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Research Article

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Anxiolytic effect of *Curculigo orchioides* on the elevated plus maze and light dark model

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ABSTRACT

In ancient to modern system of medicine Curculigo species has been recommended for relief of different central nervous system disorders. Under this reserach study the hexane, dichloromethane, ethyl acetate, methanolic and water extracts of Curculigo orchioides root were evaluated for anxiolytic activity using Elevated Plus Maze model (EPM) and Light Dark model (LDM) in mice. The methanolic and aqueous extract exhibited potential anxiolytic effects at 400 mg/kg., p.o. in both methods whereas hexane, dichloromethane and ethyl acetate extract were less effective. Preliminary phytochemical studies of the extracts revealed the presence of flavanoids, tannins and phenolic compounds that may contribute to the anxiolytic activity of Curculigo orchioides.

Keywords: *Curculigo orchioides*, Elevated Plus Maze, Light Dark model, Amaryllidaceae, Kali musli

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INTRODUCTION

In present health scenario, anxiety is the most frequent psychiatric condition commonly found. A number of the population suffers from anxiety at various stages during their life [1]. In recent years there has been high concern over alternative medicines and plant derived medications that affect the “mind.” During last few years, there has also been an increase in usage of alternative medicines by the patients for such ailments [2]. Physicians in Europe and Asia are using these medicines to explore the traditional remedies and to find out a suitable cure for these ‘mind affecting diseases’. *Curculigo orchioides* is one such plant that has been extensively used in Ayurvedic formulations for the treatment of mental illness [3].

Curculigo orchioides Gaertn. (Kali musali) is one of the highly useful plants in the indigenous systems of medicine, belongs to Amaryllidaceae family. *Curculigo orchioides* Gaertn is a small herb, up to 30 cm high with tuberous root stock, occurring wild in sub-tropical Himalayas from Kumaon eastwards. Rhizome of *Curculigo orchioides* is used as antioxidant [4], spermatogenic [5], hepatoprotective [6], immunostimulant [7], anticancer [8], antibacterial [9], antiosteopoprotic [10] and hypoglycaemic [11] etc. *Curculigo orchioides* contain three steroids (i)sitosterol, (ii)stigmasterol [12] and (iii)yuccagenin [13]. Lycorine is the only alkaloid isolated and known so far in *Curculigo orchioides* Gaertn [13]. Five phenolic compounds have been isolated and characterized from *Curculigo orchioides* Gaertn. These are (i) curculigoside (5- hydroxy-2-O-D-glucopyranosyl benzyl-2, 6-dimethoxy benzoate) [14], (ii) curculigine A, (iii)orcinol glucoside [15], (iv) corchioside A and (v) flavanone glycoside–I (glycoside–5, 7-

dimethoxy-dihydromyricetin-3-O-L-xylopyranosyl (4-1)-D-glycopyranoside)[16]. In context with uses of *Curculigo orchioides* for the treatment of CNS and immune system disorders, it is hypothesized that this plant may possess anxiolytic activity. Objective of the present study is to investigate possible anxiolytic activity of *Curculigo orchioides*.

EXPERIMENTAL SECTION

Plant material:
Curculigo orchioides Gaertn was procured from the forest area of Jabalpur and Chhindwada (M.P., India) in March 2010. The sample was authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. The voucher specimen reference number for the same is (NISCAIR/RHMD/consult/2009-10/1396/198). After authentication, plant material was collected in bulk, washed under running tap water to remove adhering material, air dried under shade & sunlight and pulverized in a mechanical grinder. The coarse powder was passed through sieve no. 40 and taken for further studies.

Preparation of extract:
The powder of dried rhizome of *Curculigo orchioides* was subjected to continuous soxhlet extraction with different organic solvents as Hexane, Di chloro methane, Ethyl acetate, Methanol and Aqueous. Exhausted extraction with each of the solvent was ensured. The five extracts were dried using rotary vacuum evaporator and the dried extracts were preserved in vacuum desiccator, containing anhydrous $CaCl_2$. All five extracts were examined for presence of different chemical constituents. The yield of the Hexane (COH), Di chloro methane (CODCM), Ethyl acetate (COEA), Methanol (COM) and Aqueous extract (COA) were 8.32, 9.36, 7.08, 12 and 10.36% respectively.

Animals:
Swiss albino mice of either sex (20 – 25 gm) were procured from Radharaman College of Pharmacy, Bhopal, India and used for the experiment. The animals were allowed to standard diet and water *ad libitum*. The experiment was approved from Institutional Animal Ethical Committee (IAEC) and experiments were carried out in accordance with the ethical committee guidelines laid down by the local committee regarding the care and use of animals for experimental procedures (Reg. No. IAEC/RCP/March – 2012/06).

Preparation of dose:
Animals were divided into different groups of six mice each. Diazepam (Calmpose 2mg/10ml injection, Ranbaxy Laboratories, India) was suspended in normal saline. Different extracts (COH, CODCM, COEA, COM and COA) were separately suspended in a vehicle comprising 1%v/v Tween 80 in normal saline. Normal saline was used as control.

Elevated plus maze model (EPM):
The anxiolytic activity was measured using elevated plus maze test [17]. The plus maze apparatus consisting of two open arms (16X5cm) and two closed arms (16X5X12) having an open roof, with the plus maze elevated (25 cm) from the floor was used to observe anxiolytic behavior in the animals [18]. The animals were fasted 18 hrs prior to the experiment. Extracts of *Curculigo orchioides* were administered orally using tuberculin syringe fitted with oral canula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus maze apparatus 45 minutes after the administration of the dose. Each mouse was placed at the center of the elevated plus maze with its head facing the open arm during this 5 minute experiment. The behavior of the mouse was recored as (a) preference of the mouse for its first entry into the open arm or closed arm (b) the number of entries into the open or closed arm (c) average time spent by the mouse in the open arm. (Average time=total duration in open arm/number of entries). During the entries experiment, the animals were allowed to socialize. All precaution was taken into consideration to insure that no external stimuli could invoke anxiety in the animal. Similar observations were recorded for the standard group (diazepam 2mg/kg) as well as control group (normal saline).

Light dark model (LDM):
The light-dark transition test was introduced in 1985 and frequently has been used to identify novel anxiolytic compounds [19]. The model uses the fear of a novel, brightly-lighted area and the avoidance behavior to enter the lighted area by hiding in a dark compartment. Anxiolytic agents increase the percent time spent in the lighted area without affecting the number of transitions between the two compartments, which would be an indicator for a stimulant [20]. Animals were handled and dose schedule was in the same manner as for the EPM. Typical

dimensions of the compartment are generally one third for the dark compartment and two thirds for the light compartment with an exterior size of (46X27X30cm) (lXbXh).The box was equally lighted with 40 watt bulb. The opening between the two compartments is not more than 7 cm. Mice were individually placed in the center of the lighted area facing the entrance to the dark compartment. The behavior of the mouse was recored as (a) First preference of mouse to lighted or dark area, (b) Total time spent in each compartment, (c) Total number of transitions between the compartments. Similar observations were recorded for the standard group (diazepam 2mg/kg) as well as control group (normal saline).

Statistical analysis

Results are expressed as mean ± S.E.M. and statistical difference were analyzed using student's t- test and results were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Anxiety are defined as the response of a subject to real or particular threats that may impair its homeostasis, this response may include physiological and/or behavioral changes. Measuring anxiety like behavior in mice has been mostly undertaken using a few classical animal models of anxiety such as the Elevated plus maze (EPM) and Light dark model (LDM). All these procedures are based upon the exposure of subject to unfamiliar aversive place [21]. Increase in time spent and number of entries of animal in the open arm and light area indicates anxiolytic activity of drugs [22].

In present study, significant increase in time spent on open arm and light area proves anxiolytic activity of *Curculigo orchioides*. The study results showed that COH, CODCM and COEA were found to be less potent anxiolytic than COM and COA extract of *Curculigo orchioides* in EPM as well as in LDM. In other words we may conclude that Methanol extract (COM) and Aqueous extract (COA) showed maximum anxiolytic activity at doses 400mg/kg., p.o. in EPM as well as in LDM. The mean time spent by the mice in the open arms and lighted area after oral administration of various extracts of *Curculigo orchioides* are shown in Table-1 and Table-2. With the above results, we found that all extracts showed significant activity at a dose of 400mg/kg. So for comparative study of all extract in both models, 400mg/kg dose was choosen. The comparative anxiolytic profiles of different extracts of *Curculigo orchioides* at 400mg/kg dose are depicted in Figure-1 and Figure-2. Preliminary phytochemical analysis of *Curculigo orchioides* showed presence of flavonoids, phenols and tannins and which might be contributing in part to the observed pharmacological effects. These phytochemicals have been reported to show potent activity against various CNS disorders. Tannins have also been shown to possess activity against many CNS disorders [23] including Alzheimer's disease [24] and epilepsy [25].

Table 1: Effect of different extracts of *Curculigo orchioides* on anxiolytic response in the Elevated plus-maze model test in mice

Treatment	Dose mg/kg	Average time spent in open arm (sec±S.E.M.)
Control	Vehicle	5.49± 0.330
Diazepam	2.0	19.35± 0.535
COH	100	6.79±0.471
	200	10.92±0.547
	400	9.33±0.347
CODCM	100	8.92±1.033
	200	12.34±0.698
	400	18.04±0.552
COEA	100	7.63±0.744
	200	8.49±0.272
	400	10.14±0.653
COM	100	18.41±0.396
	200	22.18±0.868
	400	28.36±0.883
COA	100	16.71±0.578
	200	19.15±0.384
	400	26.05±2.156

Values are expressed as mean ± S.E.M. (standard error mean); n= 6, p.o.=per oral.
COH (Hexane extract), COEA (Ethyl acetate extract), CODCM (Di chloro methane extract), COM (Methanol extract) and COA (Aqueous extract).

Table 2: Effect of different extracts of *Curculigo orchioides* on anxiolytic response in the Light dark model test in mice.

Treatment	Dose mg/kg	Average time spent in open area (sec±S.E.M.)
Control	Vehicle	5.272 ± 0.306
Diazepam	2.0	19.42±0.272
COH	100	8.07±0.539
	200	8.67±0.408
	400	9.3±0.446
CODCM	100	5.72±0.147
	200	6.95±0.245
	400	9.24±0.482
COEA	100	12.85±0.570
	200	10.48±0.735
	400	14.32±1.058
COM	100	15.46±0.672
	200	20.36±0.385
	400	31.13±0.597
COA	100	16.06±0.310
	200	11.00±0.500
	400	29.54±0.775

Values are expressed as mean ±S.E.M.(standard erro r mean); n= 6, p.o.=per oral.
COH (Hexane extract), COEA (Ethyl acetate extract), CODCM (Di chloro methane extract), COM (Methanol extract) and COA (Aqueous extract).

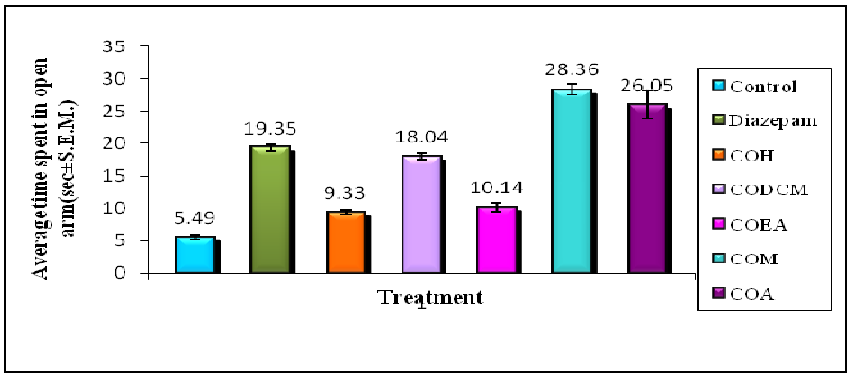

Figure: 1- Comparative anxiolytic profile of different extracts of *Curculigo orchioides* at 400mg/kg dose in EPM.

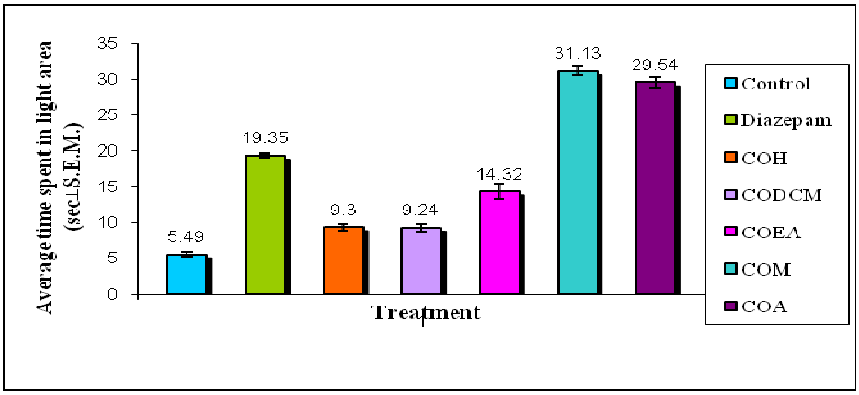

Figure: 2- Comparative anxiolytic profile of different extracts of *Curculigo orchioides* at 400mg/kg dose in LDM.

CONCLUSION

It is concluded from the present study that Methanol extract (COM) and Aqueous extract (COA) showed maximum anxiolytic activity at doses 400mg/kg., p.o. in Elevated plus maze as well as in Light dark model, which can be attributed to presence of flavonoid, tannins and phenols. Further pharmacological investigations are required to identify the active constituents of the plant extract responsible for the anxiolytic effects.

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