

**TOXICOLOGICAL STUDIES OF ALCOHOLIC EXTRACT OF  
*CURCULIGO ORCHIOIDES* ON ALBINO RATS**

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**Summary**

The rhizomes of *Curculigo orchioides* Gaertn. (Amaryllidacea) is an important Ayurvedic as well as Unani drug. It is present in several drug formulations used in the treatment of menorrhagia and other gynaecological problems. The present study was aimed at assessing the toxicological effects of the rhizomes of *Curculigo orchioides* on albino rats. The acute toxicity study did not show any toxic symptoms or death, up to a maximum administered dose of 2000 mg/kg. The biochemical studies conducted on female albino rats to assess possible health hazards likely to arise from repeated exposure, did not show any toxic effects on heart, kidney and liver.

**Key words:** Curculigo orchioides, Acute toxicity, Biochemical parameters.

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## Introduction

*Curculigo orchioides* (Fam: Amaryllidaceae) is a small perennial herb with elongated tuberous rootstock, found wild in sandy regions of hotter parts of India and Srilanka<sup>[1]</sup>. The rhizome is an important Ayurvedic as well as Unani drug, for a wide variety of ailments, especially as a general tonic, as an aphrodisiac and in the treatment of menorrhagia<sup>[2]</sup>. A number of formulations containing *Curculigo orchioides* e.g., Progen<sup>®</sup> and Maharishi Amrith Kalash<sup>®</sup> are being promoted for use in conditions like irregular menses, menopause, breast cancer and infertility<sup>[3,4]</sup>. It also has reported estrogenic activity<sup>[5]</sup>.

The rhizomes were known to contain resin, tannin, mucilage, fat and ash containing oxalate of calcium. Various flavonoid, phenolic and triterpenoid glycosides were isolated from the rhizomes of *Curculigo orchioides*.  $\beta$ -sitosterol and crystalline needles of sapogenin have also been detected<sup>[6,7]</sup>.

Inspite of its widespread use in Ayurveda, no information is available on its acute toxic effects and possible adverse effects on heart, liver and kidney on prolonged use. Hence the present study was undertaken to evaluate the acute toxic effects and effects on biochemical parameters of blood and urine.

## Materials and methods

**Materials:** Rhizomes of *Curculigo orchioides* were collected from the field areas of Manjeshwar in the month of December. The plant was identified and confirmed by Dr. Noeline J. Pinto, Head of the Department of Botany, St. Agnes College, Mangalore. A voucher specimen (V.no. NGSM 3561) has been deposited in the pharmacognosy department of NGSM. Institute of Pharmaceutical Sciences, Mangalore. The collected rhizomes were cleaned from adhering soil and other materials, and then it was dried under shade for two weeks. The dried rhizomes were chopped and pulverized in an electric grinder. They were dried under shade, powdered and subjected to Soxhlet extraction with 80% ethanol. The extract was concentrated to get a brownish sticky mass. The yield was 8.4%.

The extract was suspended in 0.6% w/v sodium carboxy methyl cellulose (Sod. CMC) for use in animal studies.

## Animals and experimental set-up

**Acute toxicity studies:** The acute toxicity study was carried out in adult female albino rats (Wistar breed) by 'up and down method' according to OECD guidelines 425<sup>[8]</sup>.

Healthy adult female albino rats were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were fasted overnight prior to dosing. Following the periods of fasting, rats were weighed and divided into different groups, each consisting of 5 animals. The drug extract was administered in a single dose by gavage using a stomach tube, at fixed dose levels of 175, 550 and 2000 mg/kg (Note: according to OECD guideline 425, a starting dose level of 175 mg/kg can be used, if no estimate of the substance's lethality is available).

Following the dosing, the animals were observed individually once during the first 30 minutes, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days. The parameters observed during the study period include ANS profiles (e.g. heart rate, salivation, respiration, pupil size diuresis, righting reflex and diarrhoea), CNS profiles (e.g., spontaneous motor activity, sedation, convulsions and tremors), body weight and deaths.

**Effect on biochemical parameters:** A 30 days toxicity study was conducted to assess possible health hazards likely to arise upon repeated exposure over a limited period of time by observing changes in various biochemical parameters of serum and urine<sup>[9]</sup>.

Albino rats weighing 150-200 g were taken and divided into four groups, each group consisting of four animals.

Group 1 (Control): Received 0.6% w/v Sod. CMC suspension at a dose of 10 ml/kg.

Group 2 (Test): Received aqueous suspension of alcoholic extract of *Curculigo orchioides* in 0.6% w/v Sod. CMC at a dose of 300 mg/kg.

Group 3 (Test): Received aqueous suspension of alcoholic extract of *Curculigo orchioides* in 0.6% w/v Sod. CMC at a dose of 600 mg/kg.

Group 4 (Test): Received aqueous suspension of alcoholic extract of *Curculigo orchioides* in 0.6% w/v Sod. CMC at a dose of 1200 mg/kg.

All these were administered orally daily for 30 days. At the end of the exposure period (i.e., on 31<sup>st</sup> day), blood was collected by cardiac puncture from the animals. The blood was coagulated at 37<sup>o</sup>C for 30 minutes and serum was separated by centrifuging at 15,000 rpm for 10 minutes. Then the serum was estimated for the following parameters: total cholesterol, serum high density lipoprotein (HDL), serum glutamic-oxaloacetic transaminase (SGPT), serum glutamic-pyruvic transaminase (SGOT), serum alkaline phosphatase (ALP), serum creatinine, serum total proteins, serum triglycerides (TG) and lactate dehydrogenase (LDH); as per standard methods<sup>[10]</sup>. Urine analysis was conducted on 24 hours samples to determine urine creatinine.

This study was conducted in accordance with the latest CPCSEA guidelines and the experimental protocol was approved by Institutional Animals Ethics Committee.

### **Statistical Analysis**

One-way ANOVA followed by Dunnet's t-test (using GraphPad Prism 4 software) was used to analyze the difference in biochemical parameters between control group and *Curculigo orchioides* alcoholic extract treated groups.

## Results

Acute toxicity study conducted caused no death even at the maximum administered dose (i.e., 2000 mg/kg). However, there was a dose dependent increase in the magnitude of certain autonomic responses, such as piloerection, increase in scrotum size, CNS profiles such as sedation, decreased spontaneous motor activity and skeletal muscle relaxation, staggering posture and purgation.

Biochemical analysis of serum of rats treated with *Curculigo orchioides* has shown statistically significant increase in, alkaline phosphatase ( $P < 0.01$ ) and globulin ( $P < 0.05$ ) at 1200 mg/kg dose (Table 1). Changes observed in other biochemical parameters were statistically insignificant.

## Discussion

The extract of *Curculigo orchioides* is being evaluated for its safety aspect.

Since the acute toxicity study conducted in female albino rats by oral route did not show any toxic symptoms or death, up to a maximum administered dose of 2000 mg/kg, the extract can be regarded as 'practically nontoxic'<sup>[11]</sup>. However, the drug showed a dose dependent increase in the magnitude of some pharmacological actions; such as sedation, which is identical to earlier observations made<sup>[12]</sup>.

The 30 days study to assess possible health hazards likely to arise from repeated exposure over a limited period of time did not show any toxic effects on heart, kidney and liver (Since LDH, SGOT & SGPT, serum/urine creatinine, the marker enzymes for cardiac, hepatic and renal toxicity respectively were not elevated). The earlier pharmacological studies have also shown hepatoprotective and antiinflammatory activity<sup>[13,14]</sup>. Alcoholic extract of *Curculigo orchioides* at the dose of 1200 mg/kg elevated serum alkaline phosphatase and globulin levels, which can be attributed its estrogenic activity, since estrogenic compounds known to increase plasma levels globulin and alkaline phosphatase<sup>[15]</sup>. Earlier pharmacological studies on alcoholic extract of *Curculigo orchioides* has shown significant estrogenic activity at a dose of 1200 mg/kg<sup>[5]</sup>.

The preliminary acute toxicity studies revealed the non-toxic effect of the drug. Further the short-term toxicity studies also gave clean chit to the use of this drug for various menopausal symptoms, and prevention of osteoporosis.

Table 1: Effect of alcoholic extract of *Curculigo orchioides* on biochemical parameters of blood and urine

Group	Treatment	Dose (mg/kg)	Cholesterol (mg%)	HDL (mg%)	SGOT (U/L)	SGPT (U/L)	Serum Creatinine (mg%)	Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	ALP (IU/L)	TG (mg%)	LDH (U/L)	Glucose (mg/dL)	Urine creatinine (mg/day)
1	Control	-	56.7 ± 7.9	34.4 ± 5.5	218 ± 5.58	65.5 ± 6.76	0.73 ± 0.04	5.68 ± 0.34	2.8 ± 0.07	2.88 ± 0.33	4.25 ± 0.48	51 ± 4.81	158.2 ± 5.53	103.77 ± 1.63	4.69 ± 0.22
2	<i>Curculigo orchioides</i> extract (p.o)	300	54.5 ± 5.6	33.4 ± 3.2	216 ± 3.8	65.5 ± 4.42	0.75 ± 0.1	5.96 ± 0.51	2.6 ± 0.04	2.34 ± 0.46	4.32 ± 0.45	49 ± 5.23	156.6 ± 4.21	101.47 ± 0.63	4.81 ± 0.31
3		600	56.1 ± 3.3	33.8 ± 4.1	217 ± 2.5	64.3 ± 3.2	0.74 ± 0.04	6.0 ± 0.38	2.7 ± 0.08	3.9 ± 0.61	4.45 ± 0.54	50 ± 6.1	157.2 ± 5.14	102.67 ± 0.45	4.45 ± 0.45
4		1200	58.5 ± 8.3	32.7 ± 2.7	218 ± 1.66	66.1 ± 5.25	0.76 ± 0.06	6.1 ± 0.35	2.7 ± 0.09	4.38 <sup>a</sup> ± 0.43	6.51 <sup>b</sup> ± 0.75	48 ± 5.32	159.5 ± 16.63	101.84 ± 1.31	4.63 ± 0.91

<sup>a</sup>  $P < 0.05$  compared to control group

<sup>b</sup>  $P < 0.01$  compared to control group

Note: Changes in other biochemical parameters were statistically insignificant.

Values are mean ±S.E.M of 4 animals in each group. Data were analysed by One-way ANOVA followed by Dunnet's t-test

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