

**Evaluation of anxiolytic activity of ethanolic extracts from the leaves of
Trichosanthes cucumerina L. in mice**

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ABSTRACT

*The present study was undertaken to evaluate the anxiolytic activity of ethanolic extract of leaves of *Trichosanthes cucumerina* L. in mice. The anxiolytic activity was evaluated by Elevated plus maze, Y-maze, Hole-board, Actophotometer, and Marble-burying behavior models. The efficacy of the extract (200 and 400 mg/kg, p. o) was compared with the standard anxiolytic drugs Diazepam (2 mg/kg, p. o) and Fluoxetine(10mg/kg, p. o). The result showed that the extract significantly increased the number of entries and time spent in the open arm in the elevated plus maze. The results also showed that the extract significantly increased the number of head dipping and line crossing, decreased the numbers of visits to the three arms, locomotor score and number of marble-buried in Hole-board, Y-maze, Actophotometer and Marble-Burying Behavior Model respectively. Present study confirms that the extract showed significant anxiolytic activity at both dose levels which is comparable with standard anxiolytics drug Diazepam.*

Keywords: *Trichosanthes cucumerina* linn, Elevated plus Maze, Y-maze, Hole-board, Marble-Burying Behavior Model.

INTRODUCTION

Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat [1]. Anxiety affects one-eighth population worldwide and has become an important research area in the field of psychopharmacology [2]. Benzodiazepines (BZDs), barbiturates, tricyclic antidepressants (TCA's) have been used for long time to treat anxiety disorders. The serious side effects associated with these drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (BZD's, barbiturates and alcohol), sexual dysfunction, anticholinergic, antihistaminic effects (TCA's) have limited their use in patients [3]. Due to this many pharmaceutical companies are conducting studies to find an alternative medicine or plant-derived medications with more specific anxiolytic effects [4].

Trichosanthes cucumerina L. Var. *Cucumerina* Belongs to the family Cucurbitaceae and is distributed throughout India, Bangladesh, Sri Lanka, Burma, Malaysia and Australia [5]. It is a perennial climber with an attractive white flower. It is highly bitter in taste; the bitter taste may suppose to contain medicinal properties [6].

It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like anti-diabetic [11], hepatoprotective [12], cytotoxic [13], anti inflammatory [14], larvicidal effects [7]. It is being used in various treatments as a cardio tonic, antipyretic, antipyretic, useful for intestinal worms and leaf juice rubbed over the liver in remittent fever [8], skin disease [9], Appetizer, laxative, aphrodisiac and blood purifier [10] and antifertility activity [15].

No major investigated reports were found for its CNS activity; therefore, we undertook the present study to determine the anxiolytic activity of leaves of *Trichosanthes cucumerina* L. by using different animal models for anxiety.

MATERIALS AND METHODS

1.1. Collection and Authentication of Plant Material

The leaves of *Trichosanthes cucumerina* linn of family Cucurbitaceae were collected from Nagaon district, Assam. The plant material was identified and authenticated by Dr. Sasikala Ethirajulu, Asst. Director (Pharmacognosy), Siddha Central Research Institute, Arumbakkam, Chennai-600106. A voucher specimen was submitted at C.L.Baid Metha College of Pharmacy, Chennai-97.

1.2. Preparation of ethanolic extract of *Trichosanthes cucumerina* L.

The collected leaves of plants were dried under room temperature without exposure to sun light. The dried plant was powdered and soxhleted with ethanol (95%). The ethanolic extract was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50-60°C) to obtain the crude extract. The extract was stored in refrigerator at 4°C until used for treatment.

1.3. Phytochemical Screening

The extract was subjected to preliminary phytochemical screening by the methods previously described by Kokate and Jayaraman J [16, 17]. 500mg of ethanolic extract was dissolved in 100ml of ethanol and filtered through Whatmann filter paper No 1. Thus, the filtrate obtained was used as test solution for the following preliminary screening tests like alkaloids, flavonoids, glycosides, lignin, phenols, saponins, sterols and tannins.

1.4. Drugs and Chemicals

Diazepam (Ranbaxy Laboratories Ltd., Mumbai) used as the standard anxiolytic drugs. Ethanol was provided from the store of C. L. Baid Metha College of Pharmacy, Chennai and was of analytical grade. Distilled water was used as vehicle.

1.5. Animals

Inbred Swiss 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. Institutional Animal Ethical Committee (IAEC) approved the protocol of the study with reference number IAEC/XXIX/03/CLBMCP/2009-2010, Dated 20/04/2010.

1.6. Acute Oral Toxicity Study

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 [18].

1.7. Experimental design

On the 1st day of the experiment, the animals were divided randomly into four groups of six animals in each.

Group I: Control, Received the vehicle, (1%Tween-80)

Group II: Received 200 mg ethanolic extract/kg b. w

Group III: Received 400 mg ethanolic extract/kg b.w

Group IV: Standard drug, Diazepam 2mg/Kg b.w/ Fluoxetine (10 mg/kg b.w)

The extracts were suspended in the vehicle (1% Tween-80) and all the treatments were given orally by using intragastric catheter at dose (10ml/kg b.w).

1.8. Elevated plus Maze Model [19]

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 EETC (200 and 400 mg/kg) and vehicle were administered for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment. The standard drug was given at a dose of 2 mg/kg p.o. 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.

1.9. Y – Maze Model [20]

Y- Maze is made of black painted wood or grey plastic. Mice were treated with the EETC (200 and 400 mg / kg p.o.) or vehicle for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment and kept individually in one arm of the apparatus. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. For a period of 10 min. the total numbers of visits to different arm were measured.

1.10. Hole –Board Model [21]

The Hole-board apparatus was used as described earlier. The apparatus consists of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. The EETC(200 and 400 mg/kg) and vehicle were administered for 5 days p.o. once daily and the last dose was given on the 5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. For a period of 10 min. the number of line crossing and number of head dipping were calculated.

1.11. Locomotor Activity [21]

The locomotor activity was measured by using an Actophotometer. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. The EETC (200 and 400 mg/kg) and vehicle were administered for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment and the

animals were kept in the Actophotometer individually. The locomotor activity was measured for a period of 10 min.

1.12. Marble-Burying Behavior Model [30]

In this method animals were individually placed in transparent; poly carbonate cages (22 x 32 x 13.5 cm) containing a 5 cm layer of saw dust and 24 glass marbles (1.5 cm in diameter) were evenly distributed on the saw dust in the cages. The EETC (200 and 400 mg/kg) and the vehicle were administered once daily p.o. for 5 days and the last dose was given on the 5th day, 60 min prior to experiment. The standard drug was Fluoxetine was given at a dose of 10 mg/kg p.o. 60 min prior to the experiment and kept in the cages for a period of 30 min. and the number of marbles at least two-third buried in the saw dust was recorded.

1.13. Statistical Analysis

The data were expressed as mean \pm standard error mean (SEM). The data were analyzed by using Graph pad software version5 by 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 AND DISCUSSION

2.1. Phytochemical Screening

The preliminary phytochemical analysis of EETC showed that the plant contains carbohydrates, phenolic compounds, flavanoid, protein, terpenoids and sterols.

2.2. Acute toxicity Study

Acute oral toxicity studies revealed the non-toxic nature of EETC. 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.

2.3. 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.

2.4. 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 EETC showed a significant decrease in the number of visits in the three arms of the Y-maze which was comparable with the standard Diazepam.

2.5. Hole-Board Model

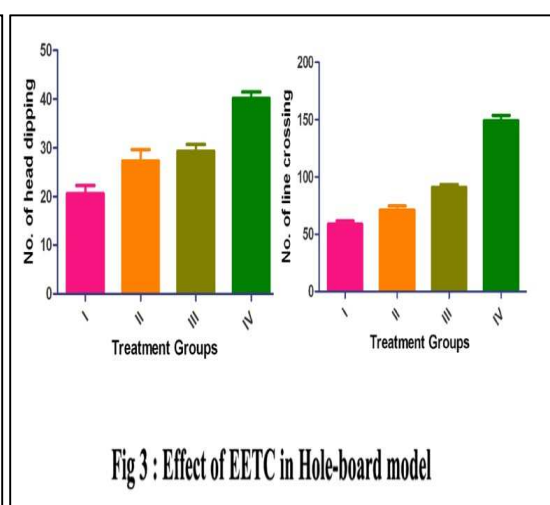
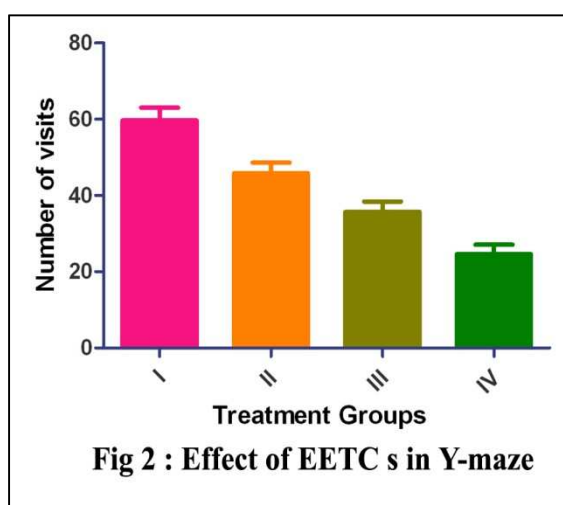
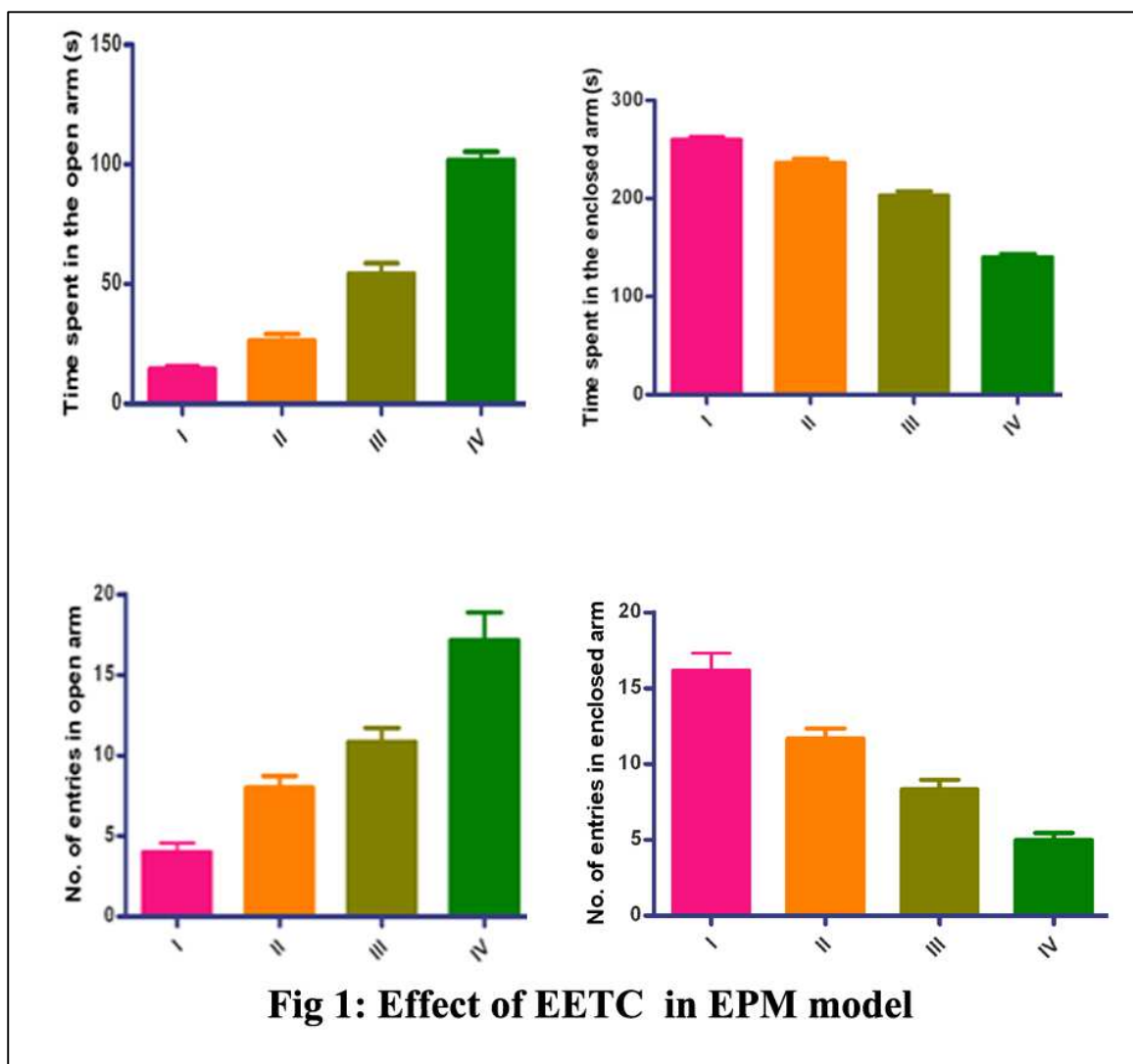
The number of line crossing and head dipping was increased significantly in case of Diazepam treated animals as compared to the control animals. The EETC at both dose levels showed an increase in the number of line crossing and head dipping significantly as compared to the control animals.

2.6. Locomotor Activity

A significant decrease in the locomotor score was observed for Diazepam when compared to the control animals. Both the doses of EETC showed significant decrease in the locomotor score when compared to the control animals.

2.7. Marble-Burying Behavior Model

A significant decrease in the number of marble buried was observed for the standard Fluoxetine when compared to the control animals. The EETC at both dose levels showed significant decrease in the number of marble buried which was comparable with the standard Fluoxetine.



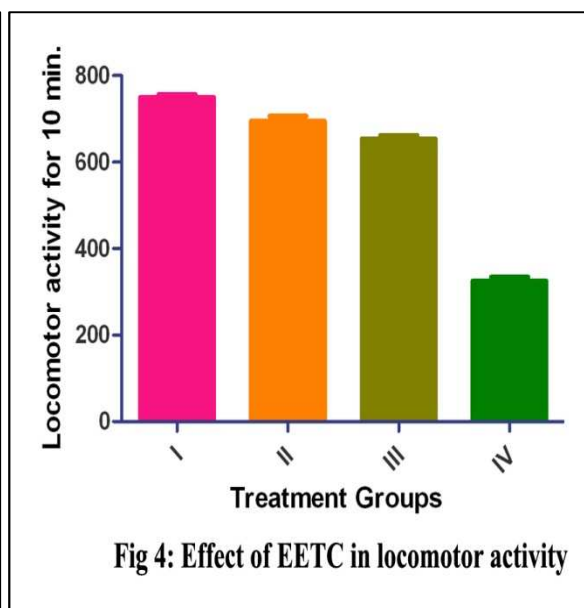
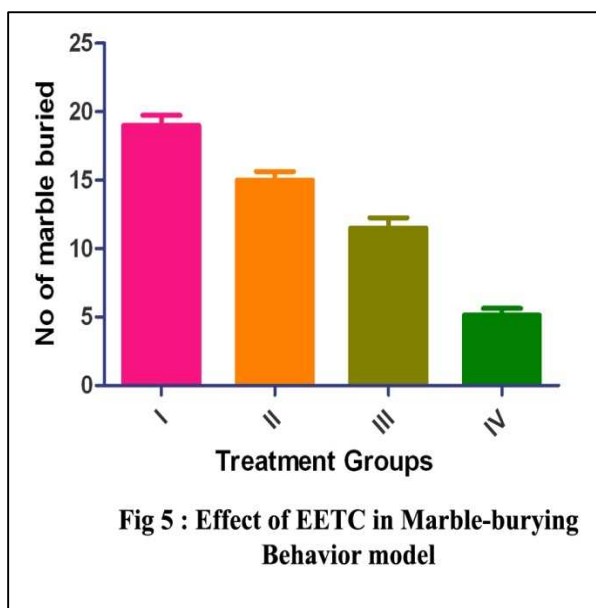


Table-1: Effect of EETC on animals in EPM model

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	14.50± 1.33	259.8 ± 3.81	4.33± 0.49	16.17± 1.13
II	EETC 200 mg/kg	26.33± 2.81*	236.8±3.64**	8.66± 0.55*	11.67± 0.66**
III	EETC 400 mg/kg	54.33± 4.33***	202.8± 4.76***	11.67± 0.88***	8.33± 0.61 ***
IV	Diazepam 2mg/Kg	102.0± 3.31***	140.3 ±3.44***	17.00± 0.85***	5.0± 0.45***

Table-2: Effect of EETC on animals in Y-maze model

Group	Treatment	Number of visits
I	Vehicle	59.67 ± 3.38
II	EETC 200 mg/kg	45.83 ± 2.79**
III	EETC 400 mg/kg	35.67 ± 2.78***
IV	Diazepam 2mg/Kg	24.67 ± 2.44***

Table-3: Effect of EETC in Hole-board model

Group	Treatment	No. of head dipping	No. of line crossing
I	Vehicle	20.7 ± 1.33	61.5 ± 1.41
II	EETC 200 mg/kg	26.2 ± 1.42*	73.5 ± 1.38*
III	EETC 400 mg/kg	29.8 ± 1.17***	114 ± 2.55***
IV	Diazepam 2mg/Kg	42.2 ± 1.58***	162 ± 5.66***

Table-4: Effect of EETC on animals in locomotor activity

Group	Treatment	Locomotor activity for 10 min.
I	Vehicle	749.5 ± 6.85
II	EETC 200 mg/kg	694.7 ± 11.52**
III	EETC 400 mg/kg	653.7 ± 8.036***
IV	Diazepam 2mg/Kg	324.8 ± 9.83***

Table-5: Effect of EETC on animals in Marble-burying Behavior model

Group	Treatment	No of marble buried
I	Vehicle	19.00 ± 0.73
II	EETC 200 mg/kg	15.00 ± 0.63**
III	EETC 400 mg/kg	11.50 ± 0.76***
IV	Fluoxetine 10mg/Kg	5.16 ± 0.47***

Values represented in (Mean ± S.E.M. (n=6), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, p compared vs. Group I

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic, serotonergic neurotransmission in etiology, expression and treatment of anxiety [22, 23]. The adrenergic and dopaminergic systems have also been shown to play a role in anxiety [24]. Despite the widespread traditional use of *Trichosanthes cucumerina* linn for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrated that the ethanolic extract of *Trichosanthes cucumerina* linn has anxiolytic activity in mice in several animal models of anxiety like by EPM, Y-maze, Hole-board and Actophotometer .The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA_A-benzodiazepine complex [25]. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances such as the benzodiazepines [26]. In EPM, normal mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics [27].

In this study, we observed that EETC (200 and 400mg/kg) induced significant increases in the both number of entries and time spent in the open arms and the number of entries and time spent in the closed arms were reduced in the EPM model. 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 EETC Hole-board model a significant increase in the exploratory head-dipping and line crossing behavior were observed after treatment with 200 and 400 mg/kg of EETC, thus reinforcing the hypothesis that it has anxiolytic activity.

Locomotors activity is considered as an index of alertness and a decrease in that indicates a sedative effect [28]. Both the doses 200 and 400 mg/kg of the extract showed a decrease in the locomotors score, thus indicating the sedative effect of the extract.

The marble-burying behavior model has been suggested as a useful model for evaluating anti-obsessive-compulsive disorder drugs because no change in the intensity of marble-burying behavior occurred during repeated testing (this is considered as compulsive behavior [31]. Both the doses 200 and 400 mg/kg of the extract decreased significantly the number of marble-buried. It may possible that the mechanism of anxiolytic action of EETC could be due to the binding of any of tthe 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 [29].

CONCLUSION

From the above observations we can conclude that Ethanolic extract of leaves of *Trichosanthes cucumerina* linn possesses anxiolytic activity at both the dose level which is comparable with the

standards. However further studies are required to know the exact mechanism of action of EETC as anxiolytics.

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