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Research Article

EVALUATION OF ANXIOLYTIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *HYBANTHUS ENNEASPERMUS* LINN. IN SWISS ALBINO MICE

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ABSTRACT

Hydro alcoholic Extract of dried whole plant of *Hybanthus ennespermus* Linn, (HAEHE) was studied for anxiolytic behavior in swiss albino mice. The Elevated plus maze, Elevated T-maze, Vogel conflict test and Isolation induced aggression models were used for the assessment of Anxiolytic activity. The efficacy of the extract (200 and 400 mg/kg) was compared with the standard anxiolytic drugs Diazepam (2mg/kg) and Fluoxetine (10mg/kg). The result showed that the extract significantly increased the number of entries and time spent in the open arm in the Elevated plus maze, increased baseline latency and decreased avoidance and escape in the Elevated T-maze, decreased number of shocks licks in the Vogel conflict test, fighting and biting in the Isolation induced aggression models. These results suggest that the HAEHE plant has anxiolytic effect.

Keywords: Anxiolytic, *Hybanthus enneaspermus* Linn, Elevated Plus Maze, Elevated T-Maze, Vogel conflict, Isolation induced aggression.

INTRODUCTION

According to the World Health report, approximately 450 million people suffer from a mental or behavioural disorder, yet only a small minority of them receives even the most basic treatment. These amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020. In the search for new therapeutic products for the treatment of neurological for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a different plant species in a variety of animal models. Anxiety a state of excessive fear is characterized by motor tension, sympathetic hyperactivity, and apprehension and vigilance syndromes. Anxiety may interfere with intelligence, psychomotor function and memory. The benzodiazepines are considered the drug of choice in the treatment of anxiety. Unfortunately, there are several side effects. The complexity of daily life in modern society frequently leads to varying degree of anxiety. Anxiety disorders have been found to be associated with severe adverse effect among medical patients in both developed and developing countries. These considerations implicate the search for newer anxiolytic agents that have a fast onset of action with less side effects and a wides safety margin. It has lead scientists to investigate plants. Various plants are being used in complementary and alternative medicines for management of anxiety[1].

Hence in the present study, HAEHE Linn were evaluated for the potential anxiolytic effect using Elevated plus Maze, Elevated T-Maze, Vogel conflict test, Isolation induced aggression model and to compare the effect with Diazepam or Fluoxetine.

The plant *Hybanthus ennespermus* Linn (Violaceae) is commonly known as spade flower. It is a small tropical plant that is available in all over world[2]. The plant is reported to possess tonic, diuretic and demulcent properties. The root is diuretic and administered as an infusion in gonorrhoea and urinary affections. Sandals employ the root in bowel complaints of children. The leaves and tender stalks are demulcent and used as a decoction; mixed with oil, they are employed in preparing a cooling liniment for the headache[3]. An infusion of the plant is given in case of cholera[4].

Hybanthus ennespermus Linn are widely used in traditional medicine as aphrodisiac and blood tonic. Pharmacological studies that have been carried out on *Hybanthus ennespermuss* Linn are hypoglycemic[5], anti-arthritic[6], anti-microbial[7], anti-bacterial[8] and anti plasmodialactivity[9]. But no scientific work has been done on its anxiolytic activity. In the present study, an effort has been made to establish the scientific validity to the anxiolytic property of this plant.

MATERIALS AND METHODS

Collection and Authentication of plant material

The plant of *Hybanthus ennespermus* Linn is whole plant was collected from Chennai 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, Chennai.

Preparation of Hydroalcoholic Extract of whole plant of *Hybanthus enneaspermus* Linn

The plant were 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 was dried at 45 ^{0}C in hot air oven till solid to semisolid mass was obtained and was stored in airtight containers in refrigerator below 10 ^{0}C. These extracts was suspended in distilled water and used for further studies.

Animals

Female Swiss albino mice weighing 20-25 g were used. They were housed in polypropylene cages in standard laboratory condition of temperature 25+ 2^{0}C with a 12h: 12h light: dark cycle. They were fed standard pellet diet and water ad libitum. Animals were acclimatized for seven days. The animals were divided into four groups of six mice each. Group I served as a control and received equivalent amount of normal saline. Group II was administered of Diazepam (ampoules 5mg/ml, Calmpose, Ranboxy, diluted in normal saline before use) or Fluoxetine (Prodep, Sun Pharma, diluted in normal saline use) in a dose of 10mg/ kg body weight orally. Group III received HAEHE in a dose of 200 mg/kg. Group IV were given same extracts in a dose of 400 mg/kg. All the extracts and control and standard were given orally by oral needle.

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MATERIALS AND METHODS

Elevated plus maze[10, 11]

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 HAEHE (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. 30 min before starting the experiment. After proper treatment each mouse was placed at the centre 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.

Elevated T-Maze Model[12, 13]

The elevated T-maze was made of wood and had three arms, one arm open arm and two closed arms of equal dimensions (50×10 cm). One arm, enclosed by 40 cm high walls was perpendicular to two opposed arms. The whole apparatus was elevated 50 cm above the floor. The mice trained by without walls in the T-arm for two days before starting experiment. Each mouse was placed at the T-arm with its head facing the three arms after giving the drug. The HAEHE (200 and400 mg/kg) and vehicle were administration for 7 days once daily p.o and the last dose was given on the 7th day, 60min prior to experiment. The standard drug was given at a dose of 10mg/kg p.o 60 min before starting the experiment. During the 5 min experiment, the behaviour of the mouse was recorded as the time of baseline latency, escape and avoidance the closed arm after opening. Mice were allowed to socialize.

Vogel conflict Test[14]

Mice were water deprived for 24 hr, two hours before testing each mouse was placed in a polypropylene cage (25×25×20 cm) and allowed to locate the metallic drinking spout of the water bottle, the whole exercise taking usually less than 2 min. Mice were treated with the HAEHE (200 and 400 mg/kg p.o) and vehicle were administered for 7 days once daily p.o. and the last dose was given on the 7th day 60min before starting the experiment. The standard drug Fluoxetine was given at a dose of 10mg/kg p.o. 60 min before starting the experiment and mice were placed in the cage for a period of 3 min. electric shock (0.45mA, 2 sec) was given through the spout on each contact of the snout of the mice with the metallic spout. The total number of no of shocks and licks was recorded during the 9 min observation period.

Isolation Induced aggression model[15]

Each mouse was isolated in cages of 28 cm×20 cm×16 cm for 6 weeks. Isolated mice were pre-screened for aggressive behavior prior to the experiment. An introducer mouse was introduced into the isolated mouse's cage for 3 min, and the isolated mice exhibiting bite marks for more than 20s were used for the test experiments on the following day. Two isolated mice that were pre-treated with drugs were placed in a neutral cage, which was the same size as their home cages as previously reported. Mice were treated with the HAEHE (200 and 400 mg/kg p.o) and vehicle was administered for 7 days once daily p.o. and the last dose was given on the 7th day 60min before starting the experiment. The standard drug Fluoxetine was given at a dose of 10mg/kg p.o. 60 min before starting the experiment and mice were placed in the cage for a period of 3 min. An assessment of the aggressive behavior (fighting and biting) of two isolated mice was conducted for 3 min as the attack duration as previously reported after minor modification.

Statistical analysis

The data obtained from each response were subjected to one-way analysis of variance (ANOVA) and inter group comparisons were made by Dunnett test. The data are expressed as + SEM for each treatment group. Statistical significance was considered at less than $p< 0.05$.

RESULTS

The effect of HAEHE was determined at two different doses 200 mg/kg and 400 mg/kg, Compared with Control and standard drug.

Effect of HAEHE in Elevated plus maze Model

HAEHE produced significant anxiolytic activity in swiss albino mice. This extract in doses of 200 and 400mg/kg increased the time spent and number of entries in open arm as compared to the control animals. This effect of HAEHE is fairly comparable with the standard Diazepam (2mg/kg) in mice. (Table.1) (Fig.1and 2)

Table 1: Effect of HAEHE in the elevated plus maze using swiss albino mice

Group	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	38.33±3.333	171.7±10.54	3.1±0.3651	7.667±0.3333
II	Diazepam 2mg/kg/ i.p	195±3.651***	48.17±10.99***	6.50±0.4282***	3.50±0..4282***
III	HAEHE 200 mg/kg/p.o	68. 33 ±9.456**	102.8±20.94*	4.667 ±0.2108**	5.1±0.5774*
IV	HAEHE 400 mg/kg/p.o	164.2±6.509***	74.17±19.93**	7.1±0.3651***	3.833±1.078**

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

Fig.2: Effect of HAEHE in number of open/closed arm entries in EPM Model

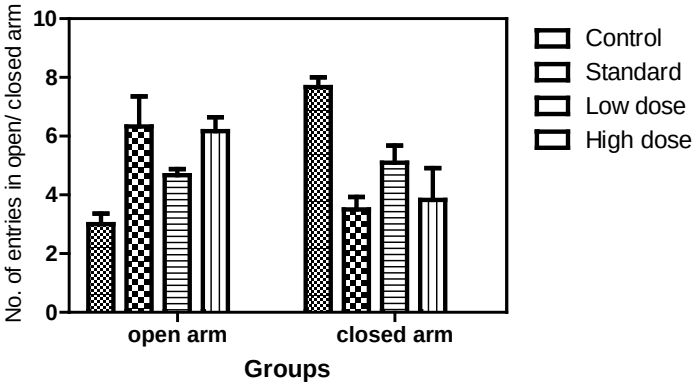

Fig. 2: Effect of HAEHE in number of open/closed arm entries in EPM Model

Effect of HAEHE in Elevated T-Maze Model

A significant increase in the baseline latency and decrease in the avoidance and escape was observed for Diazepam (2mg/kg) when compared to the control animals. HAEHE in doses 200 and 400mg/kg showed significant when compared to the control animals. (Table.2) (Fig.3)

Table 2: Effect of HAEHE in the elevated T- maze using swiss albino mice

Group	Treatment	Baseline Latency	Avoidance	Escape
I	Vehicle	2.833± 0.3073	8.333± 0.3333	7.667± 0.3333
II	Diazepam 2mg/kg/ i.p	5.833±0.6009***	3.000± 0.4472***	2.500±0.2236***
III	HAEHE 200 mg/kg/p.o	4.667± 0.3333*	7.000± 0.3651*	7.333±0.2108^{NS}
IV	HAEHE 400 mg/kg/p.o	5.167± 0.3073**	5.833± 0.3073***	5.333± 0.7601**

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

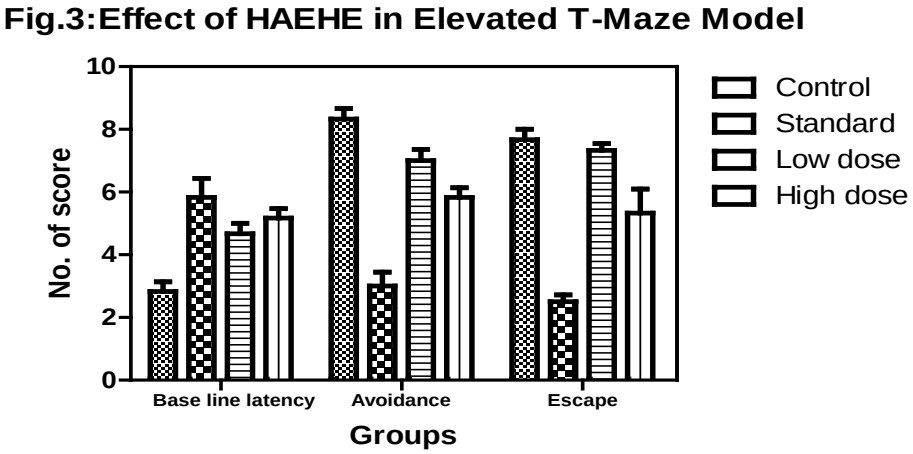

Fig. 3: Effect of HAEHE in Elevated T-Maze Model

Effect of HAEHE in Vogel conflict test

The HAEHE produced significant anxiolytic activity in mice. Both the doses of 200 and 400mg/kg increase in the number of shocks and licks were compared to control animals. The effect of HAEHE comparable with the standard Diazepam (2mg/kg) in mice. (Table.3) (Fig.4)

Table 3: Effect of HAEHE in Vogel conflict test using swiss albino mice

Group	Treatment	No. of shocks for 9 mins	No. of licks for 9 mins
I	Vehicle	5.333± 0.9189	19.00 ±1.155
II	Fluoxetine 10mg/kg	14.67 ±0.9189***	33.33± 1.994***
III	HAEHE 200 mg/kg	9.500 ±1.057*	25.17± 1.014*
IV	HAEHE 400 mg/kg	11.67± 1.256**	27.83 ±1.797**

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

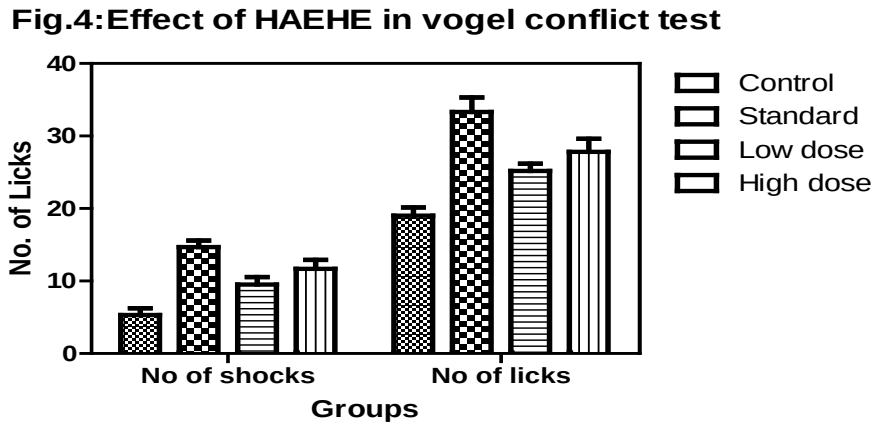

Fig. 4: Effect of HAEHE in vogel conflict test

Effect of HAEHE in Isolation induced aggression Model

A significant decrease in the number of aggressive score like fighting and biting was recorded for the standard Fluoxetine (10mg/kg) as compared to the control animals. Significant decreases in the aggressive score were also recorded for both the doses of 200 and 400mg/kg HAEHE as compared to the control animals. (Table.4) (Fig.5)

Table 4: Effect of HAEHE in Isolation induced aggression Model using swiss albino mice

Group	Treatment	Fighting	Biting
I	Vehicle	17.33 ±1.994	8.50±1.978
II	Fluoxetine 10mg/kg	4.333 ±0.4944***	2.167±0.3073**
III	HAEHE 200 mg/kg	10.83±1.621*	6.333 ±1.542^{NS}
IV	HAEHE 400 mg/kg	8.167±1.922**	4.333 ±0.8433*

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

Fig.5: Effect of HAEHE in Isolation induced aggresion Model

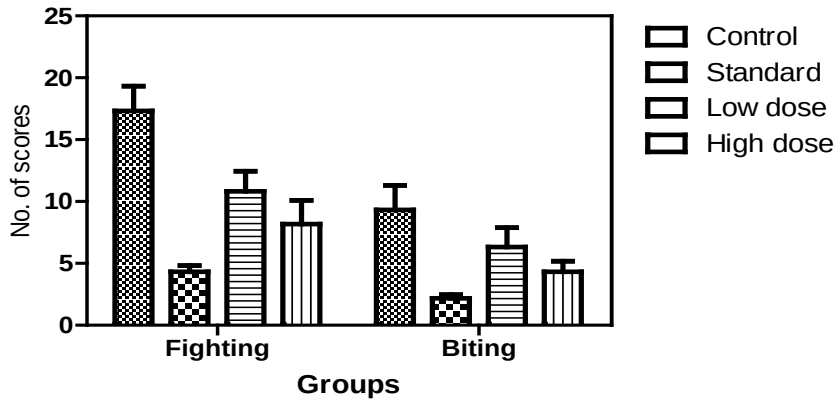

Fig. 5: Effect of HAEHE in Isolation aggresion Model

DISCUSSION

BZDs are the major class of anxiolytics. The other drugs which are also used to treat anxiety include SSRIs, SNRIs, and Azapirones. All these drugs have some unwanted side effects due to which search for newer anxiolytic with fewer side effects is going on.

Therefore, there is a clear need for new animal models that are sensitive to non-BZD anxiolytics and/or that address specific types of anxiety disorders, including those resistant to BZDs.

Several different behavioral paradigms have been suggested to reflect pharmacologically sensitive anxiety levels in animals. These include a variety of tests related to exploratory activity, social interaction behaviors, unlearned fear and punishment responses and. To the extent that such tests have led to the identification of clinically useful anxiolytics, they truly reflect, at least in part, some measure of relative anxiety state[16]. The present work demonstrated that the HAEHE had anxiolytic activity in mice in several animal models like EPM, Elevated T-maze, Vogel conflict test, and Isolation induced aggression behavior models.

The EPM model is principally based on the observations that exposure of mice to an elevated and open maze results in approach-avoidance conflict, which is manifested as an exploratory-cum-fear drive. The fear due to height (acrophobia) induces anxiety in mice when placed on the apparatus. The ultimate manifestation of anxiety and fear then is exhibited by decrease in motor activity, which is measured by the time spent by mice in the open arms. This EPM model is considered one of the most widely validated tests for assaying anxiolytic substances such as the benzodiazepines[17]. In Elevated plus maze study, we observed that HAEHE (200 and 400 mg/kg) induced significant increases in the both number of entries and time spent in the open arms compared to the closed arm entries.

In the Elevated T-maze apparently generates different types of fear/anxiety, resulting in complex variability of anxiolytic agents[18]. The apparatus was modified by closing the entrance to one of its enclosed arms, resulting in an elevated T-maze. To separate learned from unlearned fear, both inhibitory avoidance of the open arms and one-way escape from one of the open arms were measured[19]. The results obtained in this model showed that baseline latency increased, avoidance and escape are decreased significantly for all groups when compared to the control animals, which supports the anxiolytic activity of HAEHE.

Vogel conflict test is a paradigm based on the conflict between water appetite and punishment. The anxiolytic-like effect of a test drug is indicated by the increase in the number of shocks during a lick-shock paradigm. However, this increase in the number of shocks may be due to enhancement of drinking behavior by an increase in thirst or a decrease in the response to electric shock due to an increase in shock tolerance. Vogel conflict test have been employed for the identification and characterization of anxiolytic agents[20]. Both the dose HAEHE (200 and 400 mg/kg) showed a significant increase in the number of shocks and licks, thus reinforcing the hypothesis that it has anxiolytic activity.

Neurochemical and pharmacological evidences indicates the involvement of serotonergic mechanism in the control of aggressive behaviour in animals[21]. Isolation-induced aggression in mice is an animal models offensive aggression with excellent predictive validity toward human aggression. In this aggression behavior characterized by the initiative of the aggressor and intended damage to the opponent[22, 23].

In the frame work of evolutionary theory, these behaviors are understood to survival of the fittest, to disperse and generally to improve the probability of individual and species survival. Although "aggressive" behavior is associated with certain somatic and psychiatric disease states[24]. The present study showed that both the dose HAEHE (200 and 400 mg/kg) showed reduced aggressive behavior score like the number of fighting and biting in the isolation-induced aggression model.

Earlier reports on the chemical constituents of the plants and their pharmacology of the plants containing Flavanoids, Saponins and tannins possess activity against many CNS disorders[25].

Phytochemical tests of HAEHE reversed the presence of saponin and Flavanoids. It may possible that the mechanism of anxiolytic action of HAEHE could be due to the binding of any of this phytochemical GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the $GABA_A$ receptor[26]. The plant *Hybanthus enneaspermus* Linn also contains flavones which may responsible for its anxiolytic activity.

The HAEHE showed anxiolytic effect which is comparable with the standard Diazepam and Fluoxetine. So the anxiolytic of HAEHE might involve an action on GABAergic or serotonergic effect or due to its mixed aminergic potentiating effect. However further studies are required to know the exact mechanism of action of HAEHE as anxiolytics.

CONCLUSION

The present study, anxiolytic activity was assessed by using elevated plus maze, elevated T-maze, vogel conflict test, isolation induced aggression model in which the extract showed a considerable significant effect. From the above observation it could be predicted that the anxiolytic effect of HAEHE, may be due to its action on GABAergic serotonergic effect. Overall, the present study suggests of HAEHE as anxiolytic.

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