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EVALUATION OF ANXIOLYTIC ACTIVITY OF HYDROALCOHOLIC ACTIVITY OF *TEPHROSIA PURPURIA* (L) PERS ON SWISS ALBINO MICE

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ABSTRACT

The present work is to evaluate the anxiolytic activity of a hydroalcoholic extract of *Tephrosia purpuria* (L) Pers (HAETP) in mice using the elevated plus-maze (EPM), elevated zero-maze (EZM), Y-maze and hole-board models. Furthermore, the anxiolytic effects of HAETP were compared to a known active anxiolytic drug diazepam. The extract, administered orally, in two different doses of HAETP 200 mg/kg and 400 mg/kg, was able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze and elevated zero-maze, as well as decrease the visits by mice in the Y-maze, also significantly increase nose poking, line crossing and rearing in hole-board. This effect was comparable to that of the diazepam (2.0 mg/kg i.p.). These results indicate that hydroalcoholic extract *Tephrosia purpuria* (L) Pers is an effective anxiolytic agent.

INTRODUCTION: Anxiety disorders are among the most common mental disorders besides depressive disorders with approximately one eighth of the world population affected at some point in their life [1]. Major drug classes for the treatment of anxiety disorders are Benzodiazepines (BZDs), Selective serotonin-reuptake inhibitors (SSRIs), Tricyclic antidepressant, β-blockers, and Azapirones [2].

All this drug classes currently used are associated with side effects that very occurrence and severity. Like BZDs produce undesirable effects such as drowsiness, ataxia, and sedation, muscle relaxation, insomnia, hepatotoxicity and in addition. They adversely interact with other CNS depressants, particularly alcohol [3, 4]. For this reason; many researchers have been evaluating new compounds from herbs with fewer undesirable effects. It has been suggested that various traditional herbal medicines also possess anxiolytic activity such as *Bacopa monnieri* Linn, Ginseng, *Ginkgo biloba* Linn, *Piper methysticum* forst, and *Salvia officinalis* Linn. Research has also focused on the development of drugs with fewer side effects, such as sedation, muscle relaxation, and drug dependence.

Tephrosia purpurea (L) Pers. Belongs to family papilionaceae. Popularly known as “Sarapunkha” in Sanskrit, “Wild indigo” in English and “Kolinji” in Tamil. *Tephrosia purpurea* (L) Pers. has been used for centuries in the Indian traditional medicine. It possesses various pharmacological activities like anti-diabetic [6], anti-epileptic [7], anti-carcinogenic [5, 8], anti-microbial [9], anti-biotic [10], anti- inflammatory [11], analgesic [11], anti-ulcer [12], anti- hyperlipidemic [13], immunomodulatory [14], hepatoprotective [15], and wound healing [16].

A survey of literature on *Tephrosia purpuria* (L) Pers. has revealed only a few pharmacological reports of the plant. No major investigated reports were found for its CNS activity. Therefore, we undertook the present study to determine the anxiolytic activity of whole plant of *Tephrosia purpuria* (L) Pers. by using different animal models for anxiety.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The plant of *Tephrosia purpuria* (L) Pers. whole plant was collected from Namakkal district at the month of July 2010 and identified by Dr.Sasikala Ethirajulu, M.sc,Ph.D; Asst.Director, Pharmacognosy department, Siddha central research institute, Arumbakkam, Chennai and a voucher specimen was deposited at C. L. Baid Metha College of Pharmacy for future reference. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

Preparation of extract of *Tephrosia purpuria* (L) Pers: The whole plant was cut into pieces and shade dried at room temperature. The dried plant were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into soxhlet apparatus and extracted with aqueous methanol in ratio 6:4, at a temperature range of 60-70˚C. The extracts were dried at 45^{o}C in hot air oven till liquid to semisolid mass was obtained and was stored in airtight containers in refrigerator below 10^{o}C. These extracts was suspended in distilled water and used for further studies.

Phytochemical Screening [17, 18]: The extract was subjected to preliminary phytochemical screening by the methods previously described by Kokate and Jayaraman J.

Drugs and Chemicals: Diazepam (Ranbaxy Laboratories Ltd., Mumbai) were used as the standard anxiolytic drugs. Methanol was purchased locally and was of analytical grade. Distilled water was used as vehicle.

Preparation of Test Doses: The extracts were suspended in the vehicle in such concentrations as to administer 200 and 400 mg/kg doses to mice through per oral route.

Animals: Inbred adult albino mice (20-25 gm) of either sex were obtained from the animal house of C. L. Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited, Bangalore) 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 (IAEC/XXX/11/CLBMCP/2010 dated 22.09.2010)**.**

Acute Toxicity Study [19, 20]**:** The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioral, Neurological toxicity and mortality 14 days.

Elevated plus Maze Model [21, 22]**:** The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof. The HAETP (200 and 400 mg/kg) and vehicle were administered for 7 days once daily p.o. and the last dose was given on the 7th day, 60 min prior to experiment. The standard drug Diazepam was given at a dose of 2 mg/kg i.p. 60 min before starting the experiment. After proper treatment each mouse was placed at the center of the maze with its head facing the open arm.

During the 5 min experiment, the behavior of the mouse was recorded as: the number of entries into the open or closed arms and time spent by the mouse in each of the arms. An arm entry was defined as the entry of all four paws into the arm. After each trial, the elevated plus-maze apparatus was wiped clean with ethanol (10%) solution.

Elevated Zero-Maze Model [23, 24]**:** All the animals were analyzed for anxiety levels by using elevated zero maze. This test is a pharmacologically validated assay of anxiety in animal models that is based on the natural aversion of mice to elevated zero maze. It is composed of a 6 cm wide ring with outer diameter of 45 cm containing 4 equal quadrants of alternating walled (closed) or unwalled (open) sections. The entire ring is elevated to the height of 40 cm. Mice were treated with the HAETP (200 and 400 mg/kg p.o.) and vehicle for 7 days once daily p.o. and the last dose was given on the 7th day, 60 min before starting the experiment. The standard drug Diazepam was given at a dose of 2 mg/kg i.p. 60 min before starting the experiment.

After proper treatment each mice were placed at the walled region of the maze with its head facing the open arm. During the 5 min experiment, the behavior of the mice was recorded as time spent in open and closed arm and total number of entries in open and closed arm. An arm entry was defined as the entry of all four paws into the arm. After each trial, the elevated zero-maze apparatus was wiped clean with ethanol (10%) solution.

Y-Maze Model [25]**:** Y-maze is made of black painted wood or grey plastic. Each arm is 40 cm long, 13 cm high 3 cm white at the bottom, 10 cm wide at the top and converges at an equal angle. Mice were treated with the HAETP (200 and 400 mg/kg p.o.) and vehicle for 7 days once daily p.o. and the last dose was given on the 7th day, 60 min before starting the experiment. The standard drug Diazepam was given at a dose of 2 mg/kg i.p. 60 min before starting the experiment. For a period of 5 min. the total numbers of visits to different arm were measured. After each trial, the Y-maze apparatus was wiped clean with ethanol (10%) solution.

Hole-board model [21]**:** The hole-board apparatus consists of Perspex box (60×60×35cm) with four equidistant holes 2 cm diameter in the floor. The floor of the box was positioned 12 cm above the ground and divided in to nine (20×20 cm) squares. Mice were treated with the HAETP (200 and 400 mg/kg p.o.) and

vehicle for 7 days p.o. once daily the last dose was given on the 7th day, 60 min before starting the experiment. The standard drug Diazepam was given at a dose of 2mg/kg i.p. 60 min before starting the experiment. The numbers of line crossing, numbers of head dipping and rearing were calculating for 5 min. After each trial, the Hole-board apparatus was wiped clean with ethanol (10%) solution.

Statistical Analysis: The data were expressed as mean±standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett's't'- test, p values less than 0.05 were considered as significance.

RESULTS:

Phytochemical Screening: The preliminary phytochemical analysis of HAETP showed that the plant contains carbohydrates, flavonoids, flavanones, glycosides, saponins, sterols, proteins, tannins, phenols but alkaloids and steroids are absent.

Acute toxicity Study: Acute oral toxicity studies revealed the nontoxic nature of HAETP. There was no morbidity observed or any profound toxic reactions found at a dose of 2000 mg/Kg p.o. which indirectly pronouns the safety profile of the plant extract.

Elevated plus Maze Model: The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard Diazepam (**table 1, fig. 1a-b**).

TABLE 1: EFFECT OF HAETP ON MICE IN EPM MODEL

Groups	Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	No. of entries in open arm	No. of entries in enclosed arm
I	Vehicle	36.33±5.61	200.2±8.82	5.83±1.01	16.67± 1.70
II	Diazepam 2mg/kg	151.3±7.53***	104.5±11.27***	12.83±1.1***	6.83±1.01***
III	HAETP 200mg/kg	125.2±12.4***	148.8±12.04***	10.00±1.15*	11.17±1.53*
IV	HAETP 400 mg/kg	179.2±8.23***	94.33±5.07***	11.00±1.06**	7.83±1.22***

n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's't' test)

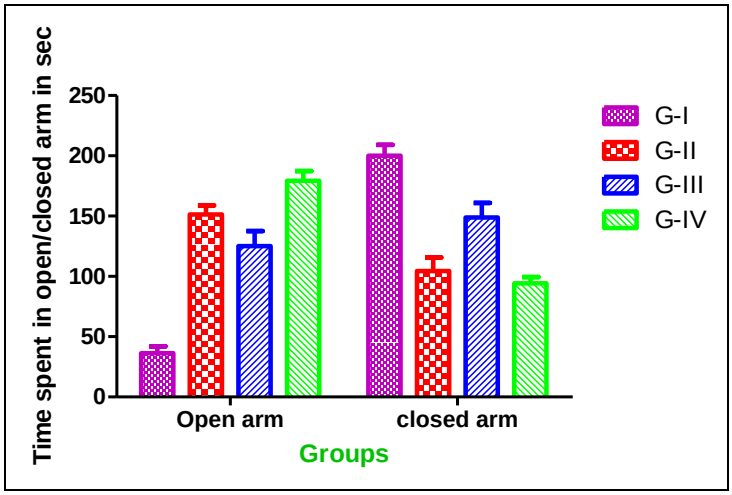

FIG. 1a: EFFECT OF HAETP IN TIME SPENT IN OPEN/CLOSED ARM IN EPM MODEL

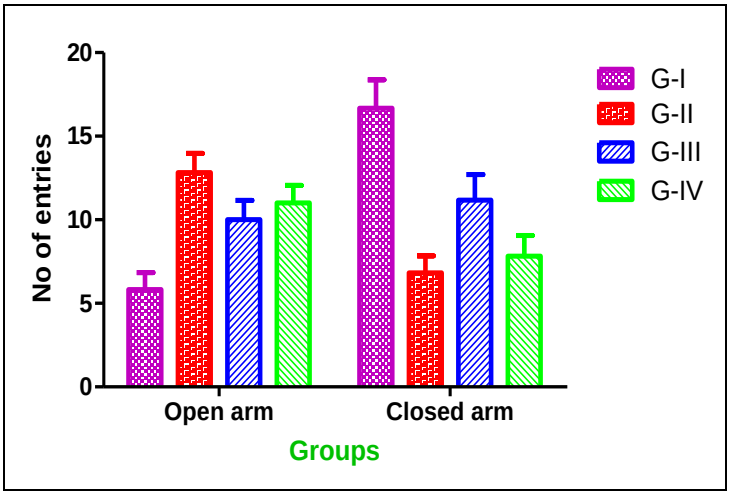

FIG. 1b: EFFECT OF HAETP IN NO OF ENTRIES IN OPEN/CLOSED ARM IN EPM MODEL

Elevated Zero Maze Model: The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard Diazepam (**table 2, fig. 2a-b**).

TABLE 2: EFFECT OF HAETP ON MICE IN EZM MODEL

Groups	Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	No. of entries in open arm	No. of entries in enclosed arm
I	Vehicle	40.50±11.89	234.5±12.61	1.33±0.61	2.33±0.61
II	Diazepam 2 mg/kg	115.5±6.84***	169.7±5.65***	8.00±1.06***	9.00±1.06***
III	HAETP 200 mg/kg	88.00±10.18**	180.7±10.18**	6.50±1.38**	7.50±1.38*
IV	HAETP 400 mg/kg	112.8±8.68***	166.8±7.55***	7.33±1.05***	8.33±1.05**

n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)

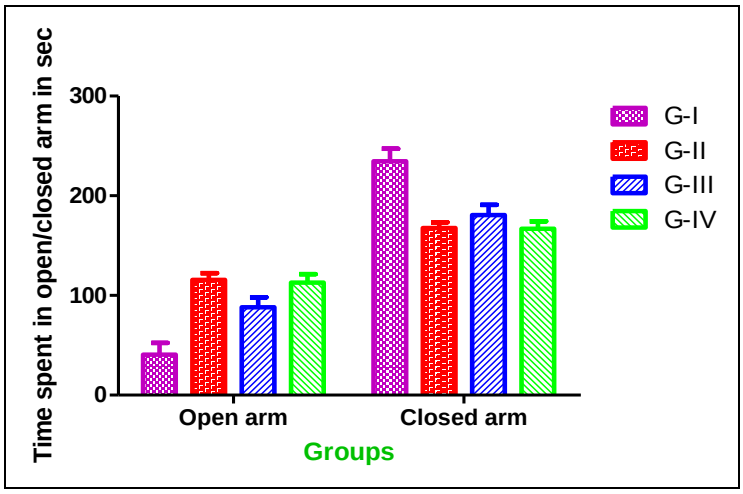

FIG. 2a: EFFECT OF HAETP IN TIME SPENT IN OPEN/CLOSED ARM IN EZM MODEL

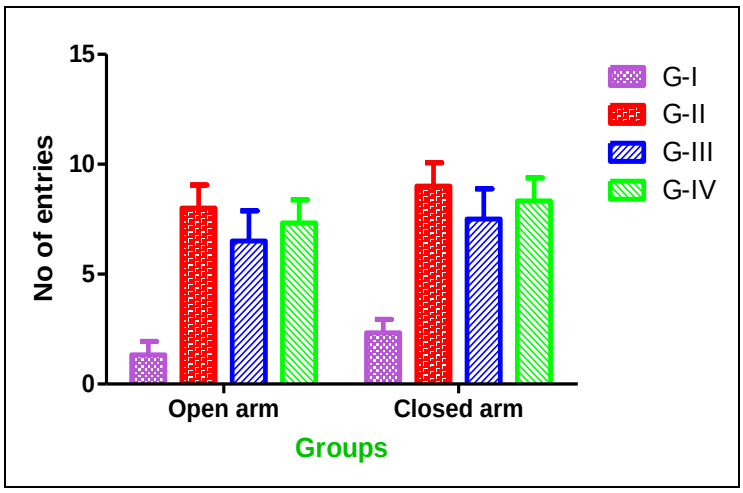

FIG. 2b: EFFECT OF HAETP IN NO OF ENTRIES IN OPEN/CLOSED ARM IN EZM MODEL

Y-Maze Model: A significant decrease in the number of visits in the three arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. Both the doses of HAETP showed a significant decrease in the number of visits in the three arms of the Y-maze which was comparable with the standard Diazepam (**table 3, fig. 3**).

TABLE 3: EFFECT OF HAETP ON MICE IN Y-MAZE MODEL

Groups	Treatment	Number of visits
I	Vehicle	44.17 ± 3.28
II	Diazepam 2mg/kg	26.83 ± 2.07***
III	HAETP 200 mg/kg	34.17 ± 2.92*
IV	HAETP 400 mg/kg	16.83 ± 2.60***

n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's't' test)

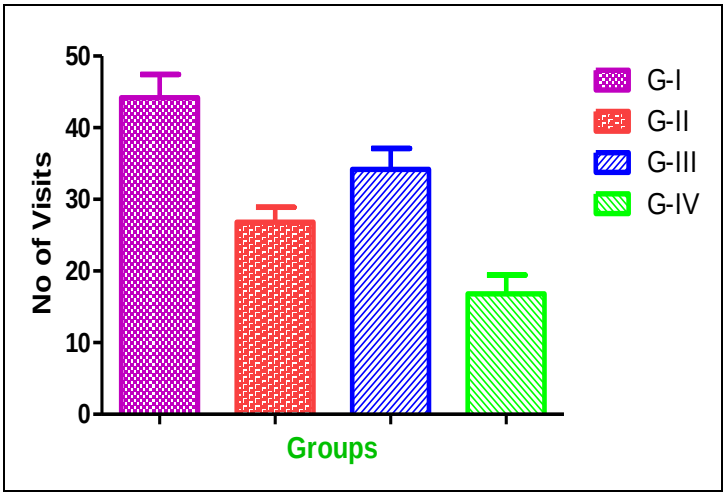

FIG. 3: EFFECT OF HAETP ON MICE IN Y-MAZE MODEL

Hole-Board Model: The number of line crossing, nose pocking and rearing was increased significantly in case of Diazepam treated animals as compared to the control animals. The HAETP at both dose levels showed an increase in the number of line crossing and nose pocking significantly as compared to the control animals (**table 4, fig. 4**).

TABLE 4: EFFECT OF HAETP ON MICE IN HOLE-BOARD MODEL

Groups	Treatment	No. of line Crossing	No. of nose pocking	No. of rearing
I	Vehicle	44.83 ± 5.71	15.33±1.70	12.83±1.10
II	Diazepam 2mg/kg	102.5±10.18***	31.67±2.29***	22.17±1.32***
III	HAETP 200mg/kg	73.00±2.60*	26.33±3.25*	18.50±1.33*
IV	HAETP 400mg/kg	89.33±6.71**	31.00±2.28***	20.17±2.15**

n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's't' test)

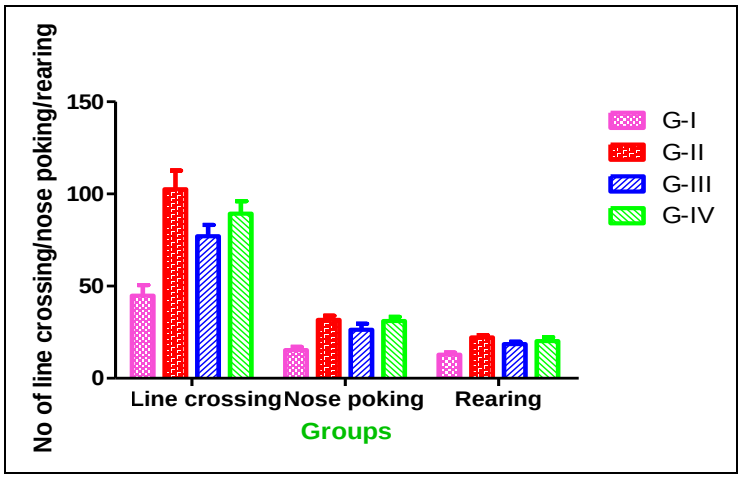

FIG. 4: EFFECT OF HAETP ON MICE IN HOLE-BOARD MODEL

DISCUSSION: Anxiety, a symptom accompanying various central nervous system disorders and a disorder by itself, is characterized in humans by a tense and physically exhaustive alertness[28]. 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. Major drug classes for the treatment of anxiety disorders are Benzodiazepines (BZDs), Selective serotonin-reuptake inhibitors (SSRIs), Tricyclic antidepressant, β-blockers, and Azapirones[2]. All this drug classes currently used are associated with side effects that very occurrence and severity. Like BZDs produce undesirable effects such as drowsiness, ataxia, and sedation, muscle relaxation, insomnia, hepatotoxicity and in addition. They adversely interact with other CNS depressants, particularly alcohol [3, 4].

Despite the widespread traditional use of *Tephrosia purpuria* (L) Pers for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrated that the hydroalcoholic extract of *Tephrosia purpuria* (L) Pers had anxiolytic activity in mice in several animal models of anxiety like by Elevated plus-maze, Elevated zero-maze, Y-maze and Hole board models.

EPM is currently the first choice test for anxiolytic drugs and has been validated for mice. In this study, we observed that HAETP (200 and 400mg//kg) induced significant increase in the both number of entries and time spent in the open arms, compared to the closed arm. In the Elevated Zero-maze model, we observed that HAETP (200 and 400mg//kg) induced significant increase in the both number of entries and time spent in the open arms, compared to the closed arm.

The results obtained in the Y-maze model showed that the number of visits in the three arms decreased significantly for all groups when compared to the control animals, which supports the anxiolytic activity of HAETP. In the Hole-board model a significant increase in the exploratory line crossing, nose pocking and rearing behavior were observed after treatment with 200 and 400 mg/kg of HAETP, thus reinforcing the hypothesis that it has anxiolytic activity. Earlier reports on the chemical constituents of the plants and their pharmacology suggest that Plants containing flavonoids, saponins and tannins possess activity against many CNS disorders [26].

Phytochemical tests of HAETP revealed the presence of saponins and flavonoids. It may possible that the mechanism of anxiolytic action of HAETP could be due to the binding of any of these phytochemicals to the $GABA_A$-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the $GABA_A$ receptor [27].

The plant *Tephrosia purpuria* (L) Pers also contains flavones which may responsible for its anxiolytic activity. So the anxiolytic activity of HAETP might involve an action on GABAergic transmission or effects on serotonergic transmission or due to its mixed aminergic potentiating effect.

CONCLUSION: From the above observations we can conclude that hydroalcoholic extract of *Tephrosia purpuria* (L) Pers possesses anxiolytic activity at (200 and 400 mg/kg) both the dose level which is comparable with the standards. However, it requires further studies to elucidate the possible mechanism involved and its use in human beings.

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