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RESEARCH ARTICLE

ACUTE ORAL TOXICITY STUDY OF *CURCULIGOORCHIOIDES* IN FEMALE ALBINO WISTAR RATS

[1,*]Bibu, J. K. and [2]Usha, P. T. A.

[1]Assistant Professor, Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Mannuthy – 680 651, Kerala Veterinary and Animal Sciences University
[2]Professor & Head, Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Mannuthy – 680 651, Kerala Veterinary and Animal Sciences University

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ABSTRACT

The methanolic extract of *Curculigoorchioides Gaertn.*(Family: Hypoxidaceae) was evaluated for its acute oral toxicity by administering as a single oral dose to female albino Wistar rats. The plant extract was administered orally in a sequential manner to five female rats at the limit dose level of 5000 mg/kg body weight. On the day of dosing, all the animals were observed for mortality and clinical signs for first 10 min, 30 min, 1 h, 2 h, 4 h and 6 h after dosing and thereafter twice daily for mortality and once a day for clinical signs, for 14 days. The body weight of rats was recorded and weekly body weight gain was calculated. After the observation period of 14 days, the surviving animals were sacrificed and subjected to complete necropsy. All treated animals survived throughout the study period. The animals did not show any major adverse clinical signs. The overall weight gain of animals during the 14 day observation period was found to be normal. On necropsy, no major gross pathological changes were observed. Based on the findings of the present study, the methanolicextract of *Curculigoorchioides*, after oral administration as a single dose to female albino Wistar rats was found to be safe upto 5000 mg/kg body weight.

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INTRODUCTION

Curculigoorchioides Gaertn. is a tiny herbal plant widely distributed in India, China, Malaya, Japan and Australia. In India, the tuberous root of this plant is considered to be tonic, alterative, demulscent, diuretic, restorative and is used in poultice for itch and skin diseases (Lakshmi *et al.*, 2003). Numerous phytochemicals have been identified from the rhizomes of this plant which include cycloartane glycosides (Yokosuta *et al.*, 2010) and phenolic glycosides (Wu *et al.*, 2005). Anticancer, hepatoprotective, anti-oxidant and immunomodulator properties have been found in experimental models (Latha*et al.*, 1999, Dhar *et al.*, 1968). Eventhough the pharmacological activity of the rhizomes of the plant has been studied, its toxicological profile at higher doses hasn't been studied yet. Hence the objective of this study was to assess the toxicological profile ofmethanolic extract of rhizomes of *Curculigoorchioides* on single administration via oral route to female albino Wistar rats. The animals were observed for 14 days or more, depending on the occurrence of toxic symptoms.

****Corresponding author: Bibu, J.K.***
Assistant Professor, Department of Veterinary Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Mannuthy – 680 651, Kerala Veterinary and Animal Sciences University

The results of acute toxicity study would be useful for selection of doses for repeated dose toxicity studies and may also provide preliminary information on the target organs of toxicity.

MATERIALS AND METHODS

This study was performed as per the Organisation for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals No. 420, Section 4: Health Effects "Acute Oral Toxicity - Fixed Dose Procedure" adopted on 17th December 2001.

Procurement, Authentication and Preparation of Plant material

The rhizomes of *C. orchioides* were procured from Amala region of Thrissur district and authenticated by Raw Material Herbarium and Museum Department of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (NISCAIR/RHMD/Consult/2012-13/2096/103/ 01) and a voucher specimen was deposited at Fr. Gabriel Herbarium Specialised on medicinal plants at Amala Ayurveda Hospital and Research Centre, Amalanagar, Thrissur with specimen no. 131.

The rhizomes of *C. orchioides*were air- dried at room temperature and coarsely powdered using an electric pulverizer. The powders obtained were extracted using a Soxhlet apparatus with methanol. The methanolic extract was then concentrated on a rotary vacuum evapourator under reduced pressure and temperature (55^{o}C) and kept under refrigeration for the complete evapouration of the solvent. This extract was further used for the study.

to dosing, were used in this study. They were randomly assigned to the cages and the individual animal was fur marked with picric acid. The females were nulliparous and nonpregnant. The animals were fed with pelleted feed *ad libitum* supplied by M/s Krish Scientist's Shopee, Bangalore, India and U.V. purified water *ad libitum.* Veterinary examination was done before allocation of animals to groups and after the completion of acclimation period.

Table 1. Mortality record - acute oral toxicity study of methanolic extract of rhizomes of *C. orchioides*

Sighting study					
Group	Dose (mg/kg)	Sex	Animal ID	Mortality	
				Absolute	Relative %
I	5000	Female	1FH	0	0
Main study					
I	5000	Female	2FB	0	0
			3FT	0	0
			4FW	0	0
			5FHB	0	0

Table 2. Clinical signs observed – Acute oral toxicity study of methanolic extract of rhizomes of *C. orchioides*

Sighting study					
Group	Dose (mg/kg)	Sex	Animal ID	Observed Signs	Period of signs in days From - To
I	5000	Female	1FH	Nil	0 - 14
Main study					
I	5000	Female	2FB	Nil	0-14
			3FT	Nil	0-14
			4FW	Nil	0-14
			5FHB	Nil	0-14

Table3. Body weight and percent body weight gain - Acute oral toxicity study of methanolic extract of rhizomes of *C. orchioides*

Sighting study:Group: I, Dose: 5000 mg/kg body weight						
Animal ID	Body weight Day 0	Body weight Day 7	% body weight gain Day 0-7	Body weight Day 14	% body weight gain Day 7- 14	% body weight gain Day 0- 14
1FH	159	187	17.61	217	16.04	36.48
Main study:Group: I, Dose: 5000 mg/kg body weight						
2FB	154	182	18.18	210	15.38	36.36
3FT	157	192	22.29	212	10.42	35.03
4FW	157	186	18.47	220	18.28	40.13
5FHB	155	176	13.55	202	14.77	30.32

Table 4. Gross pathology findings – Acute oral toxicity study of methanolic extract of rhizomes of *C. orchioides*

Sighting study: Group: I, Dose: 5000 mg/kg body weight		
Animal ID	Fate	Gross pathology findings
1FH	TS	NAD
Main study: Group: I, Dose: 5000 mg/kg body weight		
2FB	TS	NAD
3FT	TS	NAD
4FW	TS	NAD
5FHB	TS	NAD

TS = Terminal sacrifice
NAD = No abnormality detected

Test system

Healthy adult female rats of 8-12 weeks of age were purchased from Small Animal Breeding Station, Mannuthy. Institutional animal ethics committee approval was obtained prior to procurement and initiation of experiment.The animals which were acclimatized to laboratory conditions for one week prior

Experimental procedure and observations

The rats were deprived of feed overnight before and 3 h after the administration of the plant extract. Water was not withheld during this period. The extract was solubilized in demineralized water and it was administered by gavage to rats

using an intubation needle of appropriate size fitted on to a syringe. The experiment procedure consisted of a sighting study and a main study. For the sighting study, one animal was dosed at a dose rate of 5000mg/kg orally. For main study, four animals were dosed at a dose rate of 5000 mg/kg orally. The animals were observed individually for first 10 min, 30 min, 1 h, 2 h, 4 h and 6 h after dosing and thereafter twice daily for mortality and once a day for clinical signs, for 14 days.

The body weight of rats was recorded and weekly body weight gain was calculated. After the observation period of 14 days, the surviving animals were sacrificed and subjected to complete necropsy.

RESULTS AND DISCUSSION

The study was designed to determine the acute oral toxicity profile of methanolic extract of *Curculigoorchioides* in female albino Wistar rats. Rats are one of the recommended rodent species by the regulatory authorities for conducting preclinical toxicity studies among rodents. Also, it is one of the most sensitive species for expression of toxic responses. The sighting study did not show any mortality and major abnormal clinical signs at the dose level of 5000 mg/kg body weight and the animal survived throughout the study period (Table 1 and Table 2). The treated animal showed overall normal body weight gain during 14 day observation period (Table 3). Based on this, the main study was continued at the dose level of 5000 mg/kg body weight. In the main study, all animals survived throughout the study period. The treated animals did not show any major abnormal clinical signs. The overall weight gain of animals during the14 day observation period was found to be normal. The gross pathological examination did not reveal any major abnormalities (Table 4).

Similar results were observed by Ramchandani *et al*. (2014) when they tested the methanolic extract of *Curculigoorchioides* rhizomes extract at a dose rate of 2000 mg/kg orally as per Organisation for Economic Cooperation and Development (OECD) guidelines 423 in mice.

Based on the findings of the present study, the methanolic extract of *Curculigoorchioides,* after oral administration as a single dose to female albino Wistar rats was found to be safe upto5000 mg/kg body weight.

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