)	Body weight (g)	Calculated dose (ml)
1	Head	175 mg/kg	240	2.4
2	Neck	550 mg/kg	210	2.1
3	Back	2000 mg/kg	230	2.3
4	Base of the tail	2000 mg/kg	250	2.5
5	No mark	2000 mg/kg	220	2.2

Table 2: Effect of *A. grossus* ethanolic extract 2000 mg/kg on physical and behavioral Sign and symptoms

Signs and symptoms	Basal	30 min	1 h	2 h	3 h	4 h	24 h	48 h
General impression	N	N	N	N	N	N	N	N
Increased motor activity	–	–	–	–	+	+	–	–
Convulsion: Tonic	–	–	–	–	–	–	–	–
Clonic	–	–	–	–	–	–	–	–
Straub's reaction	–	+	+	+	+	–	–	–
Muscle spasm	–	–	–	–	–	–	–	–
Catatonia	–	–	–	–	–	–	–	–
Opisthotonus	–	–	–	–	–	–	–	–
Hyperesthesia	–	–	–	–	–	–	–	–
Decreased motor activity	–	–	–	–	–	–	–	–
Muscle relaxation	–	–	–	–	–	–	–	–
Anesthesia	–	–	–	–	–	–	–	–
Arching and rolling	–	–	–	–	–	–	–	–
Lachrymation	–	–	–	–	–	–	–	–
Diarrhea	–	–	–	–	–	–	–	–
Writhing	–	–	–	–	–	–	–	–
Salivation								
Viscid	–	–	–	–	–	–	–	–
Watery	–	–	–	–	–	–	–	–
Respiration								
Stimulation	–	–	–	–	–	–	–	–
Depression	–	–	–	–	–	–	–	–
Failure	–	–	–	–	–	–	–	–
Skin color								
Blanching	–	–	–	–	–	–	–	–
Cyanosis	–	–	–	+	+	+	–	–
Vasodilatation	–	–	–	–	–	–	–	–
Grip strength	N	N	N	N	N	N	N	N
Visual placing response	N	N	N	N	N	N	N	N
Tail pinch response	N	N	N	+	+	+	N	N
Auditory response	N	N	N	N	N	N	N	N
Mucus membrane	N	N	N	N	N	N	N	N
Piloerection	–	–	–	–	–	–	–	–

N: Normal, –: Absent, +: Present

produce any observable toxic effect except for mild decrease in motor activity, irritation, Straub's reaction, and ataxia in animal receiving the dose 2000 mg/kg and thus it could be concluded that the test substance is without any toxic potential even at the dose of 2000 mg/kg in animals and the LD_{50} value of *A. grossus* (L.f.) Goetgh. and D.A. Simpson was found to be more than 2000 mg/kg body weight.

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