INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

EVALUATION OF ANXIOLYTIC ACTIVITY OF *CARUM COPTICUM* BY USING ELEVATED PLUS MAZE AND OPEN FIELD METHOD

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Article Received on:27/02/2011 Revised on:29/03/2011 Approved for publication:10/04/2011

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ABSTRACT
The clinical applications of benzodiazepines as anxiolytics are limited by their unwanted side effects. Therefore, the development of new pharmacological agents is well justified. Among medicinal plants, *Carum copticum* has been recommended for relief of anxiety and insomnia in Iranian folk medicine. Nevertheless, no pharmacological studies have thus far evaluated its effects on central nervous system. Therefore, the aim of this study was to examine if the aqueous extract of *Carum copticum* seeds has anxiolytics effect in mice. The anxiolytic effect of aqueous extract (200, 400mg/kg) was examined in male albino mice using elevated plus-maze and open field as an animal model of anxiety. In the elevated plus-maze, aqueous extract at 200 mg/kg showed an anxiolytic effect by increasing the time spent on open arms and the percentage of open arm entries, compared to control group. These results suggest that the aqueous extract of *Carum copticum* seeds has anxiolytic effect.
KEYWORDS: Anxiety, insomnia, elevated plus-maze, open field method

INTRODUCTION

Human anxiety is defined as feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat[1]. Anxiety effect one-eighth population worldwide and has become important research area in field of psychotherapy[2]. Benzodiazepines, barbiturates, tricyclic antidepressant, have been used for the long time to treat anxiety disorders. The serious side effects associated with these drugs namely rebound insomnia, sedation muscle relaxation, withdrawal and tolerance, sexual dysfunction[3]. Due to this many pharmaceutical companies are conducting studies to find an alternative medicine or plant derivative medications with more specific anxiolytic effect[4]. Several indigenous drugs are being evaluated because of their easy availabilities, lack of side effects and cost-effectiveness. Traditional medicines used by about 60 % world population in rural areas in the developing countries and in the developing countries where use modern medicines predominant.

The plant *Carum copticum* of the family Umbelliferae is the well known plant in the Indian medicines system. And has historically been used stimulant, carminative, the oil is also preferred as deodorant in mouth washes, tooth pastes, and gargles and as flavoring agent for disinfectant soaps[5].

The survey of literature on *Carum copticum* has revealed that only a few pharmacological reports were found for its CNS activity; therefore we undertook the present study to determine anxiolytic activity of seeds of *Carum copticum* by using different animal model for anxiety[6].

MATERIAL AND METHODS

Collection and Authentication of Plant Material

The plant material was collected from local distributer of Karad and plant material were identified and authenticated by the Department of Botany at Deccan Education Society's Willingdon College, Sangli.

Preparation of Aqueous Extract of *Carum copticum*

Air dried seeds was homogenized to a fine powder. Hundred grams of powdered *Carum copticum* was infused in 500 ml cold distilled water for 24 h, brought to the boil, then removed from the heat source and allowed to infuse for 15 min. The extract was filtered, then concentrated over the water bath and brought to dryness under vacuum[7].

Animals

Male albino mice weighting 25–35 g were purchased from the Animals House, Krishana Institute of Medical Sciences Karad. Mice were housed in cages of 5 at 22 ± 1 ◦C in a 12-h light/dark cycle. Tap water and food pellets were available as labium. Groups of 5 mice were randomly assigned to different treatment groups and tested in a counterbalancing order[7, 8]. Animals were

naive to experiment conditions. All experiments were carried out in a quiet room under dim red light between 9:00 a.m. and 2:00 p.m.

Drugs

Diazepam hydrochloride (4 mg/kg) and Ondansetron (0.5mg/kg) were used as a reference drugs[7]. All solutions were prepared freshly on test days and administered oral in a volume of 0.1ml/25g body weight of mice[8]

Acute Toxicity Study

The procedure was as per OECD 425 guidelines. The extracts was administered orally at a dose 2000mg/kg body weight to the different groups of mice and observe for the signs of behavioral, neurological toxicity and mortality for 14 days[9].

ELEVATED PLUS-MAZE MODEL OF ANXIETY

Anxiolytic activity was measured using the elevated plus maze test. The maze consisted of two open (30 cm × 5 cm × 0.2 cm) and two closed (30 cm × 5 cm × 15 cm) arms, extending from a central platform (5 cm × 5 cm) and elevated to a height of 45 cm above the floor. The entire maze was made of clear Plexiglas. Mice were individually placed on the center of the maze facing an open arm, and the number of entries and the time spent in closed and open arms were recorded during a 5-min observation period. Arm entries were defined as entry of all four paws into an arm. The percentage of open arm entries (100 × open/total entries) was calculated for each animal[7, 10].

OPEN FIELD APPARATUS

An 'open field apparatus'; suitable for mice were made comprising of a floor space of 40 cm. x 40 cm with 30 cm. high walls. The floor was coloured black and the floor area was divided into 9 equal squares by white lines. A mouse was placed at the center 9 of the field and was left for 2 minutes for acclimatization with the apparatus. Thereafter, for the next 5 min., the following parameters were noted[11]:

a) Time spent in the central square

b) Ambulation (No. of squares crossed)

c) Rearing (No. of times the animal stands on the rear paws)

Statistics

The results were expressed as mean ± S.E.M. of two independent experiments**.** For quantitative data statistical analysis was initially performed by using a one way analysis of variance (ANOVA) by dunnet test. $P<0.001$ was considered to be significant.

RESULT

Dunnet – t- test revealed that administration of diazepam (4mg/kg) significantly increased the amount of time spent in the open arms and percentage of open arm entries compared to vehicle treated group, (Table no. 3)

Our results show that aqueous extract of seeds of *Carum copticum* at 200 mg/ kg and 400 mg/kg significantly increased both the time spent in open arm and percentage of open arm entries compared to control group of animal.(Table no.2)

In the open field test aqueous extract of *Carum copticum* alter the number of crossing, it significantly increased the number of crossings and decreases grooming and rearing (Table no.1) at the doses 200mg / kg and 400 mg/ kg resp. as compared to controls.

Time spent in Central Square in the control and standard (Diazepam) group were 1.7 ± 1.42 and 7.5±4.81 sec respectively. And at dose 200mg / kg and 400 mg/ kg of *Carum copticum* showed a significant increase in time spend similar to diazepam group as compared to control group.

For the both parameters, the maximum effect seems to be reached with the smaller dose. *Carum copticum* alters number of crossing, significantly decreases grooming and rearing as compared to control.

DISCUSSION

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms[12].

In the present we examined the effect of aqueous extract of *Carum copticum* on anxiety by using elevated plus maze and open field method. The result revealed that the aqueous extract of *CarumCopticum* should statistically significant antianxiety activity at dose level 200mg/kg and 400mg/kg. *Carum copticum* showed anxiolytic activity in dose-related manner. *Carum copticum* increase the time spent in open arm and decrease the time spent in close arm indicating anxiolytic activity. Several studies have shown that 5-HT_3 antagonist ondensetron has benzodiazepines like anxiolytic effect[13]. The role of serotonine in anxiety is now well established and has been conclusively shown that increase in central serotonargic activity leads to anxiety, whereas decrease in brain 5-HT activity results in anxiolysis[14]. The alteration in the time spent in open arm is considered more sensitive to the drug effect than the number of entries[15]. Our studies showed significant increases in the number of entries in both open and closed arms. Further, decrease in the ratio of entries also indicates anxiogenic activity of *Carum copticum*. Both diazepam and

ondensetron increased number of entries in open and closed arms, total number of entries and decreses the ratio of entries in open arm to total number of entries. This observation confirms the anxiolytic effect of *Carum copticum*.

In the open field test, when animals are taken from their home cage and placed in a novel environment, they express their anxiety and fear by decrease in ambulation and exploration, rearing and grooming behaviors, and increase in defecation due to heightened autonomic activity. These behavioral changes are attenuated by classical anxiolytics and augmented by anxiogenic agents[16]. There was a significant increase in the number of square crossed in diazepam group compared to control. Though there was slight increase in the number of squares crossed by mice in *Carum copticum* treated groups (200, 400 mg/kg) as compared to control.

Defecation is also a good indicator of emotionality in animals, and research shows that high emotionality is related to an increase in defecation, with various doses of *Carum copticum* showed reducing defecation[17].

Grooming behavior generally increases with fear or anxiety in rodents and is an index of behavioral adaptation to a stressful situation[18]. Anxiolytic drugs decrease grooming in an open-field test[19] and in the present experiments groups receiving *Carum copticum* did reduce grooming.

CONCLUSION

- Using elevated pulse maze test and open field method the aqueous extract of *Carum copticum* displayed a pronounce anxiolytic activity.
- The maximum effect of aqueous extract of *Carum copticum* seems to be reaches at a dosage of 400 milligram per kg using higher dose levels explorations of open arms was not further increased.
- Aqueous extract of *Carum cpticum* showed a profile similar to that observed with diazepam and ondansterons as anxiolytic effect.
- Anxiolytic effect of the extract reached statistical significance at the 200 mg/ kg and 400 mg/ kg these findings raised the possibility that anxiolytic effect of extract may be exerted by different phytoconstituents possibly acting through different receptors[20].
- Therefore its usefulness in clinical practice may be similar to that of diazepam. Further studies are needed in identify the anxiolytic mechanism and phytoconstituents responsible for the observed central effect of the aqueous extract of Carum copticum.

ACKNOWLEDGEMENTS

We would like to thank the Ashokrao Mane college of Pharmacy, Peth-Vadgaon for support for this study.

REFERENCES

1. Kulkarni SK. Reddy DS, Animal behavioral model for testing of anti-anxiety agents, Meth Find Exp Clin Pharmacol 1996; 18 (3):219-230.
2. Yadav AV, Kavale LA, Nade VS, effect of Morus alba L.(mulberry) leaves on anxiety in mice, Indian J of pharmacol 2008;40:32-36.
3. Kulkarni SK, Singh K, Bishnoi M, Comparative behavioral profile of newer anti-anxiety drugs on different mazes Indian J of exp.biol 2008; 46:633-638.
4. Rabbani M, Sajjadi SE, Mohmaddi A, Evaluation of anxiolytic effect of Nepeta percica boiss. In mice e CAM 2008; 5(2): 181-186.
5. Dahanuka SA, Kulkarni AR, Rege NN, Pharmacology of medicinal plants and natural products, Indian J Pharmacology 2000; 232:81-118.
6. Kirtikar KR, Basu BD, Indian medicinal plants, Vol-II, 5th ed. Delhi: periodical expert's book agency 1991; 1225-1226.
7. Emamghoreishi M, Khasaki M, Aazam MF, Coriandrum sativum: Evaluation of its anxiolytic effect in the elevated plus-maze, J of Ethnopharmacology 2005;96:365–370
8. The Ayurvedic pharmacopoeia of India, 1(1), 40-44.
9. OECD guideline for testing of chemicals; Acute Oral Toxicity-Up-and-Down Procedure:425 adapted 17th dec 2001;1-26
10. Vogel H G, Drug Discovery and Evaluation; 2nd ed: 542-575.
11. Rauniar GP1, Deo S2, Bhattacharya SK3, Evaluation of anxiolytic activity of tensarin in mice, Kathmandu University Medical Journal 2007; Vol. 5, No. 2, Issue 18: 188-194.
12. Kumar S. and Sharma A., Anti-anxiety activity studies of various extracts of Turnera aphrodisiaca Ward. J. Herb. Pharmacother, 2005; 5: 13-21.
13. Pillow S. File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus maze: a novel test of anxiety in rats. Pharmacol Biochem Behav 1986; 24:525-9.
14. Clement Y and Chapouthier G, Biological bases of anxiety. Neuroscience and Biobehavioral Reviews, 1998; 22 (5): 623-633.
15. Graeff FG, Guimares FS, de Andrade TG and Deakin JF, Role of 5-HT in stress, anxiety and depression. Pharmacol. Biochem. Behav1996; 54: 129–141, (1996).
16. .Bhattachary S.K. and Satyan, K.S. Experimental methods for evaluation of psychotropic agents in rodents: In Anti-anxiety agents Indian J. Exp. Biol 1997; 35: 565-575.
17. Angrini M, Leslie JC, Shephard RA, Effects of propranolol, buspirone, pCPA, reserpine, and chlordiazepoxide on open-field behaviour. Pharmacol Biochem Behav 1998; 59:387-397.
18. Kametani H, Analysis of age-related-changes in stress-induced grooming in the rat--differential behavioral profile of adaptation to stress. Ann. NY Acad. Sci. 1988; 525:101-113.
19. Moody TW, Merali Z, Crawley JN, The effects of anxiolytics and other agents on rat grooming behavior. Ann. NY Acad. Sci. 1993; 90:281-290.
20. Manley CH, Psychophysiological effect of odor. Crit. Rev. Food Sci. Nutr. 1993; 33:57-62.

Table 1: The effect of aqueous extract of *Carum copticum* on the number of rearings and the number of squares traversed in the open field apparatus in mice

Sr. No.	Treatment	No. of rearings (Mean ±SEM)	No. of squares traversed(mean±SEM)
1.	Vehicle	30.1 ± 0.11	66.80 ± 6.97
2.	200mg/kg	28.1± 0.11	120.20 ± 4.1
3.	400mg/kg	22.68 ± 11.1	150.80 ± 3.11
4.	Diazepam	20.7 ± 0.12	90.60 ± 6.4

n= 5 Animal in each group.
AECP: Aqueous extract of *Carum copticum*
P < 0.001 compared to vehicle treated group.
P < 0.001 compared to diazepam treated group.
P <0.001 compared to ondensetron treated group.

Table 2: Effect of aqueous extract of caram copticum on time spent in open and closed arm of the elevated plus maze in mice

Sr. No.	Treatment	Time spent in closed arm (mean± SEM)	Time spent in open arm (Mean± SEM)
1.	Vehicle	210.0 ±1.0	20.1 ± 0.1
2.	AECP(200mg/kg)	190 ± 2.0	30.2 ± 0.3
3.	AECP(400mg/kg)	180 ±12.0	39.1 ± 0.24
4.	Disepam	187.8 ± 6.3	48.7 ± 3.9
5.	Ondansetron	180.0 ± 8.1	72.2 ± 5.9

n= 5 Animal in each group.
AECP: Aqueous extract of *Carum copticum*
P < 0.001 compared to vehicle treated group.
P < 0.001 compared to diazepam treated group.
P <0.001 compared to ondensetron treated group.

Table 3: Effect of aqueous extract of *Carum copticum* on the number of entries in open and closed arms of the elevated plus maze method in mice

Sr.No.	Treatment	Number of entries in		Ratio of entries in open arm/ total entries (mean±SEM)
		Open	Closed	
1.	Vehicle	2.4 ± 1.6	15 ± 3.08	0.199 ±0.11
2.	AECP(200mg/kg)	5.6 ±1.1	5.8 ± 3.8	0.112 ± 0.03*
3.	AECP(400mg/kg)	8.2 ± 1.4	10.2 ± 0.83	0.109 ± 0.035*
4.	Dizepam	7.0 ± 0.7	10.8 ± 2.5	0.41 ± 0.02
5.	Ondensetron	10.4 ± 1.2	10.2 ±1.3	0.49 ± 0.01

n= 5 Animal in each group.
AECP: Aqueous extract of *Carum copticum*
*P < 0.10 compared to vehicle treated group.
P < 0.001 compared to diazepam treated group.
P <0.001 compared to ondensetron treated group.

Source of support: Nil, Conflict of interest: None Declared