IN-VIVO ANTINOCICEPTIVE ACTIVITY OF DIFFERENT FRACTIONS OF *CURCULIGO ORCHIOIDES* GAERTN. RHIZOME

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Summary

Curculigo orchioides (Hypoxidaceae), crude hydroalcohalic rhizome extract (HAECO) and their alkaloidal and nonalkaloidal fractions (AHAFCO, NAHAFCO) were evaluated for antinociceptive activity by modified hot plate method at different dose level 100, 300 and 500mg/kg in albino wistar rats. Oral administration of 100, 300 and 500 mg/kg of rhizome extracts and fractions of *C. orchioides* were used for the above study and acetylsalicylic acid (100 mg/kg) was used as reference drug. Hydroalcohalic extract was fractioned into alkaloidal and non alkaloidal extracts. The result showed dose dependent action of all extracts and fractions, among these alkaloidal fractions were showed more potent antinociceptive effect than other extracts and fractions. The results of the various phytochemical tests indicated the plant to be rich in various biologically active compounds which could serve as potential source of crude drugs. Therefore, the hydroalcoholic extract and their fractions from *C. orchioides* have potential application as an antinociceptive for the prevention and treatment of pain, and the present findings support its traditional local uses.

Key words: *Curuligo orchioides*, hydroalcohalic extract, alkaloids, antinociceptive activity.

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Introduction

Curculigo orchioides Gaertn. (Hypoxidaceae) is popularly known as black musali in India. It is found in all parts of India from near sea level to 2300m altitude, especially in rock crevices and laterite soil. It is also distributed in Sri Lanka, Japan, China, Malaysia and Australia. The rhizome, as well as the tuberous roots of the plant has been extensively used in indigenous systems of medicine for treatment of various diseases, in general debility, deafness cough, asthma, piles, skin diseases, impotence, jaundice, urinary disorders, leucorrhoea, menorrhagia, cancer, jaundice, asthma and wound healing[1] and also Rootstock is the officinal part and it enters into different Ayurvedic formulations[2]. It is used extensively in ayurvedic formulations for treatment of wide variety of ailments, especially as a general tonic and as an aphrodisiac. It is also used as edible flour by many tribal people to increase the root energy. It is also used as a general tonic, diuretic, demulcent and as an aphrodisiac. In the folklores it is used in lumbago, kidneys tonic, chronic nephritis, impotency, bed wetting, hypertension, arthritis. It improves complexion and is useful in general debility, deafness, cough, asthma, piles, diarrhoea, gonorrhoea, skin diseases and jaundice[3-5].
The active compounds that have been reported are flavones, glycosides, steroids, saponins, triterpenoids and other secondary metabolites. This plant have valuable source of natural products for maintaining human health[6-16]. Therefore, such plants should be investigated to better understand their properties, safety profiles and levels of efficiency against radiant heat induced pain in modified hot plate method on wistar rats. To the best of our knowledge, there are no reports of different fractions (alkaloidal and non alkaloidal) on analgesic activity of *C. orchioides.* Hence, we report here the effect of various fractions of extracts of *C. orchioides* on modified hot plate model in wistar rats.

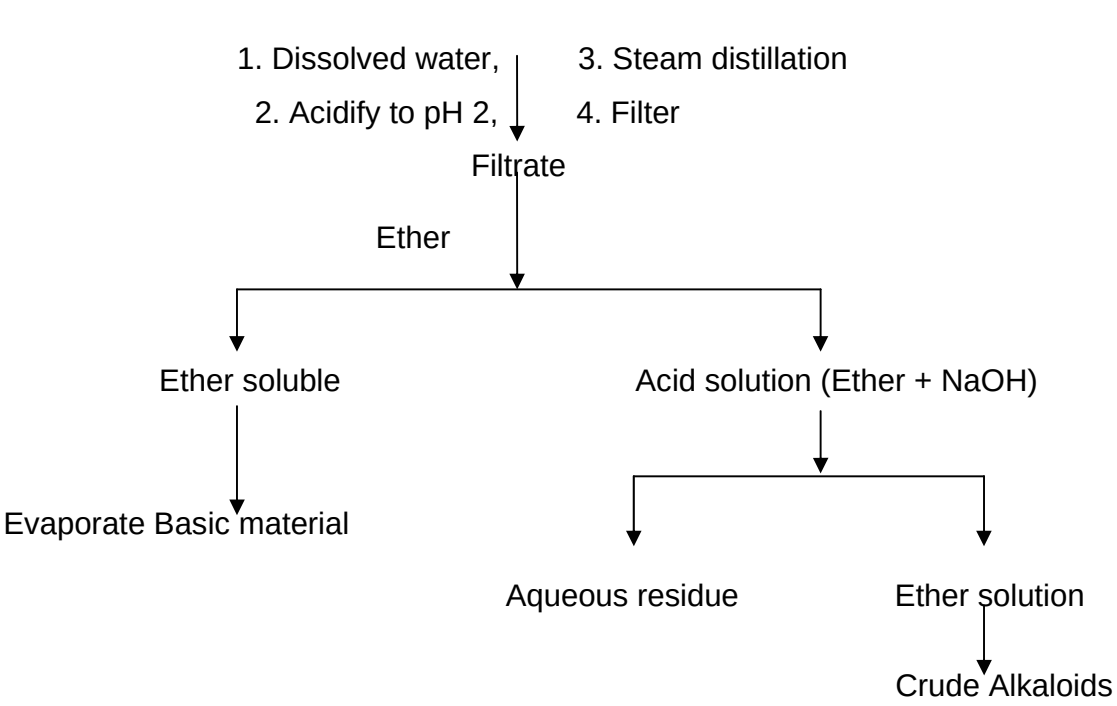
Materials and Methods

Sample Collection: The *Curculigo orchioides* rhizomes were collected from Dehradun, (U.K.) India. The authentication was done by Dr. Prashant Chaddha, Scientist, Botanical survey of India (BSI) Forest Research Institute, Dehradun, Uttrakhand, India, on 07-09-2009 with letter Ref no. : BDS-112739.

Preparation of Extracts: The rhizome of the *Curculigo orchioides* was shade dried. The shade dried rhizomes were grind in to coarse powdered and weight before extraction. Before extraction, powered rhizomes were defatted by petroleum ether. Defatted rhizome powders were extracted separately in the soxhlet extraction apparatus using ethanol (70%). The resultant hydroalcoholic extract was concentrated using rotary vacuum evaporator. The extracts were then dried and stored in vacuum desiccators (yield of the rhizome extract was 5.6% w/w).

Extraction of Alkaloids: The solvent is distilled off from crude hydroalcohalic extract of rhizome of plant *curculigo orchioides* and treated with inorganic acid. When the basic (alkaloids) are extracted as their soluble salts. The aqueous layer containing the salt of alkaloids and soluble plant impurities is made basic with NaOH. The insoluble alkaloids are set free precipitate out. The solid (ppt.) so obtained is then extracted with ether when alkaloid pass into solution and impurity left behind[17].

Separation of Alkaloids from ethanol extract of *curculigo orchioides*



Test Samples and Standards: Suspension of the hydroalcohalic extract of the rhizome (HAECO) and fractions (AHAFCO, NAHAFCO) of *Curculigo orchioides* were prepared in sodium carboxy methyl cellulose (CMC, 0.5%) using distilled water. Acetylsalicylic acid (100 mg/kg) was used as standards. Gastric administration of all drugs was accomplished via oral gavage.

Biological Evaluation

Experimental animals: Albino rats of Wister strain of both sexes weighing (150–200 g) were maintained under controlled conditions of light (12 hr) and temperature 25±2^{0}C in the animal house of GRD (PG) IMT, Dehradun, 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 after obtaining the approval from the institutional animal ethical committee.

Experimental Design: 55 Wistar rats of either sex were taken and divided into 11 groups, each group contained 5 rats. All groups received drugs by p.o route. 0.5% Carboxyl methyl cellulose (CMC) used as suspending agent and received 10 ml/kg volume except normal group. Group I (control group) – Received 0.5% sodium C.M.C in distilled water (5 ml/kg) body weight (5 ml/kg) body weight. Group II (standard group) **–** Received Aspirin (100 mg/kg).

For hydroalcohalic extract: Group III (Test group 1) – Received hydroalcohalic extract (HAECO) suspension in 0.5 % sodium C.M.C. (100 mg/kg). Group IV (Test group 2) – Received ethanolic (HAECO) extract suspension in 0.5 % sodium C.M.C. (300 mg/kg). Group V (Test group 3) –Received ethanolic extract suspension in 0.5 % sodium C.M.C. (500 mg/kg).

For alkloidal fractions: Group VI (Test group 4) – Received alkaloidal (AHAFCO) extract suspension in 0.5 % sodium C.M.C. (100 mg/kg). Group VII (Test group 5) – Received alkaloidal extract suspension in 0.5 % sodium C.M.C. (300 mg/kg). Group VIII (Test group 6) –Received alkaloidal extract suspension in 0.5 % sodium C.M.C. (500 mg/kg).

For non alkloidal fractions: Group IX (Test group 7) – Received non-alkaloidal (NAHAFCO) extract suspension in 0.5 % sodium C.M.C. (100 mg/kg). Group X (Test group 8) – Received non alkaloidal extract suspension in 0.5 % sodium C.M.C. (300 mg/kg). Group XI (Test group 9) –Received non alkaloidal extract suspension in 0.5 % sodium C.M.C. (500 mg/kg).

Antinociceptive Activity: The antinociceptive potential of the ethanolic extract and different fractions (HAECO, AHAFCO and NAHAFCO) of *curculigo orchioides* rhizomes was done by Eddy's modified hot plate method. The hydroalcoholic extract, and alkaloidal, nonalkaloidal fractions was given in three different doses viz., 100 mg/kg, hot plate 300 mg/kg & 500 mg/kg to different animal groups. The results for test groups were compared with that of standard group acetylsalicylic acid (Aspirin 100 mg/kg, oral). The test is very useful for discriminating between centrally acting morphine-like analgesics and non-opiate analgesics.

Procedure for Analgesic Activity: Groups of 5 rats (Wistar strain) of both sexes with a weight between (150-200g) were used for each dose. The animal 24 h, fasted before experiment work. Before administration of the test drug or the standard the normal reaction time is determined. The escape reaction which is the endpoint of this test can be regarded as a complex phenomenon mediated by the brain. Therefore the observation of the escape reaction can be regarded as a true assessment of the influence of the drug on the brain. The test compounds and the standard are administered orally. The animals are submitted to the same testing procedure after 15, 30, 60 and eventually 120 min. For each individual animal the reaction time is noted.

The modified hot-plate test method was employed in this study[18]. A 600 ml test beaker was placed on thermostat hot plate. The temperature was regulated to 50±1°C. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced nociceptive pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken (reaction time). Each mouse serves as its own control. Before treatment, its reaction time was taken thrice at 1 h interval. The results for test groups were compared with that of standard group (Aspirin 100mg/kg, p.o).

Statistical analysis: The data's were expressed as mean ± SEM, statistical analysis was performed by one way ANOVA followed by Tukey-Kramer multiple comparison test, p values <0.05 were considered as significant.

Results

Hydroalcohalic extract and fractions (HAECO, AHAFCO and NAHAFCO) of *C. orchioides* significantly increased the reaction time in modified hot-plate test and showed significant activities when compare with control groups ($p<0.05$). 500 mg/kg doses of all test samples showed maximum antinociceptive effect than other doses 300 and 100 mg/kg. Table 1 shows the results of the hot plate test. Three doses of hydroalcoholic (HAECO) extracts, alkaloidal and nonalkaloidal fractions (AHAFCO, NAHAFCO) of *C. orchiedes* increased the reaction time in a dose-dependent manner to the thermal stimulus. The highest nociception inhibition of thermal stimulus was exhibited at a higher dose of the extracts 500 mg/kg of alkaloidal rhizome fraction (AHAFCO), which is comparable to the acetylsalicylic acid. The result of acute toxicity test of the extract also suggests a remote risk of acute intoxication.

Table 1 Antinociceptive activity of different fraction of *curculigo orchioides*

Groups	**Dose**	**Response time in seconds (Mean* ± S.D.)**				
		initial	after 15 min	after 30 min	after 60 min	after 120 min
	mg/kg					
Control	---	16.35±0.866	16.47±0.86	16.96±0.934	17.07± 0.958	17.28±1.009
Standard	100	15.068±2.713	26.98±3.663 [a]	28.916±4.40 [a]	30.856±4.430[a]	27.98±3.663 [a]
Antinociceptive activity of crude ethanolic extract						
Test I	100	16.61±1.70	22.45±5.51 [c]	22.83 ± 7.50 [ns]	24.53 ± 4.94 [b]	17.80±5.69 [ns]

Test II	300	15.18 ± 1.03	24.32±2.609 [b]	26.52 ±2.06 [b]	27.44 ±2.249 [a]	22.68±2.57 [ns]
Test III	500	16.40 ± 0.58	26.52 ± 0.90 [a]	28.68 ± 1.26 [a]	30.72 ± 1.26 [a]	25.70±0.919 [b]
Antinociceptive activity of Alkaloidal extract						
Test IV	100	17.80 ± 0.61	26.02 ± 0.69 [a]	27.22 ±0.563 [a]	28.30 ± 0.49 [a]	21.22±0.516 [b]
Test V	300	15.64 ± 0.97	25.86±0.676 [a]	27.84 ±0.478 [a]	29.54 ± 0.63 [a]	24.90±0.644 [a]
Test VI	500	16.34 ± 0.53	26.66 ± 1.19 [a]	28.62 ± 0.91 [a]	30.90 ± 0.88 [a]	25.72±0.238 [a]
Antinociceptive activity of Non-Alkaloidal extract						
Test VII	100	18.06± 0.207	25.42 ± 0.47 [a]	27.1±0.54 [a]	29.06± 0.207 [a]	21.20±0.474 [b]
Test VIII	300	16.82 ±0.376	27.58± 0.31 [a]	29.9±0.18 [a]	31.24± 0.364 [a]	26.16±0.665 [a]
Test IX	500	15.84 ±0.328	26.0± 0.158 [a]	27.5±0.33 [a]	29.56± 0.482 [a]	25.08±0.589 [a]

SEM- Standard Error Mean, n: five animal in each group; value are mean ± SEM; *** pa< 0.001, pb <0.001, * pc<0.05, when compared to control.

Discussion

The extracts and fractions (HAECO, AHAFCO and NAHAFCO) prolonged the reaction latency to pain thermally-induced in rats by the hot plate. These suggest that the extract may possess antinociceptive activity probably mediated through a common mechanism. Analgesic activity is commonly possessed by the nonsteroidal anti-inflammatory drugs (NSAIDs) due to their effect principally by inhibiting the synthesis of prostaglandin[19]. These prostaglandins cause or responsible for stimulation of pain recptors and sensitize the skin to painful stimuli probably because they sensitize pain receptors to mechanical and chemical stimulation such as the pain producing effect of mediators (e.g. histamine, kinins) which are released in tissue injury and inflammation. Inhibition of prostaglandin synthesis may account for the antinociceptive activity of the extract and fractions. Pain and inflammation is associated with many pathophysiologies of various clinical conditions like arthritis, cancer, tissue injury and vascular diseases[20-22]. A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain and inflammation. The extract demonstrated significant anti-nociceptive activity at three different dose levels in modified hot plate animal models of pain.

Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception and the analgesic effect of the NSAIDs has also been attributed to effects at peripheral or central neurons[23,24].

These findings justify traditional use of this plant in the treatment of pain conditions and validate its claim of being used for the said purpose in folklore medicine. It can be concluded that hydroalcoholic extracts and fractions possesses analgesic property, which are probably mediated via inhibition of prostaglandin synthesis which may be of potential benefit for the management of pain disorders.

Aspirin offer relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process. Therefore, it is likely that *Curculigo orchioides* might suppress the formation of these substances or antagonize the action of these substances and thus exerts its antinociceptive activity[25].

Conclusion

From this study it can be concluded that as hydroalcohalic extract, alkaloidal and non alkaloidal fractions (HAECO, AHAFCO and NAHAFCO) of *curculigo orchioides* marked antinociceptive activities with reference antinociceptive drug asprin. The presence study establishes the effectiveness and pharmacological for use of *curculigo orchioides* as an antinociceptive drug. The drug may be further explored for with phytochemical profile to identify the active constituent responsible for antinociceptive activity. Also the medical approach of the drug for use the treatment of pain in traditional system of medicine substantiated.

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