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RESEARCH ARTICLE

Antianxiety Effect of Alcoholic Leaf Extract of *Plectranthus Amboinicus in Mice*

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ABSTRACT

This study was performed to investigate the anxiolytic effects of alcoholic extract of ***Plectranthus amboinicus*** (AEPA) in mice using the elevated plus-maze model (EPM), light dark model and hole board test. The extract administered orally in three different doses of 250mg/kg, 500mg/kg and 750mg/kg, were able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze, also increases the time spent by mice in the illuminated side of the light–dark test, dose of 500mg/kg and 750mg/kg showed more significant increase in nose poking and decrease locomotion in hole board test, in comparison with control animals. This effect was comparable to that of the diazepam (1.0mg/kg p.o.). These results indicate that AEPA is an effective anxiolytic agent.

Keywords: Anxiolytic-like effect, *Plectranthus amboinicus,* Elevated plus maze, Diazepam.

1. INTRODUCTION

Anxiety usually refers to the experience of fear, apprehensiveness, nervousness, panic, restlessness, tension, and agitation. Manifest symptoms include trembling, fainting, headaches, and sweating, possibly elevated blood pressure, and changes in other psychophysiological indices such as heart rate, muscle tone, and skin conductance[1].

Neurotransmitters involved in anxiety generation include serotonin, dopamine, noradrenaline, GABA, Corticotropin releasing factor (CRF), Melanocyte stimulating hormone (MSH), neuropeptides and neurosteroids[2] benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects[3]. The recognition of anxiolytic effects of non-benzodiazepine azapirones agents, which act as 5HT1A partial agonists and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the 5-HT1A receptor[4]. Although the azapirones display nanomolar affinity for 5HT1A receptor sites[5]. In traditional system of medicine, *Plectranthus amboinicus* is used in the treatment of renal calculi, malarial fever, hepatopathy, chronic asthma, hiccough, helminthiasis and epilepsy. The plant is credited with antifungal, antileptospiral, rheumatoid arthritis and nephroprotective activity. However, the underlying mechanism anxiolytic needs to be investigated. The present investigation was undertaken to explore the anxiolytic effect of action of the AEPA in animal models[6].

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Leaves of *Plectranthus amboinicus* were collected from Bangalore, Karnataka, and were authenticated by Dr. Kempegowda head of the Department Botany Bangalore University, and a voucher specimen has been deposited at the herbarium for further reference.

2.2. Extraction and Preliminary Phytochemical Screening [7,8]

Freshly collected Leaves of *Plectranthus amboinicus* were dried in shade and pulverized to get a coarse powder. A weighed quantity of the powder (980gms) was passed through sieve number 40 and subjected to hot solvent extraction in a Soxhlet apparatus using ethanol at a temperature range of 60- 800 C. Before and after every

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extraction the powder bed was completely dried and weighed. The filtrate was evaporated to dryness at 400C under reduced pressure in a rotary vaccum evaporator. A brownish black waxy residue was obtained. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents present in them.

2.3. Experimental models

Adult male Swiss albino mice (20–30gms) were used. They were housed in groups in polypropylene cages (11cm × 17cm × 28cm) with wood shavings as bedding, under controlled conditions of light (12h light–dark cycle, light on at 8 a.m.) and temperature (22±20C). The animals had free access to water and food except 1 h before and during the experiments. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).*IAEC ref. no IAEC/KCP/2011-12 dated 17.12.2012*

2.4. Acute toxicity studies [9,10]

Acute toxicity tests were performed in mice. All animals were fasted overnight before treatment and were given food 1 h after AEPA treatment. A single high dose (2000 mg/kg), as recommended by the OECD guidelines, was administered orally to mice. General behavior was also observed at 1, 3 and 24 h after administration. The number of animals that died after administration was recorded daily for 14days.

2.5. Experimental design

The animals were divided into five groups of Swiss albino mice, each comprising six animals. Group I served as a control received 0.05ml/10g of saline orally, Group II mice were administered with standard drug Diazepam (1mg/kg body weight administered orally) dissolved in normal saline, Group III, IV & V received (alcoholic extract of *Plectranthus amboinicus*) AEPA 250mg/kg, 500mg/kg, 750mg/kg body weight orally for 21days respectively, after 21 days dosing period the animal's anxiety level was observed by screening methods such as elevated plus maze, light dark model, hole board test.

2.6. PHARMACOLOGICAL SCREENING:

2.6.1. Elevated plus-maze test[11]:

The elevated plus-maze comprised two open (30 cm×5 cm×0.25 cm) and two enclosed (30 cm×5 cm×15 cm) arms that radiated from a central platform (5 cm×5 cm) to form a plus sign. The maze was constructed of black painted wood. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals. The plus-maze was elevated to a height of 40 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, were counted during a 5 min test period were used as indices of anxiety. A mouse was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels.

2.6.2. Light dark test[12]*:*

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into thedark box. The apparatus was cleaned thoroughly between trials.

2.6.3. The hole-board test[13]:

The apparatus was composed of a gray wooden box (50 cm×50 cm× 50 cm) with four equidistant holes 3 cm in diameter in the floor. The centre of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 15 cm above the ground and divided into squares of 10 cm×10 cm with a water resistant marker. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The total locomotor activity (numbers of squares crossed) and the number and duration of head-dippings were recorded. A head dip was scored if both eyes disappeared into the hole.

3. STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of the mean (S.E.M.). All data are subjected to analysis of variance (ANOVA) followed by Dunnet's "t" test. P values <0.05(95% confidence limit) was considered statistically significant.

4. RESULTS:

4.1. Effect of AEPA on Elevated plus maze

In EPM saline treated animals the time spent in the open and closed arms, and entries in the open and closed arms were compared with alcoholic extract of *Plectranthus amboinicus* extract at the dose of 250mg/kg, 500mg/kg and 750mg/kg & also Diazepam (1mg/kg) showed significant ($p<0.001$) increase in the time spent in the open arms and significant ($p<0.05$) increase in number of entries in open arm (Graph 1& 3). Furthermore, AEPA 250, 500 and 750 mg/kg had decrease in time spent and number of entries in closed arm (graph 2 &4) as Diazepam showed a significant ($p<0.05$) in elevated plus-maze.

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	7.00 ± 0.2582	11.666 ± 0.333	37.666± 1.308	190.833 ± 3.049
II	Diazepam	1	12.00± 0.2582***	6.666 ±0.33 33***	81.333± 0.25***	129.66± 2.390***
III	AEPA	250	7.166 ± 0.4014	10.5 ± 0.4282	46.00± .508**	160.5 ± 2.405***
IV	AEPA	500	8.666 ± 0.2108**	9.333 ±0.4216 16***	62.00± 13***	147.166±1.701***
V	AEPA	750	11.00 ± .3651***	7.5 ± .2236***	78.833± 9***	137.5 ± 2.156***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. control

Table No.1. Effect of AEPA on EPM paradigm in mice

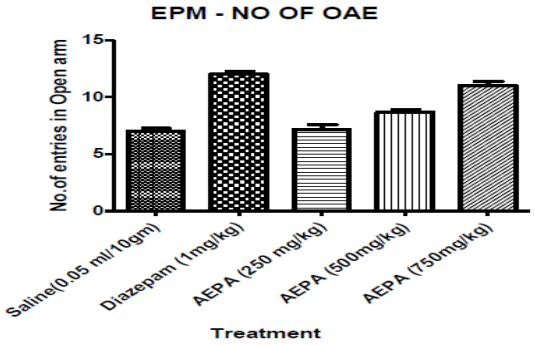

Fig 1: No. of entries in open arm in EPM

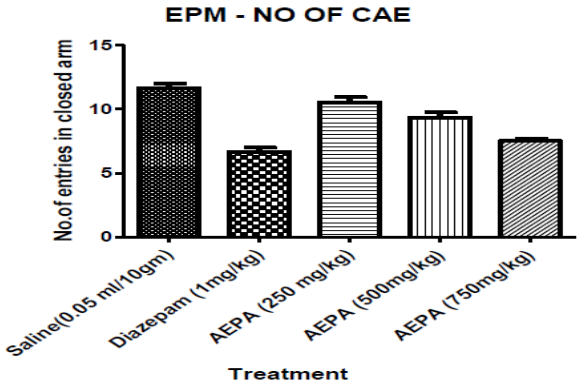

Fig 2: No. of entries in closed arm in EPM

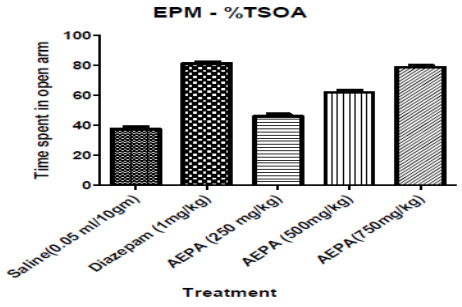

Fig 3: Time spent in open arm in EPM

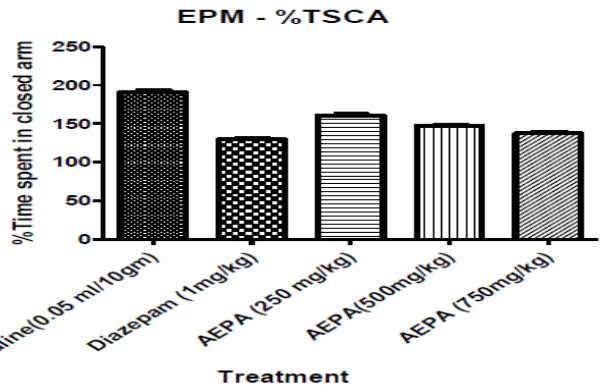

Fig 4: Time spent in closed arm in EPM

4.2. Effect of AEPA on Light dark model

In LDT, (Table No. 2) animals treated with three doses of AEPA (250, 500 and 750 mg/kg) & diazepam showed reduced time spent but increase in number of entries in dark chamber and with concomitant increase in time & number of entries in light chamber when compared with controls . Animals treated with high dose and moderate (500 and 750 mg/kg) shows more significant results when compared with low dose (250 mg/kg).

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	7.666 ± 0.2108	0.4 ± 0.3073	4.5 ± 0.2236	1.333 ± 0.2108
II	Diazepam	1	4 .0± 0.2582***	1.8 ± 0.3073*	13.0 ± 0.2582***	5.5 ± 0.3416***
III	AEPA	250	6.833± 0.3073	0.6 ± 0.4014	7.333± 0.2108***	1.5 ± 0.2236
IV	AEPA	500	5.333 ± .2108***	1.2 ± 0.3073	8.5 ± 0.2236***	2.6 ± 0.2108**
V	AEPA	750	3.50± 0.2236***	1.8 ±0.4216**	12 ± 0.2582***	4 ± 0.2582***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.2. Effect of AEPA on Light Dark transition model

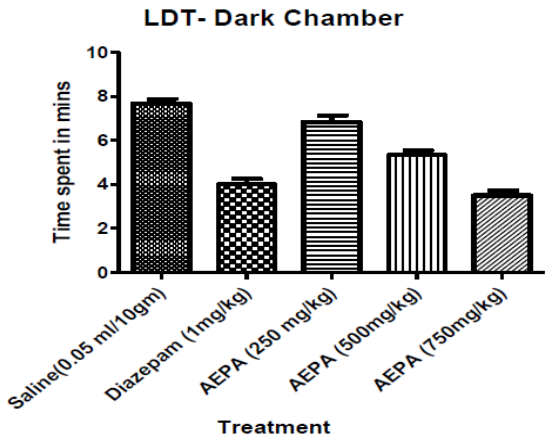

Fig 5: Time spent in dark chamber LDT

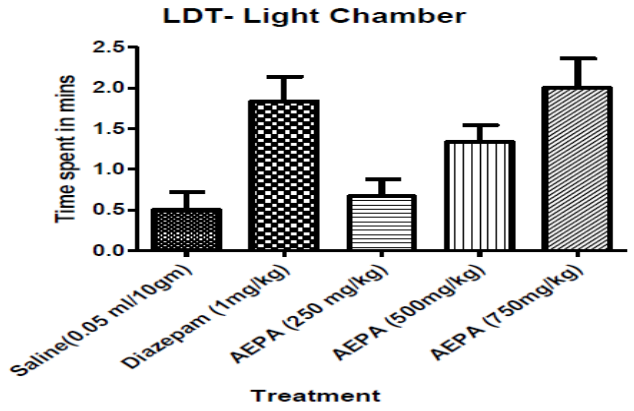

Fig 6: Time spent in light chamber LDT

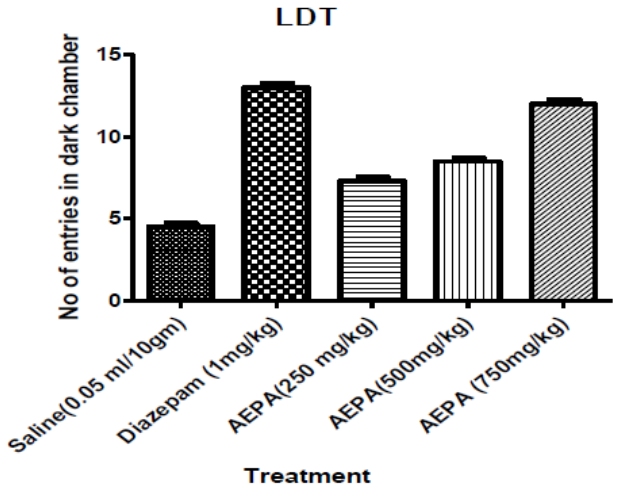

Fig 7: number of entries in dark chamber LDT

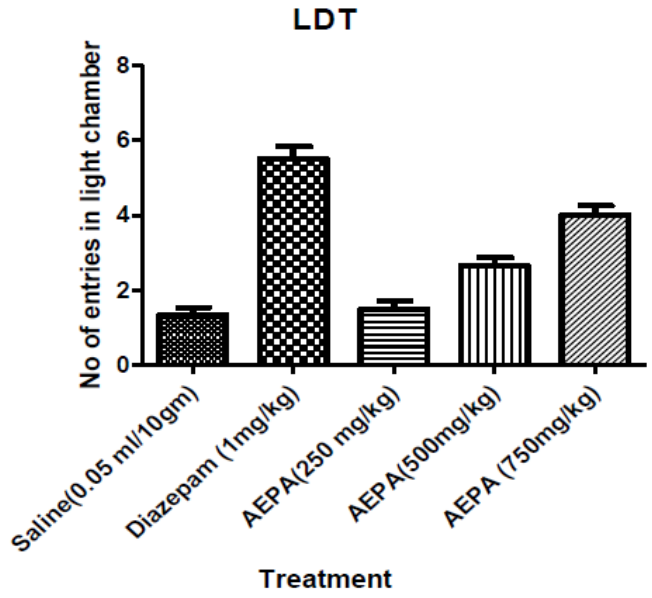

Fig 8: number of entries in light chamber LDT

4.3. Effect of AEPA on Hole board test

It has been shown that head-dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head dipping behavior. In HBT, (Table No.3) animals treated with three doses of AEPA (250, 500 and 750 mg/kg) showed significant increase in number of head-dip and decrease locomotion (Line crossing) which was significant when compared with control. Similarly, animals treated with diazepam (1 mg/kg), as expected, showed a significant increase in number of head-dip counts and Line crossing. Animals treated with high dose and moderate dose (500 and 750 mg/kg) shows more significant results when compared with low dose (250 mg/kg).

Group No.	Drug Treatment	Dose (mg/kg)	No.of head dipping	Line crossing
I	Control	0.05 ml/10 g	22.333 ± 0.4216	65.166 ± 1.447
II	Standard	1	652.5± 0.991***	41.333± 0.08198***
III	AEPA	250	32.166 ± 1.078***	61.333± 1.054
IV	AEPA	500	39.5± 0.8851***	53.833± 1.014***
V	AEPA	750	48.166 ± 1.014***	48.833± 0.8333***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.3. Effect of AEPA on Hole Board test in mice.

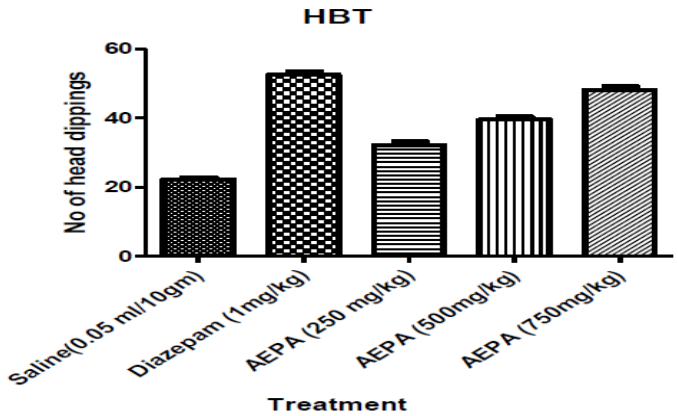

Fig 9: No. of head dip in HBT

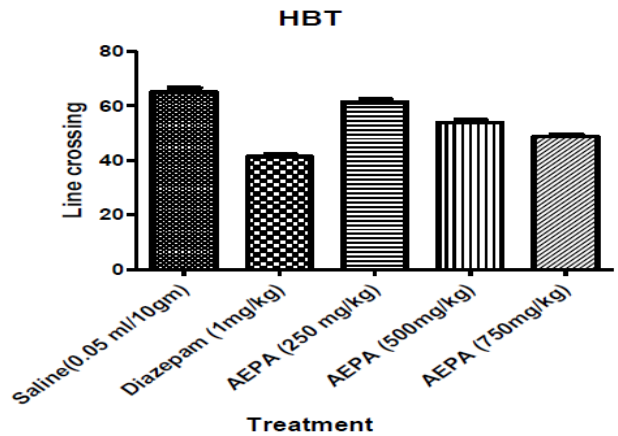

Fig 9: Line crossing in HBT

5. DISCUSSION

Anxious reaction is an adaptive reaction of an individual when confronted with danger or threat. Behavioral and physiological responses accompanying anxiety prepare an individual to react appropriately to such situation. One of the most widely used animal models for screening putative anxiolytic is the elevated plus-maze[14]. 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 [15]. In agreement with previously published reports, diazepam increased the percentage time spent on open arms and the number of entries on open arms [16]. Total number of open arm entries and number of closed arm entries are usually employed as measures of general activity. In the present study it is noted that administration of AEPA prolonged the time spent in the open arms and the number of entries into open arms.

The light/dark box is also widely used for rodents as a model for screening anxiolytic or

anxiogenic drugs, based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, a novel environment and light [17]. It has been reported that simply the measurement of the time spent in the light area, but not the number of transfers, is the most consistent and useful parameter for assessing an anxiolytic action [18]. The present study showed that AEPA could increase the time in the light area, suggesting again that AEPA possesses anxiolytic properties.

The hole-board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and/or responses to stress in animals [19]. It has been shown that head-dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in headdipping behavior[20]. In the present study AEPA increased head-dip counts and head-dip duration without changing locomotion. These results indicate that AEPA has a significant anxiolytic effect in this paradigm.

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Conflict of Interest: None Declared

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