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Original Research Article

Effect on anxiety of *Coriandrum sativum* leaf hydroethanolic extract oil and aqueous fraction in swiss albino mice

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ABSTRACT

Background: Anxiety is a protective reflex and the most common disorder. This study was done to evaluate the effect of anxiolytic property of oil and aqueous fractions isolated from hydroethanolic extracts of *Coriandrum sativum* leaf by novel freezing technique with swiss albino mice.
Methods: Hydroethanolic extract of *Coriandrum sativum* leaves was prepared. Oil and aqueous part were separated with freezing technique. Animals were divided into six groups. Ist group served as control and 1% DMSO was administered orally. IInd to Vth group were administered with *Coriandrum sativum* oil fraction and *Coriandrum sativum* aqueous fraction at doses of 400 and 800 mg/kg orally. VIth group was treated with diazepam 1mg/kg orally. After one hour of dosing, battery of test was done viz, elevated plus maze (EPM), light dark arena, photo actometer and rotarod.
Results: One-way analysis variance (ANOVA) followed by Dunnett's multiple comparison test was used for statistical analysis. Anxiolytic property was found to be in the following order diazepam>*Coriandrum sativum* aqueous 800>*Coriandrum sativum* aqueous 400>*Coriandrum sativum* oil 800>*Coriandrum sativum* oil 400 mg/kg. All the extracts were devoid of adverse effects of motor coordination.
Conclusions: *Coriandrum sativum* leaf possesses anxiolytic effect. The aqueous fraction of the hydroethanolic extract of the *Coriandrum sativum* leaf was found to be potent and further analysis may lead to identification of active compounds. The findings that the extract is non-sedating anxiolytic and is of good safety index are promising.

Keywords: Anxiolytic, *Coriandrum sativum*, Elevated plus maze, Light dark arena, Swiss albino mice

INTRODUCTION

Anxiety is a normal human emotion that serves as an adaptive function from a psychobiological perspective. However, in a psychiatric setting, feeling of fear or dreads that are unfocused or out of scale with the perceived threat often requires treatment.[1]

According to the World health report, approximately 450 million people suffer from mental or behavioural disorders, yet only a small minority of them receives even the most basic treatment.[2] This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020.[3] In the search for a new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide has progressed constantly demonstrating the pharmacological effectiveness of different plant species in a variety of animal models.

Coriandrum sativum belongs to family *Apiaceae*. It is an annual herb and according to the climatic condition, it is

cultivated as a summer or winter annual crop.[4] The plant is named after koris, the Greek word for bug as the unripe fruits have a smell that has been compared to that of bed bugs.[5] All parts of the plant are edible but fresh leaves and dried seeds are used commonly as flavouring agents for culinary purposes. In Indian traditional medicine, it is used in disorders of digestive, respiratory and urinary system as it has diaphoretic, diuretic, carminative and stimulant activities. In Iranian folk medicine, it has been indicated for dyspeptic conditions, loss of appetite, convulsions and insomnia.[6,7]

Most of the anxiolytic agents share common adverse effects like drowsiness and motor in-coordination affecting the quality of life. These facts express the necessity for research in the field of anxiety focusing on new therapeutic strategies. In this study *Coriandrum sativum* was screened for its anxiolytic property.

METHODS

Collection and authentication of plant material

Leaves of *C. sativum* (each of 250 g) were collected from local market of Mangalore, Karnataka, India and were authenticated. The voucher specimen was submitted to Department of Pharmacology, Yenepoya University for future references.

Preparation of hydroethanolic extract

Leaves of *C. sativum* was cleaned with running tap water to remove extraneous particles and extracted using hydroethanolic (30:70) in Soxhlet apparatus at 70-80^{o} C for 5 days. The extract was filtered, concentrated under reduce pressure and dried using rotary evaporator at 85 ^{o} C for 4 hours. The yield was 11.33%. The extract was frozen thrice in order to separate oil and aqueous part completely. Later the extract was stored at 4^{o}C. The extract thus obtained was dissolved in 10% DMSO for the study.

Chemicals and drugs used

Ethanol 99.9% manufactured by Honyon International Inc, China. Diazepam 10 mg tablet - manufactured by Abott Healthcare Ltd, India.

Animals

36 healthy male swiss albino mice aged 3-4 months weighing 25-30 g were used in the present study. They were procured from animal house, Department of Pharmacology, Yenepoya Medical College, Mangalore. The animals were housed in polypropylene cages, four in each cage. The animals were given standard pellet diet and water ad libitum and 12 hours light and 12 hours dark cycle was maintained. The animals were acclimatized to the laboratory conditions 1 hour before experiments. The experiment was conducted in a soundproof and dimly lighted environment. The experiment was performed between 12-5 pm. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC). Care of the animals was taken as per guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) India.

Study design

The animals were divided into six groups (n=6). Group I - control (1% DMSO 0.1 ml/10 g/oral), group II to V- test (CS oil fraction and CS aqueous fraction at doses of 400 and 800mg/kg/ oral), and group VI - standard (diazepam 1 mg/kg/oral).

After one hour of dosing, battery of test was done viz, elevated plus maze (EPM), light dark arena (LD), Photo actometer (PA) and rotarod (RR). Acute toxicity study was done at dose of 2000 mg/kg orally and animals were observed for 14 days. The anxiety level was evaluated using the following standard test.

Elevated plus maze[8,9]

The elevated plus maze combines three potential anxiogenic factors - novelty, height and open space. The cross shaped maze consists of four arms that are interconnected by a central platform. The maze is suspended 50 cm above the ground level. The mouse was placed on the central platform facing one of the enclosed arms and observed for 5 minutes. The test was recorded using a video camera attached to a computer. During the 5 minutes test, the number of open and closed arm entries, plus the time spent in open and closed arms were recorded. The arena was cleaned with spirit after each test.

Light dark arena[8,9]

The maze is divided into two parts, 1/3 with opaque walls and a cover (dark compartment) whereas the remaining 2/3 is open and illuminated (light compartment). The opening between the two compartments permits the mouse to move from one chamber to the other. The mouse was placed in the light compartment and observed for 5 minutes. The test was recorded using a video camera attached to a computer. During the test period, the time spent in light and dark compartment and number of crossings was recorded. The arena was cleaned with spirit after each test.

Assessment of locomotor behaviour

Photo actometer[8,9]

The locomotor activity can be easily measured using photo actometer, which have a square arena in which the animal moves. This apparatus operates on photo-electric cells, which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. The locomotor activity

of the mice was evaluated for 5 minutes. The arena was cleaned with spirit after each test.

Assessment of muscle co-ordination

Rotarod[10]

One of the important pharmacological actions of antianxiety agents of benzodiazepine class of drugs is muscle relaxant property. The skeletal muscle relaxation together with taming effect of these agents reduces anxiety and tension.

The loss of muscle grip is an indication of muscle relaxation. This effect can be easily studied in animals using rotarods. The difference in fall off time from the rotating rod between the control and diazepam treated animal is taken as an index of muscle relaxation.

The angle of the slope of inclined plane, or the rate of rotation of the rod should be adjusted such that a normal mouse can stay on the plane or on the rod for an appreciable period of time 2-5 minutes.

Mice were placed one by one on the rotating rod. Cut off time was 120 seconds. The 'fall off time' was noted when the mouse fell from the rotating rod. Three chances were given to each mouse.

Statistical analysis

The results were recorded as mean±SD. All the results were analysed with one-way analysis variance (ANOVA) followed by Dunnett's multiple comparison test. P value less than 0.01 was considered as significant. Graph pad prism version 3.02 was used for statistical analysis.

RESULTS

Effect of hydroethanolic extract coriandrum sativum extracts on elevated plus maze

Effects on anxiety

Parameter 1 - time spent in open arm. The anxiolytic potency of the extracts in EPM test were in following order, control<leaf oil 400<leaf oil 800<leaf aqueous 800<leaf aqueous 400<diazepam (Table 1).

Table 1: Effect of HECS on time spent in open arm of elevated plus maze number of mice in each group.

Group	Time spent in open arm
Control	15.17±4.02
Leaf oil 400	123.67±12.24**
Leaf oil 800	149.83±35.69**
Leaf aqueous 800	154.83±23.22**
Leaf aqueous 400	165.00±18.95**
Diazepam	252.33±25.54**

n=6; symbol **indicates significant values at p<0.01 vs control.

Parameter 2 - number of entries to open arm. The anxiolytic potency of the extracts in EPM test indicated by number of entries to open arm were in the following order, control<leaf aqueous 400<leaf oil 800<leaf oil 400<leaf aqueous 800<diazepam (Table 2).

Table 2: Effect of HECS on number of entries to open arm of elevated plus maze; number of mice in each group.

Group	Number of entries to open arm
Control	3.67±1.75
Leaf aqueous 400	7.50±1.87**
Leaf oil 800	7.50±2.43**
Leaf oil 400	7.83±1.47**
Leaf aqueous 800	13.00±2.37**
Diazepam	16.00±1.41**

n=6; symbol **indicates significant values at p<0.01 vs control.

Effect of HECS extracts on light dark arena

Parameter 1 - time spent in bright arena. The anxiolytic potency of the extracts in LDA test as indicated by time spent were in following order, control<leaf oil 800<leaf oil 400<leaf aqueous 400<leaf aqueous 800<diazepam (Table 3).

Table 3: Effect of HECS on time spent in bright arena of light and dark arena; number of mice in each group.

Group	Time spent in bright arena
Control	14.83±5.57
Leaf oil 800	83.00±12.96
Leaf oil 400	115.83±5.42**
Leaf aqueous 400	123.67±12.45**
Leaf aqueous 800	151.833±15.32**
Diazepam	166.67±21.41**

n=6; symbol **indicates significant values at p<0.01 vs control.

Parameter 2 - number of crossings. The anxiolytic potency of the extracts in LDA test as indicated by number of crossings were in following order, control<leaf oil 800<leaf oil 400<leaf aqueous 400<leaf aqueous 800<diazepam (Table 4).

Table 4: Effect of HECS on number of crossings of light and dark arena; number of mice in each group.

Group	Number of crossings
Control	5.02±1.67
Leaf oil 800	7.83±1.17**
Leaf oil 400	9.50±1.87**
Leaf aqueous 400	10.53±2.22**
Leaf aqueous 800	14.17±3.87**
Diazepam	15.16±3.97**

n=6; symbol **indicates significant values at p<0.01 vs control.

Effects on locomotor behaviour

Photo actometer

All oil fractions exhibited an increase in general activity which decreased with increase in dose. All aqueous fractions increased general activity with direct dose response relationship (Table 5).

Table 5: Effects of HECS on photo actometer and number of mice in each group.

Group	Dose and route	No. of movements
Control	0.1 ml/10 g/oral	82.19±11.94**
Diazepam	1 mg/kg/oral	439±22**
HECS leaf oil	400 mg/kg/oral	205±18.68**
	800 mg/kg/oral	161.83±29.95**
HECS leaf aqueous	400 mg/kg/oral	306.67±10.51**
	800 mg/kg/oral	394±12.99**

n=6; symbol **indicates significant values at p<0.01 vs control.

Effects on motor coordination

Rotarod

As seen from Table 6, all the animals treated with the extracts passed the cut off time 120 seconds which indicated that muscle coordination was unaffected (Table 6).

Table 6: Effects of HECS on rotarod.

Group	Dose and route	Retention time (cut off time 120s)
Control	0.1 ml/10g/oral	90 s
Diazepam	1 mg/kg/oral	>120
HECS leaf oil	400 mg/kg/oral	>120
	800 mg/kg/oral	
HECS leaf aqueous	400 mg/kg/oral	>120
	800 mg/kg/oral	

s-seconds.

As per EPM and LDA test, anxiolytic property of the test materials was found to be in the following order. Diazepam>CS aqueous 800>CS aqueous 400>CS oil 800> CS oil 400 mg/kg. Results from photo actometer indicate that none of the extracts possess sedative property. All the extracts were devoid of adverse effects of motor coordination.

DISCUSSION

Several herbs and spices have been used for culinary purpose since ages. Traditional belief that spices and herbs possess central nervous activity, antimicrobial and metabolic effects are supported by various preclinical and clinical studies. The practice of using these flavouring agents may have originated from zoo pharmacognosy, where the self-medication by the animals either to treat or prevent disease is guided by internal drive.[11] The present study was carried out to evaluate the anxiolytic activity of *Coriandrum sativum.* Previous studies have reported that leaf possess anxiolytic properties.[12,13] These mandates for a detailed study of this plant to assess the anxiolytic activity. The constituents responsible for anxiolytic properties may have a variable distribution pattern in the different parts of the plant. The leaves were extracted using hydroethanolic solvent because of its broad spectrum of extractive value. In order to narrow down the search further, hydroethanolic extracts of leaves were divided into aqueous and oil fractions. The anxiolytic properties were assessed using elevated plus maze and light dark arena. Evaluation of the locomotor behaviour of the test compound by photo actometer was done to categorize the extract into sedative and non-sedative. Rotarod test was performed to find their effects on muscle coordination.

Anxiolytic activity

The elevated plus maze and light dark arena test are the two commonly employed animal model for anxiety. EPM is based on three anxiogenic factors-novelty, height and exploratory behaviour. The number of entries and time spent in the open arms have been found to be increased by anxiolytics and reduced by anxiogenic agents.[13] The LDA test is based on the natural aversion of mice to brightly lit places. Anxiolytics reduce the natural aversion to light and increase the time spent in the light compartment. The result from EPM and LDA gives a complicated picture with mixed response which makes it quite difficult to come to a conclusion. Detailed analysis by combining the results from both anxiety models are required to solve this puzzle. In order to accomplish this, the parameters from EPM and LDA are grouped into exposure parameters (time spent in open arm/bright area) and exploratory parameters (number of entries into open arm/number of crossings) respectively.

Exposure behaviour

Exposure of the animals to high risk (open arm/brightly lit arena) zone indicates low anxiety and anxiolytic agents tend to potentiate this behaviour. This can also happen if the drug disturbs the alertness of the animal.

In EPM

The exposure behaviour was increased in following order. At 400 mg/kg: control<leaf oil<leaf aqueous<diazepam. At 800 mg/kg: control<leaf oil<leaf aqueous<diazepam.

In LDA

The exposure behaviour was as follows. At 400mg/kg: control<leaf oil<leaf aqueous<diazepam. At 800mg/kg: control<leaf oil<leaf aqueous<diazepam.

Exploratory behaviour

Mice tend to avoid the open areas, especially when they are brightly lit, favouring darker and or enclosed space.[14] This approach avoidance conflict results in behaviours that have been correlated with increase in physiological stress indicators.[15] Administration of benzodiazepines and other anxiolytic treatments results in increased exploration of the open arms, without affecting general motivation or locomotion.[16] In contrast, this behaviour can be augmented in case of fear induced hyperactivity leading to a false positive result.

In EPM

The exploratory behaviour was increased in following order. At 400 mg/kg: control<leaf aqueous<leaf oil<diazepam. Both oil and aqueous part of leaf extracts exhibited moderate anxiolytic property. At 800 mg/kg: control<leaf oil<leaf aqueous<diazepam.

Leaf aqueous part was more potent than oil fraction. A direct dose response relationship was observed in leaf aqueous and an inverse dose response relationship with leaf oil.

In LDA

The exploratory behaviour was as follows. At 400 mg/kg: control<leaf oil <leaf aqueous<diazepam. At 800 mg/kg: control<leaf oil <leaf aqueous< diazepam. A direct dose response relationship was observed in leaf aqueous and an inverse dose response relationship leaf oil.

At lower dose (400 mg/kg), leaf oil exhibited anxiogenic property and at higher dose (800 mg/kg) anxiolytic property. This indicates the presence of either anxiogenic and anxiolytic constituents or single constituents acting on multiple receptor types. The anxiogenic compound may have antagonized the effects of anxiolytic constituents or mechanism at low dose.[17] At high dose, anxiolytic constituents might have superseded.

Photo actometer

Photo actometer is a closed box in which the movements of animals are recorded photoelectrically to assess the locomotor activity.[18]

Table 7: Activity report of HECS using photo actometer.

Extract	Effect on anxiety	Sedative property
Leaf oil	Biphasic	Non sedative
Leaf aqueous	Anxiolytic	Non sedative

The oil fractions exhibited an increase in general activity with an inverse dose response relationship. The aqueous fractions increased general activity with direct dose response relationship (Table 7).

Rotarod

All the animals treated with the extracts passed the test at a cut off time 120 seconds which indicates that muscle coordination was unaffected.

CONCLUSION

The results obtained in this study suggests that the hydroethanolic extracts of leaves of *Coriandrum sativum* at a dose of 400 mg/kg and 800 mg/kg given orally possess anxiolytic activity but with a complex pharmacodynamic profile as expressed by varying anti-anxiety effect with dose and screening techniques used.

After the scrutiny with the individual test analysis and the combined analysis, it is proposed that Leaf aqueous possess anxiolytic properties containing one or more compounds. None of the test extracts exhibited sedative or any adverse effect on motor coordination as indicated by photo actometer and rota rod respectively. Hence extracts of *Coriandrum sativum* could be better alternative to the conventional anxiolytic agents in terms of its non-sedative action. The following study design is proposed for the continuation and identification of active constituent employing the findings of the present study. LC-MS, H-NMR, C-NMR and FT-IR analysis of the fractions should be employed to identify known constituents or for structure elucidation of novel compounds.

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