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Research Article

Anxiolytic Activity of Ethanolic Extract of Seeds of *Cuminum cyminum* Linn in Albino Wistar Rats

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ABSTRACT
The plant *Cuminum cyminum* linn. was found to be used by different traditional systems and folklore for the treatment of various CNS disorders. The aim of the present study was to investigate the anxiolytic activity of ethanolic extract of *Cuminumcyminum* in adult albino Wistar rats. The animal behavior was evaluated by anxiolytic activity using standard procedures in experimental animal models. The results revealed that ethanolic extract showed promising results in terms of statistical manner when compared with the control group. In conclusion, this plant exhibits anxiolytic activity in testing animals.

Keywords: *Cuminum cyminum*, Behavior, anxiolytic, HPLC.

INTRODUCTION

Central Nervous System (CNS) disorders are the most common disorders in the world which pose an intellectual challenge to the physicians to make the correct neurological diagnosis. Drugs acting on CNS were among the first to be discovered by primitive human and are still the most widely used groups of pharmacological agents[1]. The drugs acting on the CNS are therapeutically invaluable as they are expensive and associated with various side effects. In tradition health system of India, a large number of medicinal plants have been used for many centuries for treating various neurological diseases by using plant extracts or their active principle[2]. Among the various CNS disorders, anxiety is the most common disorder which is defined as a feeling of fear that is out of proportion to the nature of the threat[3] mainly occurs with dysfunction of serotonin, norepinephrine, GABA, glutamate and other CNS neurotransmitters and due to social, genetic and environmental factors[4]. Lifetime prevalence rates for total anxiety disorders are 16.6% in India[5]. Herbal formulations to treat anxiety are *Angelica archangelica*, *Coriandrum sativum*, *Abies pindow*, *Azadiracta indica*, *Magnolia dealbata* etc.[6]. Cumin (*Cuminum cyminum*) is a small slender, herbaceous annual, of the Umbelliferae family also known as Zeera[9]. It is drought-tolerant, and is mostly grown in Mediterranean climates and widespread in diverse ethnomedical systems from Northern Europe to Mediterranean regions, Russia, Iran, Indonasia and North America, where it is remained as an integeral part of their folk medicines. It is also in the list of Ayurvedic Pharmacopeia of India[10]. In tradition health system of India *Cuminum cyminum* has many therapeutic effects like abortive, antiseptic, bitter tonic, carminative, stomachic[7], diuretic, bactericidal and fungicidal properties and also used as gastrointestinal, gynecological, used in broncho pulmonary disorders, in respiratory disorders, for the treatment of toothache, diarrohea, liver function[12], leprosy, enlargement of spleen, ulcers, corneal opacities, 'Vate' tumors. The plant has various pharmacological properties like also anti-Alzheimer, antiepileptic, anti-oxidant, anti-inflammatory, anti-stress, anti-asthmatic, analgesic, hypo or hypercholesteramic, anticancer, antitussive[12]. However, there is no report available on anxiolytic activity of this indigenous plant in rat model. Keeping in view, we have evaluated the anxiolytic role of *Cuminum cyminum* seeds ethanol extract by using rat models.

MATERIALS AND METHODS

Plant material

The dried seeds of *Cuminum cyminum* was procured in the month of January, 2015 from Ambala (Haryana) India and were authenticated (No. 952) from Guru Nanak Dev University (G.N.D.U.), Amritsar, India.

Preparation of extract

The fresh, dried plant material was pulverized in electric grinder and the powder was used for further extraction. The dried, powdered of seeds (500g) was successively Soxhlet extracted using ethanol for 72 h each. The last trace of solvent is removed by reduced pressure distillation and then vacuum dried. A dark semi solid mass was obtained. It was stored below 4ºC until further used. When needed, the extract was suspended/dissolved in desired solvent and used. The extracts were concentrated by performing the qualitative chemical tests to determine the presence of sterols, Phenol compounds, flavonoids and saponins, respectively[13].

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Table 1: Effect of ethanolic extract of Cuminum cyminum in Elevated Plus Maze

Groups	Treatment	Dose	Time spent in open arm(s) (Mean ± S.E.M.)	No. of Entries in open arm (Mean ± S.E.M.)
1	Normal Control	-------	34.2±1.7	4.5±0.79
2	Diazepam (std)	2 mg/kg	64.8±2.3*	10.4±0.69*
3	Cumin extract	200 mg/kg	35.7±1.5	4.75±0.43
4	Cumin extract	400 mg/kg	48.9±2.0	6.1±0.34
5	Cumin extract	800 mg/kg	56.2±2.2*	7.94±0.62*
6	Cumin extract	1600 mg/kg	59.8 ±2.5*	7.59±0.67*

All values represent Mean ±S.E.M. (n=6),* P<0.05 vs standard drug.

Table 2: Effect of ethanolic extract of Cuminum cyminum in Light and Dark apparatus

Groups	Treatment	Dose	Time spent in light compartment(s) (Mean ± S.E.M.)	Number of Entries in Light Compartment (Mean ± S.E.M.)
1	Normal control	------	20.4±1.3	7.3±0.79
2	Diazepam (std)	2 mg/kg	62.4±2.9*	15.8±0.80*
3	Cuminextract	200 mg/kg	27.8±1.6	8.2±0.81
4	Cumin extract	400 mg/kg	39.5±1.8	9.5±0.75
5	Cumin extract	800 mg/kg	49.1±2.1*	11.7±0.92*
6	Cumin extract	1600 mg/kg	51.7±2.5*	11.4±0.59*

All values represent Mean ±S.E.M. (n=6),* P<0.05 vs standard drug.

Table 3: Effect of ethanolic extract of Cuminum cyminum in Actophotometer

Groups	Treatment	Dose	Count
1	Normal control	--------	107± 6.8
2	Diazepam (std)	2 mg/kg	174± 9.3*
3	Cumin ethanol extract	200 mg/kg	127± 6.3
4	Cumin ethanol extract	400 mg/kg	148± 7.9
5	Cumin ethanol extract	800 mg/kg	169±8.3*
6	Cumin ethanol extract	1600 mg/kg	171±8.2*

All values represent Mean ±S.E.M. (n=6),* P<0.05 vs standard drug.

Animals

Adult albino wistar rats of either sex weighing about 165-225gm were used with the approval of the institute animal ethics committee (MMCP/IAEC/15/25). The animals were housed under standard conditions of temperature (24 ± 28°C) and relative humidity (60-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (Lipton India, Ltd) and water ad-libitum.

Drugs

Diazepam (2 mg/kg) was used as standard drug.

Preparation of test doses

Ethanol extract was administered orally at dose of200, 400, 800 and 1600 mg/kg in the form of suspension prepared in vehicle (2 ml of simple syrup with 8 ml of Tween 80).

Experimental Design

Thirty-six animals were used for this study. The animals were divided into six groups. Each group consist of six animals.

Group 1: Vehicle control group.

Group 2: Diazepam (2 mg/kg once daily, oral) for 3 days.

Group 3: *Cuminum cyminum* linn ethanol extract (200 mg/kg once daily, oral) for 3 days.

Group 4: *Cuminum cyminum* linn ethanol extract (400 mg/kg once daily, oral) for 3 days.

Group 5: *Cuminum cyminum* linn ethanol extract (800 mg/kg once daily, oral) for 3 days.

Group 6: *Cuminum cyminum* linn ethanol extract (1600 mg/kg once daily, oral) for 3 days.

On the third day, after 45 min. of drug administration in different groups of rats the animals were taken for the following tests for the screening of anxiolytic activity.

Experimental models

Elevated Plus Maze (EPM)

The model is principally based on the observation that exposure of rat to elevated and open maze results in approach-avoidance conflict which is manifested as an exploratory-cum-fear drive. The fear is due to height induces anxiety in rat when placed on the EPM[8]. Locally fabricated elevated plus maze consisting of consisted of a matte elevated to a height of 25 cm from floor with two open arm (16 x 5 cm) and two enclosed arms (50 x 10 x 50 cm). 45 min. after the oral administration of different extracts at a dose of 200, 400, 800 and 1600 mg/kg bodyweight, the rat was placed at the centre of the maze, facing one closed arm. During a 5 min test period the following measures were taken: the time spent in the open and closed arms; and total number of arm entries[14]. Increased exploratory activity in the open arm was taken as an indication of anxiolytic activity[15].

Light dark model

The original maze was divided into two parts, 1/3 with opaque walls and a cover (dark compartment with dimensions 7 × 14 × 30 cm) whereas remaining 2/3 open and illuminated (light compartment with dimensions 30 × 14 × 30 cm) for rats. The door (10 × 10cm) between two compartments was permitted rats to move one side to another. During a 5 min test period the following measures were taken: the time spent in the light and dark compartment; and total number of compartment entries[16].

Actophotometer

The locomotor activity can be easily studied with the help of actophotometer, the rats were grouped and treated with drugs. On third day 45 min. After administration of drug animals were placed individually in the actophotometer. Basal activity score of all the rats was recorded for 5 minutes (Shafee*et al*., 2012). The digital counts, as the number of line crossings by rats due to beam interruptions were recorded. The counts correspond to locomotor activity[17].

High Performance Liquid Chromatography (HPLC)

Quercetin contents in ethanol extract of *Cuminumcyminum* were analyzed by using RP-HPLC.At first, three different buffers including methanol buffer (buffer A), acetonitrile buffer (bufferB), and 0.4% phosphoric acid buffer (buffer C), were mixed at ratios 60:20:20; 65:20:15; and 70:15:15. It was found that the whole separation course required almost 21 min and the peak of quercetin was obtained at the 15th minute and was asymmetric. Thus, we use 0.2% phosphoric acid buffer (buffer D) and mixed buffer A and buffer D at the ratios of 70:30; 60:40; and 65:35. The quercetin peak time now shifted to the fifth minutepointandthewholeseparation course required9min while the peak obtained was symmetric.Since quercetin is reported to have the maximum absorption at 250 and 360 nm wavelengths, we, therefore, compared the shapes ofquercetin peaks at these wavelengths and found that the shape of the peak was more symmetric and higher at the detection wavelength of 360 nm. We used Agilent 1120 system with TC-C18 column (250 9 4.6 mm, 5 lm). The mobile phase was methanol-0.2% phosphoric acid (65:35) solution. The flow rate was 1.0 ml min-1. The column temperature was 30ºC and the detection wavelength was 360 nm. The quercetin peaks were identified and quantified against the external reference standards[18].

Statistical analysis

The results are expressed as mean ± SEM. One–way ANOVA followed by Dennett's test used for comparison between different groups. The results are considered significant if the probability of error is $p<0.05$.

RESULTS

Preliminary Phytochemical Screening Test

The result of qualitative analysis indicated that ethanol extract of dried seeds of *Cuminum cyminum* possesses sterols, tannins, terpenes, flavonoids, alkaloids, volatile oil and phenolic compounds.

HPLC (High Performance Liquid Chromatography)

Our HPLC results confirmed the presence of quercetin 5.05% in cumin ethanol extract showed at 4.85 retention time compared with standard quercetin at same retention time 4.85.

Elevated Plus Maze

Administration of standard drug diazepam (2 mg/kg) significantly increases number of open arm entries, time spent in open arms. Plant extract (800 and 1600 mg/kg) treated rats exhibited significant ($p < 0.05$) increase in time spent and open arm entries as shown in table 1.

Light and Dark apparatus

There was significant anxiolytic activity observed with diazepam, plant extracts when compared to control. In the Light and Dark apparatus, plant extract (800 and 1600 mg/kg) treated rats showed significant ($p < 0.05$) increase in time spent and entries in the light compartment during 5 min. Interval of test as compared with control as shown in table 2.

Actophotometer

Plant extract (800 and 1600 mg/kg) treated rats showed significant ($p < 0.05$) increase in locomotor activity when compared with control as shown in table 3.

DISCUSSION

Anxiety disorders occur due to involvement of GABAergic, serotonergic, dopaminergic system involvement. People with anxiety like neurological disorders are often subjected to social isolation, poor quality of life and increased mortality. These disorders are the cause of staggering economic and social costs[19]. Benzodiazepines have been extensively, used in the last 40 years to treat several forms of anxiety, but due to their unwanted side effects, alternative treatment strategies with favorable side effect profiles. Medicinal plants are a good source to find new remedies for these disorders[20]. Despite the widespread traditional use of *Cuminum cyminum* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrates that the *Cuminum cyminum* extract had anxiolytic activity in rats. Elevated Plus Maze is used to evaluate psychomotor performance and emotional aspects of rodents[21]. Results showed that plant extracts treated rats exhibited significant increase in the time spent and number of open arm entries which reflects plant anxiolytic activity. In light dark box test, animals usually try to spend more time in dark box compared to light box out of fear of exposure to the new environment. Transitions have been reported to be an index of activity exploration because of habituation over time spent in each compartment as reflection of aversion[22]. Results showed that plant extracts treated rats exhibited significant increase in the time spent and number of entries in light compartment. Actophotometer is used to calculate motor dysfunction produced by centrally acting drugs to determine possible alterations in the motor coordination ability of the animal[23]. Our observations, gave a conclusion that the mean offline crossings by rats due to beam interruptions was significantly increased ($p < 0.05$) in animals treated with ethanolic extract of plant at a dose 800 & 1600 mg/kg as

compared to control group. From above observations, we can conclude that anxiolytic effect of ethanol extract of Cumin may be due to the presence of flavonoid called Quercetin which acts on CNS bind to the benzodiazepine site on the GABAA- receptor resulting in anxiolytic activity. GABAA receptors are heteromeric GABA-gated chloride channels. The transmembrane ion channel is opened by a stimulus generated by GABA, which allows an influx of chloride ions. This results in a decrease of the depolarizing effects of an excitatory input, thereby depressing excitability. As a result, the cell is inhibited and an anxiolytic activity is achieved[24]. Although major active components and the precise anxiolytic mechanism need to be identified.Further research and clinical trials are needed to standardize its use in humans.

CONCLUSION

From this study, seeds of *Cuminum cyminum* possess anxiolytic activity in rat model. However, further investigations are required to isolate the phytoconstituents responsible for behavioral activity and to find their mechanism of action.

CONFLICT OF INTEREST

The authors confirm that this research article content has no conflict of interest.

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