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A STUDY ON ANXIOLYTIC ACTIVITY OF AQUEOUS LEAF EXTRACT OF *PLECTRANTHUS AMBOINICUS* LINN. IN EXPERIMENTAL ANIMAL MODELS

Satish S*[1], PalakshaM N[2], Manjunatha E[3], & Vaisakh Harshan[1]

1. Department of Pharmacognosy, Karavali College of Pharmacy, Mangalore, Karnataka, India.
2. Department of pharmacology, Aditya College of Pharmacy, Surampalem, E.G.Dist-533437.
3. Sree Siddaganga College of Pharmacy, B.H.Road, Tumkur, Karnataka. India.

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For Correspondence:

Dr. Satish S.

Department of Pharmacognosy, Karavali College of Pharmacy, Mangalore, Karnataka, India

E-mail:

satish.mpharm@gmail.com

ABSTRACT

Anxiety is one of the leading causes for many cardiovascular, metabolic and Neurologic dis-orders in both developed and developing countries. AEPA has been referred in Gemmotheraphy Materica medica for the treatment of various diseases for its anxiolytic activity. Present study was undertaken to evaluate anxiolytic activity of AEPA. Evaluation of anxiolytic activity was done by using 3 doses of AEPA (150, 250 & 350 mg/kg) in two in vivo models Elevated Plus Maze Test, Light Dark Test. The Animals were treated with three doses of AEPA (150, 250 and 350 mg/kg) in all the anxiolytic models employed. In EPM, increase in the time spent at open arm was noted whereas in LDT animals showed reduced time spent in dark chamber. Aqueous Extract of Plectanthus amboinicus has significant anxiolytic activity in different models of anxiety.

INTRODUCTION

The human central nervous system is an extremely complex structure, having more than 12 billion nerve cells. Together with the endocrine system, it coordinates and regulates the functioning of all body organs[1]. Anxiety, depression and mental health problems in general and senile neurological disorders in particular, are widely prevalent in modern fast-paced life with a multitude of stressful conditions[2]. Anxiety is an exaggerated feeling of apprehension, uncertainty, uneasiness and dread. Excessive anxiety can weaken and damage the quality of life[3]. Anxiety Disorders includes Panic disorder with or without agoraphobia, Agoraphobia with or without Panic disorder, Specific Phobia, Social Phobia, Obsessive Compulsive Disorders, Post Traumatic Stress Disorder and Generalized Anxiety Disorder[4].

Plant kingdom is rich of various medicinal species having their effect on nervous system. Now a day's pharmacological spectrum and biological efficiency of such herbal drugs can be suitably established due to development of various neuropharmacological testing of herbal drugs with effect on CNS and ANS[5]. Medicinal plants being inexhaustible and major sources of new drug continue to get explored for newer ideal drugs for the treatment of almost every disease including the anxiety disorders[6].

In the present study, *Plectranthus amboinicus* Linn. (family- Lamiaceae) was selected on the basis of native knowledge on reports which is not available for the claimed activity.

MATERIALS AND METHODS

Collections of plants

The leaf of *Plectranthus amboinicus* L. belonging to the family Lamiaceae was collected from the Kasaragode district of kerala and was Authenticated by the Scientific officer (Botany) of Pilikula Nisarga Dhama, Mangalore, Karnataka and the annexure of the same is enclosed. (See Annexure A-2). It is preserved in the departmental library for future reference.

Preparation of plant material

The leaves were washed 2 or 3 times with tap water so that it was made free from all dust materials. They were cut into small pieces and made into a paste with the help of a blender. For the aqueous extract, 500 g of plant material was extracted by infusion boiled water (500 ml) for three days. The respective aqueous extracts were separated from its residues by gravity filtration. The final crude extract was obtained as dark green to black powder in percentage from dry weight (15.2% d.w). The extract was stored in refrigerator at 4 – 8 0C [7].

Experimental Animals

Swiss albino mice weighing 18-30 gm were used for the study. The mice were inbred in the central animal house of the Department of Pharmacology, Karavali College of Pharmacy, Mangalore, under suitable conditions of housing, temperature, ventilation and nutrition were used for anxiolytic activity. The animal care and experimental protocols were in accordance with CPCSEA /IAEC.

Dose fixation

A dose of AEPA 150mg/kg, 250mg/kg and 350mg/kg of body weight chosen as per the previous work[8].

Elevated Plus Maze Test

Experimental rats randomly divided into six groups and each group contains 6 rats.

Group I – Received 0.05ml/10g of Normal saline intra peritoneally.

Group II – Received 1 mg/kg Diazepam intra peritoneally.

Group III – Received 150 mg/kg AEPA orally.

Group IV – Received 250 mg/kg AEPA orally.

Group V – Received 350 mg/kg AEPA orally.

This rodent model of anxiety has been extensively used for evaluation of novel anxiolytic agents and to investigate psychological and neurochemical basis of anxiety. This test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.

The primary measures are the proportion of entries into the open arms and the time spent on the open arm expressed as a percentage of the total time spent on both open and closed arms[9]. Prior to starting the experiment, the mice were handled daily to reduce stress. Two hours after the oral administration of the test drugs and 30 min after the intraperitoneal administration of diazepam, the animal was placed in the centre of the maze, facing one of the enclosed arms. Thereafter, the number of entries and time spent in the open and closed arms were recorded during the next 5 min. An arm entry being defined when all four paws are in the arm. Number of open and closed arm entries and percent time spent in open and closed arm were measured. At the end of each trial the apparatus was wiped clean in order to eliminate any olfactory clues, which might modify the behavior of next animal. The procedure was conducted in a sound attenuated room, and observed.

Light Dark Test

Experimental rats randomly divided into six groups and each group contains 6 rats.

Group I – Received 0.05ml/10g of Normal saline intra peritoneally.

Group II – Received 1 mg/kg Diazepam intra peritoneally.

Group III - Received 150 mg/kg AEPA orally.

Group IV – Received 250 mg/kg AEPA orally orally.

Group V – Received 350 mg/kg AEPA orally orally.

Dark/light box (or black/white box) comprises one aversive (i.e. light) and one less aversive (i.e. dark) compartment. In the original setup, the dark compartment is smaller than the light one and both the compartments are separated by a partition containing an opening. Later on, modifications were made in that the two compartments were equal in size and connected by a tunnel. Transitions between the compartments and time to explore each of them are interpreted as indicators of anxiet y and are sensitive to anxiety- affecting drugs[9]. The apparatus consisted of a light and dark chamber divided by small partition containing 13 cm long x 5 cm high opening which separates the dark chamber from the light chamber. The numbers of crossings between the light and dark sites were recorded. Movements through the partition and the time spent in the dark and light chamber were counted. Male mice were placed into the cage. The number of entries in dark and light chamber and time spent in minutes in dark and light chambers were measured. The procedure was conducted in a sound attenuated room, with observed.

Statistical analysis

All the data are expressed in mean ± SEM. The significance of differences in means between control and treated animals for different parameters was determined by One-way analysis of variance (ANOVA) followed by Dennett's multiple comparison test. Significance of difference between normal and control group were evaluated by Student's *t*-test. A $p<0.05$ was considered statistically significant.

Results

Elevated Plus Maze Test

In EPM, (Table No.1) animals treated with three doses of AEPA (150, 250 and 350 mg/kg) showed increase in the time spent at open arm of the elevated plus maze which was significant at higher dose (52.83 ± 4.79; $p\leq 0.01$) when compared with control (38.33± 1.20). Similarly, animals treated with diazepam (1 mg/kg), as expected, showed a significant increase in the time spent at open arm of the elevated plus maze (76.83±2.25; $p\leq 0.001$).

More over the time spent at closed arm decreased in all three doses and which was significant at moderate and higher doses of test (154.17±4.52; p≤ 0.001, 145.33 ± 1.82; p≤0.001) when compared with control (183.00 ± 2.67). Similarly, animals treated with diazepam (1 mg/kg), as expected, showed a significant decrease in the time spent at closed arm of the elevated plus maze model (128.17± 3.16; p≤ 0.001).

Table 1. Effect of AEPA on EPM paradigm in mice

Group No.	Drug Treatment	Dose (mg/kg)	Number of entries (mean±SEM)		Time spent in sec (mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	0.05ml/10g	6.83 ± 0.40	11.66± 0.33	38.33± 1.20	183.00 ± 2.67
II	Diazepam	1	12.16± 0.40***	6.66 ± 0.33***	76.83±2.25***	128.17± 3.16***
III	AEPA	150	7.16 ± 0.40	11.33 ± 0.21	39.00± 1.71	176.00 ± 2.88
IV	AEPA	250	7.83 ± 0.30	9.83 ± 0.30**	43.50± 2.64	154.17±4.52***
V	AEPA	350	9.66 ± 0.42***	7.83 ± 0.30***	52.83± 4.79**	145.33 ± 1.82***

All values are expressed as a mean ± SEM, n=6, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test).

Animals treated with all three doses (Table No.1) showed decrease in the number of entries in closed arm of the elevated plus maze model which was significant for moderate and high dose (9.83 ± 0.30 ; p≤ 0.01, 7.8 ± 0.30 ; p≤ 0.001) when compared with control (11.66 ± 0.33). Similarly, animals treated with diazepam (1 mg/kg), as expected, showed a significant decrease in number of entries at closed arm of the elevated plus maze model (6.66 ± 0.33; p≤0.001) whereas there was increase in the number of entries in open arm of the elevated plus maze significantly for high dose (9.66 ± 0.42; p≤ 0.001) when compared with control (6.83 ± 0.40). Similarly, animals treated with diazepam (1 mg/kg), as expected, showed a significant increase in number of entries at open arm of the elevated plus maze model (12.16 ± 0.40; p≤ 0.001).

Animals treated with moderate and high dose (250 and 350 mg/kg) shown more significant increase in the number of entries and time spent at open arm of the elevated plus maze model when compared with low dose (150 mg/kg).

Light Dark Test

In LDT, (Table No. 2) animals treated with three doses of AEPA (150, 250 and 350 mg/kg) showed reduction in time spent in dark chamber and which was significant at moderate and higher dose (6.33 ± 0.33; p≤ 0.05,5.33±0.33; p≤0.001) and with significant increase in time spent in light chamber at higher dose (1.83 ± 0.30; p≤ 0.05) when compared with their

control readings (7.83± 0.30 and 0.66 ± 0.21).Similarly, animals treated with diazepam (1mg/kg) as expected showed reduced the time spent in dark chamber with increase in time in light chamber (4.0± 0.25 ; p≤ 0.001and 2.16 ± 0.30; p≤0.001).Animals treated with high dose and moderate (250 and 350 mg/kg) shows more significant results when compared with low dose (150 mg/kg).

Table 2. Effect of AEPA on Light Dark transition model

Group No.	Drug Treatment	Dose (mg/kg)	Time spent in min (Mean±SEM)		Number of Entries (Mean±SEM)	
			Dark	Light	Dark	Light
I	Control	0.05ml/10g	7.83 ± 0.30	0.66± 0.21	4.66 ± 0.21	1.33 ± 0.21
II	Diazepam	1	4 .00± 0.25***	2.16 ± 0.30***	12.83 ± 0.40***	5.50 ± 0.34***
III	AEPA	150	7.50± 0.22	0.50 ± 0.22	6.16± 0.30*	3.00± 0.25**
IV	AEPA	250	6.33 ± 0.33*	1.50 ± 0.22	7.50 ± 0.22***	2.83 ± 0.30**
V	AEPA	350	5.33± 0.33***	1.83 ± 0.30*	10.50 ± 0.42***	3.66 ± 0.21***

All values are expressed as a mean ± SEM, n=6, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test).

All animals treated with three doses of AEPA (Table No.2) showed increased number of entries in dark chamber (6.16± 0.30; p≤ 0.05, 7.50 ± 0.22 ; p≤ 0.01 , 10.50 ± 0.42; p≤0.001) and also with increase in number of entries in time in light chamber (3 ± 0.25; p≤ 0.01, 2.83 ± 0.30 ; p≤ 0.01, 3.66 ± 0.21; p≤ 0.001) when compared with their control reading (4.66 ± 0.21 and 1.33 ± 0.21).Similarly, animals treated with diazepam (1mg/kg) as expected showed increased number of entries in both dark chamber and light chamber(12.83± 0.40 ; p≤0.001 and 5.50 ± 0.34 ; p≤ 0.001).Animals treated with moderate and high dose (250 and 350 mg/kg) shows more significant results when compared with low dose (150 mg/kg).

DISCUSSION

The current available drug therapies focus on altering the availability of GABA, NA and serotonin (5-HT).The incidence of anxiety in the community is very high and is associated with lot of morbidity. Hence, it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders[10]. Despite the widely popular use of AEPA for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the administration of different doses of AEPA in mice was able to induce anxiolytic effects. In present study, unconditioned behavioural models namely Elevated plus maze test, Light dark

test were employed. These test are based on unconditioned behaviour, relying on natural behavioural reactions and do not require specific training of the animals. Sometimes these anxiety models rely on species- specific responses (e.g., social interaction) and are referred to as 'ethologically based' models.

The assessment of anxiety related behaviour in animal model is based on the assumption that anxiety in animals is comparable to anxiety in humans[11]. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform, moreover it is known that anxiolytic agent increases the frequency of entries and time spent in open arm of the EPM. In agreement with previously published reports, diazepam increased the percentage time spent on open arms and the number of entries on open arms[12]. In the present study it is noted that administration moderate and high dose (250 mg/kg & 350 mg/kg) of AEPA prolonged the time spent in the open arms and the number of entries into open arms significantly when comparing to the low dose (150 mg/kg)

Light-dark exploration have been validated pharmacologically behaviourally and physiologically. This method titrates a naturally tendency of mice to explore a novel environment, against the aversive properties of a brightly lit compartment. Within this system a number of measurable indices of anxiety exist: the number of shuttle crossing between chambers, general locomotor activity, number of rearing and amount of time spent in the dark area of the apparatus[13]. The present study showed that AEPA (150, 250 and 350 mg/kg) increase the time in the light area and decrease in dark area suggesting again that AEPA possesses anxiolytic properties.

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plant containing Flavanoids, Phenolic acids and Glycosides possess activity against many CNS disorders[15]. Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors [16]

CONCLUSION

The results suggest that *Plectranthus amboinicus* has potential role in the management of anxiety disorders. Further investigations are necessary for elucidating the exact mechanism and bioactive compounds.

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