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Research Article

EVALUATION OF ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF FOENICULUM VULGARE IN MICE MODEL

R. NAGA KISHORE*, N. ANJANEYULU, M. NAGA GANESH AND N. SRAVYA

Department of pharmacology, Geethanjali College of pharmacy, Cheeryal (V), Keesara (M), R.R. District, Andhra Pradesh, 501301
Email: rnkishore.sm24@gmail.com

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ABSTRACT

The present study was designed to investigate the anxiolytic activity of ethanolic extracts of *Foeniculum vulgare* fruit. The anxiolytic activity was evaluated by elevated plus maze, rota rod, open field test, and hole board models. The efficacy of extract (100-200mg/kg) was compared with standard anxiolytic drugs diazepam (1mg/kg). Extract administered animals showed exploratory behavior with all tests similar to diazepam. The results showed that the extract significantly increased the number of entries and time spent in the open arm in the elevated plus maze apparatus. In open field test, the extract showed significant increase in number of rearings, assisted rearing and number of square crossed. Furthermore the extract produced skeletal muscle relaxant affect assessed by rota rod. Altogether these results suggest that the ethanolic extract of *Foeniculum vulgare* may possess anxiolytic activity and provide a scientific evidence for its traditional claim.

Keywords: *Foeniculum vulgare,* Open field test (OFT), Rota rod test (RRT), Elevated plus maze apparatus (EPMT), Hole board test (HBT).

INTRODUCTION

Anxiety is the displeasing feeling of fear and concern. When anxiety becomes excessive, it may be considered as an anxiety disorder. An anxiolytic *Foeniculum vulgare(fennel)* is a drug used for the treatment of anxiety, and its related psychological and physical symptoms[1]. Anxiolytics are also known as minor tranquilizers. Anxiolytics are classified in to Benzodiazepines, Barbiturates, Azapirones. There is a need for further development of new drug molecules for its a side effects. So, Herbal treatments are to be helpful in treating anxiety. Plants like *Foeniculum vulgare (fennel), Bacopa monnieri* (Brahmi), *Lactuca virosa* (Opium Lettuce) are found to be having anxiolytic activity[2].

Word fennel developed form the Middle English fenel/fenyl. Latin feniculum, mean "hay". It is highly aromatic and flavorful herb with culinary and medicinal uses[3]. *Foeniculum vulgare* L (umbelliferae) is a perineal herb commonly used in Middle Eastern, Mediterranean, Indian and somewhat Asian cuisine. In the Indian traditional medicine it is used in disorders of digestive, respiratory, and urinary systems. Pharmacological studies demonstrated the following actions, antihypertensive, diuretic, antioxidant, antimicrobial, carminative and anti-inflammatory medicine. Plant contains active constituents like anisic acid, anisic aldehyde,D-pinene, fenchone, organic phellandrine, volatile oils(50-60%), anethole[4].

However, so far, its effects on CNS activity have not been studied. Therefore we undertook the study to evaluate the anxiolytic potential of *Foeniculum vulgare* by using different animal models and studying the effect of the plan on their exploratory behavior.

MATERIALS AND METHODS

Animals: inbred adult albino mice (25-30gms) of either sex were used for the study. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed and drinking water was provided ad libitum. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The animals were divided into four groups, each consisting of six mice and were used in all sets of experiments. Institutional animal ethical committee approved the protocol of the study.

Plant material

Fruits of *foeniculum vulgare* were collected from local market Hyderabad, Andhra Pradesh. These were authenticated at the department of pharmacognosy department.

Preparation of the ethonolic extract

The fruits of *foeniculum vulgare* were collected, washed thoroughly in water and air dried at 35-40ºC for a week. Dried fruits were pulverized by using electric grinder to obtain a fine powder. The powder was defatted with petroleum ether. Later it was extracted with ethanol extraction was done by using soxhlet apparatus. The filtrate was evaporated to dryness at 40ºC.

Drugs

Diazepam hydrochloride (Ranbaxy laboratory ltd, Mumbai) was used as reference drug. It was diluted with saline to the required strength before use.

Preparation of test doses

The extracts were suspended in the vehicle. Various strengths were prepared from a stock solution 100 mg/ml. the solutions were prepared freshly solutions were administered orally.

Acute toxicity study

The procedure was followed as per OECD 423 guidelines[4]. The extracts was administered orally at a dose of 100, 200, 400, 600, 800, 1000, 2000, mg/kg body weight. Animals were observed for 10 days to study their behavioral neurological toxicity.

Treatment schedule

The anxiolytic activity was examined by using the elevated plus maze test (EPMT), open field test (OFT), and motor coordination test assessed by rota rod test (RT), hole board test (HST)[5, 6]. The animals were divided in to four groups, with each group consisting of six male mice. First group receives normal saline, second group received diazepam (1mg/kg), third and fourth groups received plant extract (100 &S 200 mg/kg)

Elevated plus Maze test

The plus – maze consists of two open arms and two closed arms (50 x 10 x 40 cm each) elevated to a height of 50 cm Extract and diazepam (1mg/kg) were administered to groups. Thirty minutes post treatment, each mouse was placed in turn in the centre of the maze facing one of the closed arms. The cumulative times spent by each mouse in the open and closed arms of the maze were recorded for 5 min[7].

Open field test

Each animal was placed into an acrylic cage (50 × 50 × 10 cm). The arena of the open field was divided into 25 squares, the 9 inner squares in the center and 16 squares in the periphery along the

walls. Experimental room was a sound attenuated, dark room after 1hr of oral administration with vehicle, diazepam, and plant extract, animals were placed individually in one of the corner squares and number of rearings, assisted rearings and number of squares crossed were observed for 5 min[8].

Rota Rod

The effect on motor coordination was assessed using a rota-rod apparatus. Rota rod apparatus consisted of a base platform and an iron rod of 3 cm diameter and 30 cm length, with a non slippery surface. This rod was divided in to four equal sections by three disks, and then enabling four mice to walk on the rod at the same time at the speed of 22rpm observed over a period of 15, 30, 45, 75, and 90 min. Intervals between the mounting of the animal on the rod and falling off of it were recorded as the performance time. There after four mice were randomly selected to determine locomotor activity[9]. The effect on motor coordination was assessed using a rota-rod apparatus. In brief, mice were trained to remain for 5 min on the rod rotating at speed of 22 rpm.

Hole board test

The hole board is a white painted wooden board (40 cm x 40 cm) with four equidistant holes (1cm diameter x 2 cm depth). Using two thick colored lines which intersect at the centre, the board was divided into 4 equal sectional squares of 20 cm x 20 cm. Each mouse was placed in turn at one corner of the board with the animal subsequently moving about and dipping its head into the holes. The number of head dips and sectional crossings in 5 min. were recorded for individual mouse[10].

Statistical Analysis

All results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA followed by student 't' test.

RESULTS

Acute toxicity study

The extracts was administered orally at a dose of 100, 200, 400, 600, 800, 1000, 2000, mg/kg body weight. Animals were observed for 10 days to study their behavioral neurological toxicity. Animals were observed for signs of toxicity such as hyperactivity, grooming, convulsions, and hypothermia continuously for 2 hours and for mortality up to 24 hours after administration of the doses. As even the mice receiving the highest dose of *foeniculum vulgare* did not show any mortality. This indicates the safety profile of the plan extract[11].

Elevated plus maze

Administration of diazepam (0.5 m/kg) significantly increases number of open arm entries, time spent in open arms and the number of rearings in open arm. They showed a reduction in the time spent in closed arm. Plant extracts treated mice exhibited significant increase in the number of open arm entries. The number of arm entries, but decreases in time spent in closed arm as shown in the table 1.

Open field test

There was significant anxiolytic activity observed with diazepam, plant extracts when compared to control. In the open field test, plant extract showed significant increase in number of rearings, number of squares crossed and number of assisted rearings during 5 min intervals of test as compared with control as show in table 2.

Rot rod test

A significant decrease in the locomotor score was observed for diazepam when compared to the control animals. Both the doses of plant extract showed significant decrease in the locomotory score when compared to control animals as shown in able 3.

Hole board test

The number of line crossings and head dipping was increased significantly in case of diazepam treated animals as compared to control animals. The plant extracts showed significant increase in the number of line crossing and head dipping significantly as compared to control animals as shown in table 4.

Table 1: Elevated plus maze test

Treatment	Dose(mg/kg)	Time spent in open arm (s)	Entries in open arm
Saline	1ml	30.25±4.41	3.98±0.52
Diazepam	0.5	78.59±3.52	8.64±0.47*
Plant extract	100	42.83±3.97	5.48±0.39
Plant extract	200	67.92±4.80	7.32±0.21*

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

Table 2: Open field test

Treatment	Dose(mg/kg)	Number of squares crossed	Number of Rearings
Saline	1ml	40.3±2.1	10.1±1.4
Diazepam	0.5	29.5±3.6*	8.2±1.8
Plant extract	100	22.9±2.4	5.4±2.3
Plant extract	200	26.4±2.8*	9.3±1.2

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

Table 3: Rota rod test

Treatments	Time (sec) of animals remained without falling from rod					
	15min	30min	45min	60min	75min	90min
Saline	180.2±9.1	152.9±8.5	140.1±6.8	128.1±7.6	109.9±5.8	93.5±8.4
Diazepam	157.6±6.9*	142.7±6.4	138.2±6.5	112.9±6.3	97.6±4	73.1±4.4
Extract 100mg	140.8±5.7	120.4±5.8	115.1±8.2	100.3±7.2	94.7±3.1	89.3±5.5
Extract 200mg	152.1±5.8*	110.1±4.9	103.4±7	94.5±6.5	92.4±4.8	77.1±4.1*

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

Table 4: Hole board test

Treatment	Dose(mg/kg)	Number of head dipping	Number of line crossing
Saline	1ml	10.1±1.5	22.6±1.3
Diazepam	0.5	16.5±1.8	37.8±1.4*
Plant extract	100	11.1±1.6	28.4±1.9
Plant extract	200	15.9±2*	33.9±2

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

DISCUSSION

Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. The adrenergic and dopamanergic system have also been shown to play a role in anxiety. BZA have been extensively, used for 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. Despite the wide spread traditional use of *foeniculum vulgare* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrates that the *foeniculum vulgare* extract had anxiolytic activity in mice by Elevated Plus Maze, Rota rod, Open field, and Hole board models[12].

Elevated Plus Maze is used to evaluate psychomotor performance and emotional aspects of rodents. Results showed that plant extracts treated mice exhibited significant increase in the number of open arm entries. The number of arm entries, but decreases in time spent in closed arm reflects plants anxiolytic property[13].

The open field test is used to evaluate the animal emotional state. The open field model examines anxiety related behavior characterized by the normal aversion of the animal to an open area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Mice treated with extract showed increase in number of rearings and time spent in the center.

Rota rod test, the difference in the fall of time from the rotating rod between the vehicle and extract treated groups were taken as an index of muscle relaxation. Plant extract showed significant decrease in the locomotory score and fall of time of the mice from the rotating rod.

In the Hole board test, the plant extracts showed significant increase in the number of line crossing and head dipping. This indicates the anixyolytic activity of plant extract[14].

CONCLUSION

From the above observations we can conclude that ethanolic extract of *Foeniculum vulgare* possesses anxiolytic activity at both the dose level which is comparable with the standard. However further studies are required to know the exact mechanism of action of *Foeniculum vulgare* as anxiolytic.

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