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Original Article

Acute Toxicity Study and *In-vivo* Anti-inflammatory Activity of Different Fractions of *Curculigo orchioides* Gaertn. Rhizome in Albino Wistar Rats

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Abstract

The effects of hydroalcoholic extract (HE) of *Curculigo orchioides* Gaertn. rhizome (Hypoxidaceae) and its alkaloidal and non-alkaloidal fractions (AF) and (NAF) were evaluated in carrageenan-induced paw edema experimental models of inflammation. The oral administration of HE, their AF and NAF fractions were used at doses of 100, 300 and 500 mg/kg. The effect produced by the HE, AF and NAF fractions were comparable to that of indomethacin with a dose of 10 mg/kg as the reference drug. The percentage of inhibition of inflammation of all extracts was dose dependent. The crude HE showed 22.45%, 35.62% and 39.03% inhibition; AF showed 31.68%, 36.89% and 41.17% inhibition; and NAF showed 28.34%, 34.49% and 37.43% inhibition of induced hind paw edema in rats at doses of 100 mg/kg, 300 mg/kg and 500 mg/kg, respectively, while indomethacin inhibited 48.66% of the edema. The acute toxicity evaluation showed that all extract were not toxic up to 2000 mg/kg (p.o). The results suggest that the anti-inflammatory effects of the extracts as claimed in folk Indian medicine.

Keywords: Acute toxicity; *Curculigo orchioides*; Hydroalcohalic extract; Inflammation; Paw edema.
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1. Introduction

A scientific evaluation of herbs or medicinal plants according to their traditional methods of use in various diseases management can incorporate them into the complementary and alternative medicine. *Curculigo orchioides* Gaertn. (Hypoxidaceae) is a perennial herb with long cylindrical rhizomes, distributed throughout India, especially in north hillside region, as well as in other Asian countries [1]. It is popularly known as "kali musali" in India. The plant is found in near sea levels up to 2300 m, especially in moist laterite soil. The rhizome, as well as the tuberous roots of the plant has been extensively used in indigenous systems of medicine in India, Pakistan, China and some other Asian countries for the treatment of various diseases [2]. The rhizomes of this plant possess medicinal as well as other properties and is effective against different

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diseases like: cooling, diuretic, aphrodisiac, tonic hemorrhoids, leucorrhoea, pruritis, skin diseases, asthma, bronchitis and jaundice, cancer, diarthrosis wound healing, and sweet etc. [3, 4]. The active compounds reported are flavones, glycosides, alkaloids, steroids, saponins, triterpenoids and other secondary metabolites [5-7]. Therefore, the plants have long since been deemed a valuable source of natural products for maintaining human health.

Therefore, we used the rhizome hydroalcoholic extracts (HE), its alkaloidal fraction (AF) and non-alkaloidal fractions (NAF) to study their anti-inflammatory effects using hind paw edema method, and their acute toxicity Wistar rats. To the best of our knowledge, there are no reports of different fractions AF and NAF on anti-inflammatory activity of *C. orchioides*.

2. Materials and methods

2.1. Sample collection

The *C. orchioides* rhizomes were collected from Dehradun, (Uttarakhand) India. The authentication was done by Scientist at Botanical survey of India (BSI), Forest Research Institute, Dehradun, Uttrakhand, India, on 7-09-2009 with letter Ref no. : BDS-112739.

2.2. Preparation of extracts

The rhizomes of the *C. orchioides* were shade dried. The shade dried rhizomes were grinded into coarse powder, weighted and defatted by petroleum ether using maceration method for 6 hour before extraction. The dry defatted rhizome powders were extracted in the Soxhlet extraction apparatus using hydroalcoholic (ethanol 70%) solvent. The hydroalcoholic extracts were concentrated using rotary vacuum evaporator. The resultant extract was dried and stored in vacuum desiccators.

2.2.1. Extraction of alkaloids

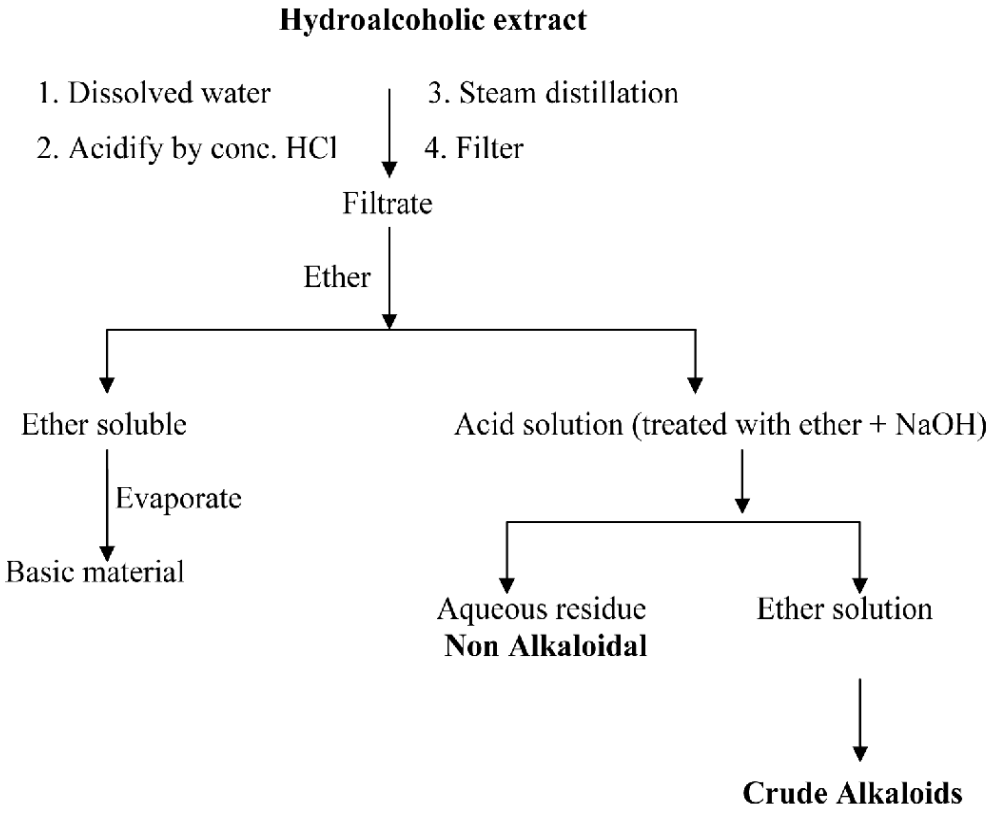
Ethanolic extract of *C. orchioides* rhizome was distilled off solvent and treated with concentrated hydrochloric acid. The basic compounds (alkaloids) were extracted as their soluble salts. The aqueous layer, containing the salt of alkaloids and soluble plant impurities, was made basic with NaOH. The insoluble alkaloids were set free and precipitated out. The solid precipitate was extracted with ether. Alkaloids passed into solution and impurities left behind, and the remaining portion of the extract was non-alkaloidal [8]. Order of separation of Alkaloids from ethanolic extract of *Curculigo orchioides* is shown in Scheme 1.

2.3. Test samples and standards

Suspension of the *C. orchioides* rhizome extracts (HE) and its alkaloid (AF) and non-alkaloid (NAF) fractions were prepared in sodium carboxyl methyl cellulose (CMC, 0.5%) in distilled water. All test samples and standard drug were administered orally to the rats. Indomethacin (10 mg/kg) was used as standard drug.

2.4. Acute toxicity test studies

Acute toxicity studies were carried out on Wistar rats according to standard procedures. Hydroalcoholic extracts at doses of 50, 100, 300, 1000, and 2000 mg/kg body weight were administered to separate groups of mice (n=5) after overnight fasting. Subsequent to administration of the extracts, the animals were observed closely for the first 3 h for any toxic manifestations such as increased locomotor activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 h. The animals were observed for a further week. The hydroalcoholic extracts were safe up to a dose of 2000 mg/kg body weight, so 100 mg/kg, 300 mg/kg and 500 mg/kg were used as moderate dose for the evaluation of anti-inflammatory activities [9].



Scheme 1. Separation of alkaloid and non-alkaloid extracts of *C. orchioides.*

2.5. Pharmacological evaluation

2.5.1. Experimental animals

Fifty five Albino rats of Wister strain of both sexes weighing (150–200 g) were maintained under controlled conditions of light (12 h) and temperature 25±2 °C in the Animal House of Guru Ram Das (PG) Institute of Management and Technology, Dehradun, for two week prior to the experiment for acclimatization. Animals had access to food and water ad libitum. All pharmacological activities were carried out as per CPCSEA (Committee for the purpose of control and supervision of experiments on animals) norms (Regn: No. 1145/a/07/CPCSEA) after obtaining the approval from the institutional animal ethical committee of department of pharmacy in GRD (PG) IMT, Dehradun.

2.5.2. Experimental design

Fifty five Wistar rats of either sex were taken and divided into 11 groups, each group was consist of 5 rats. The extracts were administered to the tests groups, indomethacin was administered to standard group and control group received only 0.5% Sodium carboxymethylcellulose (sodium CMC) suspension by oral route. Carrageenan (1% in 0.9% NaCl; 0.1 ml) was administered into the subplantar surface of the right hind paw of the Wistar rats 30 min. after the administration of the extracts or indomethacin [10]. Sodium CMC did not produce evident changes in activity response.

Group-I (control group) received 0.5% sodium CMC in distilled water (10 ml/kg body weight). Group- II (standard group) received indomethacin (10 mg/kg) suspension in 0.5% sodium CMC (10 ml/kg body weight) served as standard drug. Groups-III, IV and V (test groups 1) hydroalcohalic extract in 0.5% sodium CMC (100 mg/kg, 300 mg/kg and 500 mg/kg) at 10 ml/kg. Groups-VI, VII

Table 1. Anti inflammatory activity of different fraction of *Curculigo orchioides*.

Groups	Dose	Change in mean paw volume (in ml)				
	(mg/kg)	Initial	After 15min	After 30 min	After 60 min	After 120 min
Control	---	0.212±	0.400±	0.420±	0.430±	0.430±
		0.008	0.035	0.035	0.035	0.035
Standard	10	0.172±	0.184±	0.194±	0.202±	0.208±
		0.008	0.014[a]	0.011[a]	0.043[a]	0.008[a]
	Anti inflammatory activity of crude hydroalcohalic extracts					
Test I	100	0.206±	0.216±	0.286±	0.368±	0.276±
		0.019	0.019[a]	0.018[b]	0.022[b]	0.005[b]
Test II	300	0.198±	0.226±	0.244±	0.26±	0.276±
		0.015	0.016[a]	0.011[a]	0.011[b]	0.005[a]
Test III	500	0.198±	0.212±	0.226±	0.244±	0.262±
		0.013	0.011[a]	0.011[a]	0.005[b]	0.008[a]
	Anti inflammatory activity of alkaloidal Fractions					
Test I	100	0.194±	0.206±	0.258±	0.288±	0.298±
		0.011	0.011[a]	0.008[a]	0.008[c]	0.008[b]
Test II	300	0.192±	0.222±	0.248±	0.256±	0.266±
		0.013	0.013[a]	0.008[a]	0.0089[b]	0.008[a]
Test III	500	0.192±	0.202±	0.224±	0.238±	0.248±
		0.010	0.013[a]	0.013[a]	0.008[a]	0.004[a]
	Anti inflammatory activity of non- alkaloidal Fractions					
Test I	100	0.196±	0.208±	0.288±	0.318±	0.330±
		0.011	0.015[a]	0.015[b]	0.015[b]	0.016[c]
Test II	300	0.198±	0.208±	0.258±	0.278±	0.282±
		0.015	0.015[a]	0.014[a]	0.015[b]	0.011[b]
Test III	500	0.196±	0.214±	0.244±	0.254±	0.264±
		0.011	0.008[a]	0.009[a]	0.005[b]	0.005[a]

SEM- Standard Error Mean, n: five animal in each group; value are mean±SEM; [a]Significantly different from control (p<0.001); [b]Significantly different from control (p<0.01); [c]Significantly different from control (p<0.05). Change in mean paw volume (in ml) after administration of control, sample and standard drugs.

and VIII (test groups 2) alkaloidal extract in 0.5% sodium CMC (100 mg/kg, 300 mg/kg and 500 mg/kg) at 10 ml/kg. Groups-IX, X and XI (test groups 3) non-alkaloidal extract in 0.5% CMC (100 mg/kg, 300 mg/kg and 500 mg/kg) at 10 ml/kg.

2.5.3. Anti-inflammatory activity

For evaluation of anti-inflammatory effect, the paw volume was measured at 15, 30, 60 and 120 min after administration of the extracts or the standard drug. The percentage of inhibition of the edema induced by carrageenan was calculated with comparison to the control group (0.5% sodium CMC, 10 ml/kg) [11-13]. The anti- inflammatory activities were determined as the percentage of inhibition of the edema formed after 2 h of carrageenan administration. The percentage inhibition was calculated by using the fallowing formula:

$$\text{Inhibition \%} = (A-B/A)\times 100$$

A: Mean paw inflamation of control

B: Mean paw inflamation of test

2.5.4. Procedure

The animals were fasted for 12 hours prior to experimental work. A mark was made on the right hind paw just beyond tibiotarsal junction, so that every time the paw was dipped in the mercury (Hg) column up to the fixed mark to insure constant paw volume with the help of Plethysmograph. The initial volume was noted of each rat by mercury (Hg) displacement method. After 15, 30, 60 and 120 min of carrageenan administration, the paw volumes of all groups was measured by using Plethysmograph.

Table 2. Percentage of inhibition of inflammation of fractions of *Curculigo orchioides*.

Groups	Dose/ Body wt	Change in mean paw volume	% Inhibition
Group I (control)	10 ml/kg	0.374±0.086	0.00%
Group II (standard)	10 mg/kg	0.172±0.014	48.66%
Percentage inhibition of Inflammation of Crude ethanol extracts			
Group III (Test group I)	100 mg/kg	0.290±0.0194	22.45%
Group IV (Test group I)	300 mg/kg	0.241±0.0867	35.62%
Group V (Test group I)	500mg/kg	0.228±0.0253	39.03%
Percentage inhibition of Inflammation of alkaloidal fraction			
Group VI (Test group II)	100 mg/kg	0.255±0.0114	31.68%
Group VII (Test group II)	300 mg/kg	0.236±0.0089	36.89%
Group VIII (Test group II)	500 mg/kg	0.220±0.0130	41.17%
Percentage inhibition of inflammation of non-alkaloids extract			
Group IX (Test group III)	100 mg/kg	0.268±0.011	28.34%
Group X (Test group III)	300 mg/kg	0.245±0.039	34.49%
Group XI(Test group III)	500 mg/kg	0.034±0.028	37.43%

All groups contained 0.5% sodium CMC as suspending agent. Group-I (control group)- contained only 0.5% sodium CMC. Group-II (standard group) indomethacin (10 mg/kg). Group-III, IV and V (Test groups 1) hydroalcohalic extracts. Group-VI, VII and VIII (Test groups 2) alkaloidal extracts. Group- IX, X and XI (Test groups 3) non-alkaloidal extracts.

2.6. Statistical evaluation

All values are reported as mean±SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall p-Value was found statistically significant ($p<0.05$), further comparisons among groups were made according to Tukey's test.

3. Result and discussion

The yield of the *Curculigo orchioides* rhizome extract was 5.6% w/w. The acute toxicity of hydroalcoholic extracts was safe up to a dose of 2000 mg/kg body weight. The effects of *C. orchioides* rhizome extracts of HE, AF, and NA on paw edema induced by carrageenan are shown in Table 1. Treatment with HE, AL, and NAF of *C. orchioides* produced a diminished inflammation in rat hind paw when challenged with carrageenan induced inflammation. Percentage of inhibition of inflammation by HE, AF, and NAF are demonstrated in Table 2. Treatments with 500 mg/kg extract doses showed more effect on inflammation than other doses (300 mg/kg and 100 mg/kg) in all test groups. This indicates that HE exhibited a dose-dependent effect on inflammation i.e. 22.45%, 35.62% and 39.03% inhibition at doses level of 100 mg/kg, 300 mg/kg and 500 mg/kg, respectively. The AF fraction showed 31.68%, 36.89% and 41.17% inhibition at doses level of 100 mg/kg, 300 mg/kg and 500 mg/kg, respectively, and NAF showed 28.34%, 34.49% and 37.43% inhibition of inflammation at dose levels of 100 mg/kg, 300 mg/kg and 500 mg/kg, respectively.

Antiinflammatory activity of AF was near the activity of the standard drug indomethacin, and was more potent than other extracts HE and NAF. The extracts of *C. orchioides* showed significant anti-inflammatory activities as evidence by inhibition of paw edema when compare with control group. The results were significant at $p<0.05$ for carageenan induced right hind paw edema method. The present study shows the effectiveness of *C. orchioides* as an antiinflammatory drug, as suggested in the folk medicine.

The search for new anti-inflammatory agents from the vast array of medicinal plant sources are intensifying since they may hold promise for the discovery of therapeutic agents with beneficial effect not just in suppressing relevant aspects of the

inflammatory cascade but also on diverse disease conditions where the inflammatory response is amplifying the disease process. This present study was carried out to assess the validity of the folkloric uses of this plant in the treatment of inflammatory disorders.

According to literature, *C. orchioides* contains different phytoconstituents like flavonoids, alkaloids, steroids, polyphenols, carbohydrates etc. Anti-inflammatory effects of flavonoids, alkaloids, steroids and tannins have been reported and caused marked inhibition of carrageenan induced edema in experimental animals [14, 15]. Carrageenan induced inflammation mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings, the late phase is sustained by prostaglandins released and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [16]. The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [17], which are aimed at host defense and usually activated in most disease condition. Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in amplifying the disease process. Therefore, it is likely that *C. orchioides* might suppress the formation of these substances or antagonize the action of these substances and thus exerts its anti-inflammatory activity. The study also provides a strong evidence for the use of the rhizome of *C. orchioides* in folkloric treatment as anti-inflammatory agent. The activity may be due to the presence of one or more phytochemicals present in the extracts. In future, determination of the active phytochemicals those are responsible for the activities and also exact mechanism of actions.

4. Conclusion

In conclusion, the results of the study showed that the extract of *C. orchioides* rhizomes and its fractions shows anti-inflammatory activities, which explain on the basis of its use in traditional medicine to manage inflammation. The extract contains some biologically active constituents worthy of further investigations. Therefore, the hydroalcoholic extract of *C. orchioides* and its fractions has a potential application as an anti-inflammatory for the prevention and treatment of inflammation, and the present findings support its traditional local uses.

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