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Research Article

Acute Toxicity Study of Ethanolic Extract of the *Cyperus kyllingia* Roots

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Abstract

The present study was designed to find out LD_{50} and to ascertain the safety of ethanol extracts of roots of *Cyperus kyllingia* by acute toxicity study (IP rout) in Swiss Albino mice (20g-25g) as per guideline (OECD guidelines). The study involved intraperitoneal administration of different doses of the extract to groups of male mice. All mice, five mice per group (six group), group no. 1 was treated as control. From group 2 to group 6, were sequentially administered with CK root extract (ethanolic extract), in doses of 100 mg/kg , 200 mg/kg, 300 mg/kg, 400 mg/kg and 500 mg/kg of body weight, in that order respectively, to determine acute toxicity. Signs of toxicity and mortality were noted after 24h of administration of the extract. We had observed at dose 200 mg/kg and 400 mg/kg body wt the percentage (%) of mortality 40% and 60%. Conclusively, LD50 value of ethanol extracts of roots of *Cyperus kyllingia* was found to be 251.3014 mg/kg body weight.

Keywords: Acute toxicity, *Cyperus kyllingia*, OECD guidelines

Introduction

The Lethal Dose 50 (LD50) involves the administration of a substance to a group of animals at increasing doses in order to determine the dose kills 50% of the test subject within a set time frame. It is the study of poisonous effect of drugs and other chemicals with emphasis on detection, prevention and treatment of poisoning. It also includes the study of adverse effect of drugs, since the same substance can be a drug or a poison, depending on the dose[1]. An advantage of determine the LD50 at an early stage in the investigation of a new drug is that the doses used to established the drugs spectrum of pharmacological activity could be related to its lethal dose[2]. *Cyperus kyllinga* (Fam. Cyperacaea) commonly called Nut grass and locally "Nirbisi", is a tufted perennial weed, 5-45 cm tall, with short, horizontal creeping rhizome, 1-2 mm in diameter and cornered by orate – lanceolate scales, internodes variable in length[3]. It possesses various medicinal properties and used to treat many diseases [4].

Materials and Methods

Collection and Authentication of plant

The plant was collected from the local fields of Ichharia village (Sonamukhee forest range), Bankura,West Bengal, India and authenticated by Botanical Survey of India (BSI) (Authentication no. BSI/CNH/71/2011/Tech.II/), Shibpur, Howrah, West Bengal, India.

Preparation of ethanolic extracts

In brief, the roots were carefully washed under running tap water followed by sterile distilled water and dried at room temperature for 5 days, pulverized to a fine powder and stored in airtight bottles. The root powder was later extracted with absolute alcohol by soxhlet extraction procedure and then concentrated by distilling the solvent.

Experimental animals

Acute toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines [5]. The institutional ethical committee of Bengal College of Pharmaceutical Sciences & Research, Durgapur, West Bengal, India, approved the protocol for these experiments under number BCPSR/IAEC/002. Experiments were performed using healthy young adult female swiss albino mice, non-pregnant and weighing 25-30 g. Female mice were chosen because of their greater sensitivity to treatment [6].

Assignment of animals

The animals were randomly divided into six groups each containing five mice. They were identified by the markings them using a red color marker. Group no. 1 was unmarked (treated as control group) and the others were marked on tail.

Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with temperature controlled environment (23 $\pm$ 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.

Mode of administration

Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water). Each dose group should consist of 5 mice. Inject the drug by intraperitoneal rout and observe the animal for 14 days for death due to acute toxicity.

Administration Dose

Following the period of fasting, animals were weighed and test substance was administered via i.p. rout at a dose of 100, 200, 300, 400 and 500 mg/kg body weight. After the administration of test substance, food for the mice was withheld for 2 h.

Observation period

Animals were observed individually after at least once during the first 30 min, periodically during the first 24 hrs, with special attention given during the first 4 h and daily thereafter, for a total of 14 days.

Signs recorded during acute toxicity studies

All the mice were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes. The time of death were recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 hrs and then these were maintained for a further 14 days with a daily observation.

Statistical Analysis:

The statistical analyses were carried out using statistical package for social sciences (SPSS- computerpackage). Percentage organ-body weight ratios and mice body weights were expressed as mean ± SD (Table 2).

Table 2: Statistical Details

GROUP	LOG DOSE	MEAN	±SD	PROBIT	MEAN	±SD
2	2.00			3.36		
3	2.30			4.75		
4	2.47	2.412	± 0.2730	5.25	5.05	± 1.6072
5	2.60			5.25		
6	2.69			6.64		

Results and Discussion

The present study conducted as per the OECD guidelines. The study period of 14 days even when the limit dose was maintained at 500 mg/kg body weight. Table 1 indicates the parameters observed before and after the administration of the test substance. The writhing reflux was observed immediately upto 15 min after administration of the test substance. It was observed that at doses 100, 200, 300, 400 and 500 mg/kg body weight the percentage of dead 0, 40, 60, 60 and 100 respectively (Table 1). This is clearly indicated that between the 200 and 300 mg/kg body weight doses 50% mortality has been shown.

Table 1: LD50 output

GROUP	DOSE mg/kg. bw.	LOG DOSE	DEAD/ TOTAL	% DEAD	CORRECTED %	PROBIT
1			Group no. 1 treated as control			
2	100	2.00	0/5	0	5	3.36
3	200	2.30	2/5	40	40	4.75
4	300	2.47	3/5	60	60	5.25
5	400	2.60	3/5	60	60	5.25
6	500	2.69	5/5	100	95	6.64

So, the LD50 dose must be in between 200 and 300 mg/kg body weight. The particular dose we calculate when log dose vs. probit value was plotted (Figure 1).

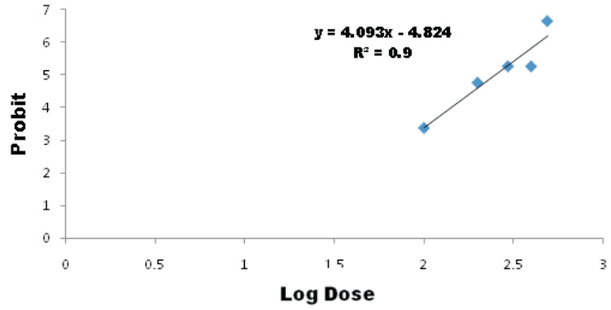

Figure 1: log dose vs. probit value graph

Probit 5 on y axis corresponds to Log dose = 2.400195
LD50 = 251.3014 mg/kg body weight (Antilog of 2.400195)
(Probit 5 means 50% death)

Conclusion

The present study was designed to find out LD50 and to ascertain the safety of ethanol extracts of roots of *Cyperus kyllingia* by acute toxicity study (IP rout) in Swiss Albino mice (20g-25g) as per guideline (OECD guidelines). The study involved intraperitoneal administration of different doses of the extract to groups of male mice.The advantage of determine the LD50 at an early stage in the investigation of a new drug is that the doses used to established the drugs spectrum of pharmacological activity could be related to its lethal dose. So the LD50 of the ethanolic extract of *Cyperus kyllingia* root is 251.3014 mg/kg body weight.

Conflicts of Interest The authors declare no competing interest.

Acknowledgement

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