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In Vivo Sedative and Anxiolytic Activities of *Thunbergia erecta* (Acanthaceae) Leaves Activate Gamma-Aminobutyric Acid (GABA) Mediated Hyperpolarization in Swiss Albino Mice

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Abstract

Background: *Thunbergia erecta* (Acanthaceae) is the most abundant medicinal plant in different parts of Bangladesh where it is known as "nilghonta". It has been used as traditional medicine for insomnia, depression and anxiety management. However, no scientific evidence of *T. erecta* belonging to neuropharmacological activity has been reported. The aim of present study was to investigate *in vivo* sedative and anxiolytic activities of methanol extract from the leaves of *T. erecta* in Swiss Albino mice. **Methods:** Sedative activity of METE was investigated using open field, hole cross and thiopental sodium-induced sleeping time test model whereas anxiolytic activity was screened by elevated-plus maze, light-dark box, hole-board and marble-burying test method in mice at 200 and 400 mg/kg doses. The acute toxicity study and phytochemical analysis of METE also carried out. Diazepam used as the positive control for the following behavioral pharmacology test. **Results:** METE exhibited significant ($p < 0.001$) sedative effect by decreasing the number of (square and hole) crossed by mice in open field and hole cross tests in a dose-dependent (200 & 400 mg/kg) manner. In thiopental sodium-induced sleeping time test METE significantly ($p < 0.001$) shortened the latency period and prolonged the sleeping time in a dose dependent (200

& 400 mg/kg) manner. Elevated plus maze (EPM) and light-dark box test results explicated that mice preferred open arms and light part instead of close arms and dark part significantly ($p < 0.001$). In hole-board and marble-burying test METE (200 & 400 mg/kg) reduced ($p < 0.001$) the number of head dipping and number of marble burying respectively. However, Phytochemical screening of METE revealed the presence of flavonoids, glycosides, tannins saponin, carbohydrates and alkaloids. **Conclusion:** The experimental result indicates *T. erecta* contains phytoconstituents that possess sedative and anxiolytic activity which traditionally used in insomnia, depression and anxiety management.

Keywords

1. Introduction

Natural products found from plants have been conveying a vital role among human being since ancient times. An enormous number of scientific reports evidenced of using the medicinal plants as natural remedies. Nowadays, the medicinal plant is used as an alternative to synthetic drugs [1].

Thunbergia erecta is vigorous, a woody shrub which belongs to Acanthaceae family and 100 species in the genus of *Thunbergia* found in several places in Bangladesh. In most of the places *T. erecta* is known as bush clockvine and king's-mantle [2]. This shrub has small, ovate leaves with entire margins borne opposite on thin, brown stems [3]. The purple flowers have a yellow throat and may appear singly or in small clusters. This plant produces rounded seed capsules that end in a beak. *Thunbergia* genus has ornamental value and is native to tropical regions of Africa, Western Africa, Madagascar, Australia and South Asia [4].

Thunbergia species have some bioactive compounds regarding their pharmacological properties already reported. The member of *Thunbergia* genus is reported to contain alkaloids, glycosides and phenolic compounds such as flavonoids, tannins, phenolic acids, rosmaric acid, feruloylmalic and coumaroylmalic acid, naphthalene, iroidglucosides, benzyl beta glucopyranoside, grandifloric acids, delphinidin and apigenin [5].

Traditionally, *Thunbergia* species leaves, stems, and roots used as anti-inflammatory and antipyretics agents [6]. It has been also reported to possess antibacterial activities against gram positive as well as gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis* and *Streptococcus pyogenes* [7]. *Thunbergia* species also exhibit antinociceptive and antitumor [8], cytotoxic and antioxidant [9], carminative [10], antidiarrheal [11] activities.

Regarding the previous reports and our phytochemical analysis, it has been revealed that *T. erecta* contains several bioactive compounds which have sedative and anxiolytic activities. The scientific basis of *Thunbergia* protrudes us to investigate the neuropharmacological activity of *T. erecta* in the management of central nervous system disorder.

2. Materials and Methods

2.1. Plant Material Collection and Extraction

Plant material (leaves of *T. erecta*) was procured from Dhaka (Gazipur region), Bangladesh during 21st July 2018. Responsible scientific officer in National Harberium of Bangladesh accomplished identification with a DACB no. 45803 and a specimen was kept for future reference.

The stem and other adulterants were removed from the leaves of *T. erecta.* The fresh leaves were separated, rinsed and allowed for air drying at ambient temperature (25°C ± 2°C) until the leaves became dry for grinding. The dried leaves were grounded to the coarse powder by dint of blender equipment and before grinding of the sample, the grinder was completely cleaned to restrict contamination with any other materials grounded beforehand. Maceration of 300 g fresh powdered leaves in 3 liters methanol was performed for seven days under occasional stirring. Seven days later, filtration of the mixture followed by concentrating using rotary evaporator (<40°C) yielded 30.8 g (yield 10.2%) of semisolid methanol extract of *Thunbergis erecta* (METE).

2.2. Experimental Animals

Young Swiss-Albino mice aged four to five weeks of either sex were brought from the animal breeding house of Jahangirnagar University (JU), Savar, Bangladesh. Standard environment condition (relative humidity 55 - 65; temperature 23°C ± 2°C; 12 hrs of light-dark cycle) facilitated with ICDDR, B provided food and water at was maintained to store the mice. Mice were given one week before the experiment to be adapted with the experiment condition. All animals were kept overnight without food prior the experiments. Mice were taken care in accordance with Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) developed by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences.

2.3. Drugs and Treatments

The Mice were separated into four different groups (n = 10) designated as control, positive control, and two test groups (200 & 400 mg/kg METE) for every experiment. For all tests, (except open field and hole-cross test) METE (test group) at 200 and 400 mg/kg body weight was given orally to test the animals 30 mins prior the experimental observation whereas, diazepam (1 mg/kg) in positive control group mice were administered intraperitoneally, 15 mins prior the observation. Diazepam for positive control mice and METE for test group mice

were dissolved in 0.9% NaCl saline solution prior administration. To minimize the solvent effect the animals in control group received 0.9% saline water (vehicle) orally and it was done at the dose of 0.1 mL/mouse 30 mins prior the test observation. In case of open field and hole-cross test, mice belongs to all group (test, positive control and control group) placed on the apparatus immediately after the treatment.

2.4. Experimental Methods

2.4.1. Phytochemical Screening

Freshly ready crude extract of *T. erecta* was used for the quantitative analysis of presence of flavonoids, glycosides, tannins, carbohydrates and alkaloids following the standard procedure [12].

2.4.2. Acute Toxicity Study

Animals were separated into several groups having (n = 10) mice in each group. Treatment was given orally to the mice at the doses of 1000, 2000, 3000 and 4000 mg/kg. After gavages, mice were preserved in different cages and given food as well as water as per need. Those mice were placed under observations for the post 72 hours of oral gavage to find out anomalies in behaviors, allergic reactions, and mortality [13].

2.4.3. Sedative Activity Test

1) Open Field Test

Special apparatus made of plywood field with black and white colored squares (72 cm × 72 cm × 36 cm) were used in open field method. The basement of open field appliance consists of cardboard separated into 16 squares (18 cm × 18 cm). This experiment was conducted at ambient temperature under the illumining condition. In this test, after treatment (described in Drugs and Treatment section) a mouse was kept in the middle of cage. The number of explorations by the mice was enumerated for 3 mins at 0, 30, 60, 90 and 120 mins afterwards the treatments [14].

2) Hole Cross Test

This model consists of a wooden cage with the middle partition which contains a hole of 3 cm diameter. The size of cage used was $30 \times 20 \times 14 \text{ cm}^3$ with 7.5 cm height. Mouse placed one end of the cage was allowed to cross the hole for going one chamber to another adjacent chamber. Then the number of hole cross was recorded for 3 mins at 0, 30, 60, 90 and 120 mins for each mouse [15].

3) Thiopental Sodium-Induced Sleeping Time Test

The mice were randomly grouped into three groups control, test groups and positive control where each group consisted of ten mice [16]. The test groups were provided with METE orally, the control group was treated with 0.9% saline water. Mice of positive control group were treated with diazepam (1 mg/kg). Thiopental sodium (40 mg/kg, i.p.) used as sleeping agent was given to all experimental animals thirty (30) mins later of sample or 15 mins later of standard

ingestion. Immediately after, the test animals were kept under monitoring for the latent period (interval between Thiopental sodium administrations and loss of righting reflex) and duration of sleeping (interval between the loss and recovery of righting reflex).

2.4.4. Anxiolytic Activity Test

1) Elevated Plus-Maze Test

The elevated plus maze test (EPM) was carried out to determine the anxiolytic behavior of mice. The apparatus was constructed of two uncovered sides (16 × 5 × 12cm) and two closed sides (16 × 5 × 12 cm) which had 50 cm elevated from the floor with an open roof [17]. Each mouse was put in the middle of the apparatus where the head of the mouse was positioned towards the uncovered sides. Effects related to the behavior of the mouse were watched for the duration of 5 mins free exploration with different parameters (time period spent in uncovered arms and closed arms).

2) Light-Dark Box Test

This test was used to determine anxiolytic-like effects in mice [18]. This apparatus consists of a completely automated box to be observed by the investigator. A rectangular box (46 × 27 × 30 cm high) that has opening at top and is separated into a tiny (18 × 27 cm) area and a big (27 × 27 cm) area along with an unveiling door (7.5 × 7.5 cm) situated at the middle of the divider at the ground level. One chamber was colored black and made dark but the large part was marked white and glaringly enlightened by a 60-W light source. The time spent in the illuminated and dark compartment recorded for 5 mins. The ambience was kept dark during the experiment. This test capitalized the conflict occurred between the propensity of mice to probe a new ambience and bright light phobia of mice [19].

3) Hole-Board Test

A wooden box with (40 cm × 40 cm × 25 cm) size and sixteen equally distant holes of 3 cm diameter situated upon the floor was used as appliance [20]. The middle of individual cleft was 10 cm away from the surrounding wall of the box. The basement of the box is situated 15 cm upwards the floor and separated into squares of (10 cm × 10 cm) with a water-protective marker. After 15 mins later of treatment with diazepam or 30 mins later of treatment with METE or vehicle each mouse placed at the middle of the hole-board and the number of head dipping by mouse was counted for next 5 mins. The dipping of head was considered for scoring if either eye became concealed into the hole.

4) Marble Burying Test

Mice were separately kept in cages made of glass material with the selected bedclothes for 30 min (habituation period) and afterwards same were put into different cages for anticipation [21]. Twenty-five (25) glass marbles were situated at evenly maintained 7 cm aside on 4 cm strata of lying stuff in the cages. Experimental mice previously habituated with the cage environments. The marble burying test was accomplished by putting each mouse in the cage and the

count of marbles greater than two-thirds clothed with bedclothes were counted. After the end of each trial, bedclothes and cages were changed by new sets and marbles were cleaned with water, dried by using towel of paper, and left to achieve to ambient temperature.

2.4.5. Statistical Analysis

The output of the work has been shown as mean ± SEM. To calculate the statistical significance one way ANOVA followed by Dunnett's post hoc test by SPSS 20 program was employed. Statistics were considered significant compared to the control group at $p < 0.001$, $p < 0.01$ and $p < 0.05$.

3. Results

3.1. Phytochemical Screening

The preliminary phytochemical screening revealed the presence of alkaloids, glycoside, flavonoid carbohydrate, saponin and tannin in methanol extract of leaves of *Thunbergia erecta* (Table 1).

3.2. Acute Toxicity Study

After oral administration of 1000 - 4000 mg/kg MESC showed no hypersensitive symptoms or fatality in mice over the observation time period (72 hr). This nontoxic profile of METE made us certain to select the dose (200 and 400 mg/kg) for this study.

3.3. Sedative Activity Study

3.3.1. Open Field Test

The count of squares elapsed decrement by mice movements concealed significantly ($p < 0.01$) by fourth (90 min) and fifth investigation (120 min) time at 200 and 400 mg/kg (Figure 1). We observed that the sedative effect achieved by the diazepam (1 mg/kg) taking group, showed parallel modification with METE (Table 2).

3.3.2. Hole Cross Test

Reduction of the count of holes crossed was significant ($p < 0.001$) from third observation (60 min) and remained at fifth observation (120 min) period which was comparable to vehicle treated group (Figure 2). In addition, METE and the positive control group (diazepam 1 mg/kg) revealed a similar pattern of modification (Table 3).

3.3.3. Thiopental Sodium-Induced Sleeping Time Test

This test showed significant ($p < 0.001$) reduction in sleep onset. Moreover, significant ($p < 0.001$) rise in term of total sleeping period was noticed in mice at the doses of 200 and 400 mg/kg when comparison was done with the control group (Figure 3). Diazepam, used as positive control resulted an analogous effect to that of METE (Table 4).

Table 1. Preliminary qualitative phytochemical screening of METE.

Phytochemical constituents	Tests	Inferences
Alkaloids	Mayer's test	+
	Dragendorff's test	+
	Wagner's test	+
	Hager's test	+
Flavonoids	Lead acetate test	+
Carbohydrates	Molisch's test	+
	Fehling's test	+
Glycosides	Modified borntrager's test	+
Tannins	Gelatin test	+
Saponins	Frothing test	+
	Foam test	+

Key: + present.

Table 2. The sedative effect of METE on the open field test in mice.

Treatment	Dose (mg/kg)	Number of squares crossed				
		0 min.	30 mins.	60 mins.	90 mins.	120 mins.
Control	0.1 ml/mouse	106.20 ± 2.53	106.60 ± 2.01	104.80 ± 2.15	101.40 ± 2.69	96.80 ± 2.08
Diazepam	1	84.80 ± 2.22	43.80 ± 1.39***	23.00 ± 1.30***	15.00 ± 1.37***	6.00 ± 0.54***
METE	200	102.00 ± 2.55	92.80 ± 1.39***	71.60 ± 0.81***	62.00 ± 1.14***	32.60 ± 1.63***
METE	400	99.00 ± 1.87	83.60 ± 1.36***	63.00 ± 1.76***	44.00 ± 1.44***	17.40 ± 0.74***

Each value is presented as the mean ± SEM (n = 10), METE = Methanol extract of *Thunbergia erecta* leaves. "***$p < 0.001$; vs. control group (Dunnett's test)".

Table 3. The sedative effect of METE on hole cross test in mice.

Treatment	Dose (mg/kg)	Number of holes crossed				
		0 min.	30 mins.	60 mins.	90 mins.	120 mins.
Control	0.1 ml/mous	20.00 ± 1.30	21.20 ± 1.06	21.80 ± 0.97	20.00 ± 1.30	18.60 ± 0.67
Diazepam	1	13.40 ± 1.56	11.40 ± 1.20***	8.00 ± 1.04***	6.20 ± 0.66***	2.40 ± 0.92***
METE	200	19.40 ± 1.96	16.80 ± 0.97*	13.40 ± 0.67***	11.60 ± 0.67***	5.80 ± 0.97***
METE	400	15.20 ± 1.49	15.60 ± 0.98**	11.00 ± 0.83***	9.20 ± 0.58***	3.40 ± 0.92***

Each value is presented as the mean ± SEM (n = 10), METE = Methanol extract of *Thunbergia erecta* leaves. "***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs. control group (Dunnett's test)".

Table 4. The sedative effect of METE on thiopental sodium-induced sleeping time test in mice.

Treatment	Dose (mg/kg)	Onset of action (min)	Duration of sleeping time (min)
Control	0.1 ml/mouse	14.47 ± 0.41	97.00 ± 1.09
Diazepam	1	7.64 ± 0.07***	186.80 ± 0.91***
METE	200	11.50 ± 0.68***	132.00 ± 1.94***
METE	400	8.50 ± 0.22***	177.60 ± 1.24***

Each value is presented as the mean ± SEM (n = 10), METE = Methanol extract of *Thunbergia erecta* leaves. "***$p < 0.001$ vs. control group (Dunnett's test)".

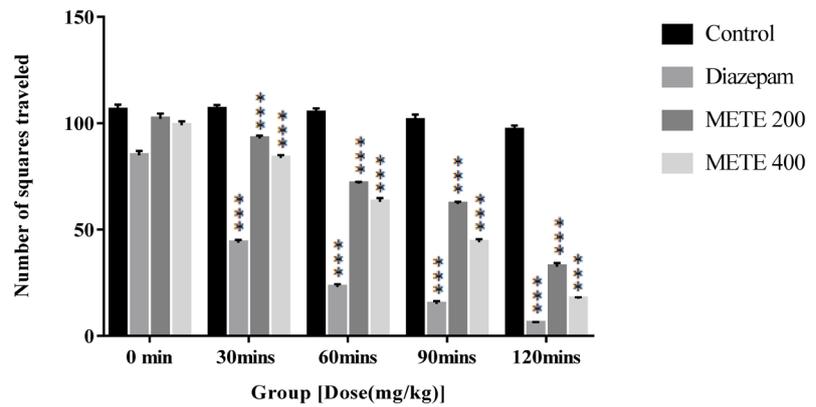**Effect of METE and Diazepam in Open field test**

Figure 1. The sedative effect of METE on the open field test (number of square crossed at 0 min, 30 mins, 60 mins, 90 mins and 120 mins) in mice.

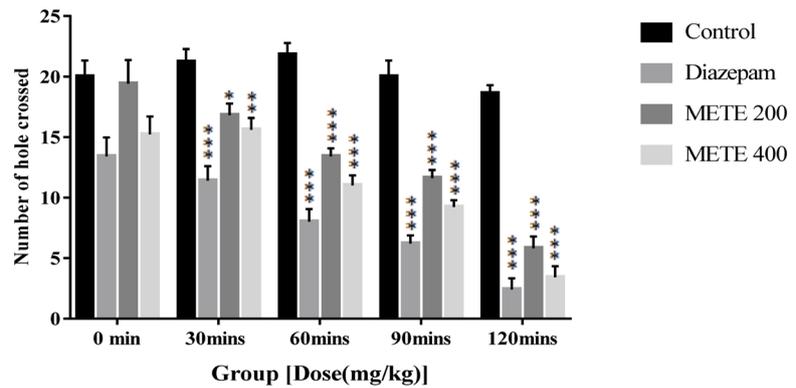**Effect of METE and Diazepam on Hole cross test**

Figure 2. The sedative effect of METE on the hole cross test (number of square crossed at 0 min, 30 mins, 60 mins, 90 mins and 120 mins) in mice.

Effect of METE and Diazepam in Thiopental sodium induce sleeping test
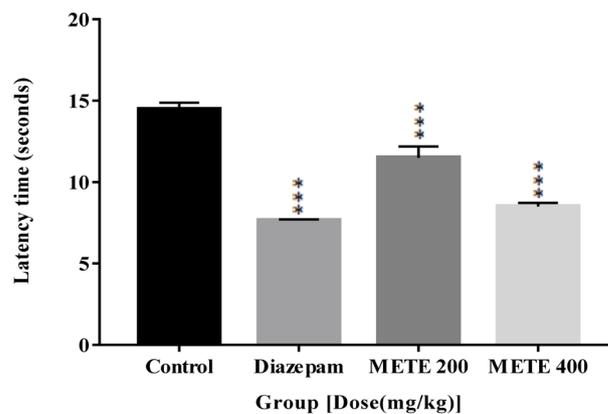

Figure 3. The sedative effect of METE on the Thiopental sodium induce sleeping test (Latency time and sleeping time) in mice.

3.4. Anxiolytic Activity Study

3.4.1. Elevated Plus-Maze Test
In this test mice opted the open side of the plus-maze when treated with METE (Figure 4). The frequency of time used in open side at 200 and 400 mg/kg were statistically significant ($p < 0.001$) and comparable to control. The positive control group (diazepam 1 mg/kg) showed the similar effect to that of METE (Table 5).

3.4.2. Light-Dark Box Test
In this test, the time stayed in light parts significantly ($p < 0.001$) aggravated at 200 and 400 mg/kg when compared with the control group (Figure 5). The same effects were noticed in mice treated with diazepam (1 mg/kg) (Table 6).

3.4.3. Hole-Board Test
In this test, the propensity for head dipping significantly ($p < 0.001$) decreased at 200 and 400 mg/kg of METE when compared with the control group (Figure 6). The similar effects were viewed in animal examined with diazepam (1 mg/kg) (Table 7).

3.4.4. Marble-Burying Test
In the marble-burying test, the behavioral condition of animal significantly ($p < 0.001$) decreased by the count of burying at 200 and 400 mg/kg of METE (Figure 7) when measured with control. Diazepam, as the positive control exerted the same effects as METE (Table 8).

Table 5. The anxiolytic effect of METE on elevated plus-maze test in mice.

Treatment	Dose (mg/kg)	Time spend in open arms (s)	Time spend in close arms (s)
Control	0.1 ml/mouse	108.80 ± 2.05	191.20 ± 2.05
Diazepam	1	243.40 ± 1.56***	56.60 ± 1.56***
METE	200	211.40 ± 2.50***	88.60 ± 2.50***
METE	400	232.60 ± 1.74***	67.40 ± 1.74***

Each value is presented as the mean ± SEM (n = 10), METE = Methanol extract of *Thunbergia erecta* leaves. "****p* < 0.001 vs. control group (Dunnett's test)".

Table 6. The anxiolytic effect of METE on light-dark box test in mice.

Treatment	Dose (mg/kg)	Time spent in the light compartment (s)	Time spent in the dark compartment (s)
Control	0.1 ml/mouse	126.40 ± 2.65	173.60 ± 2.65
Diazepam	1	260.60 ± 2.90***	39.40 ± 2.90***
METE	200	214.60 ± 1.32***	85.40 ± 1.32***
METE	400	248.00 ± 2.28***	52.00 ± 2.28***

Each value is presented as the mean ± SEM (n = 10), METE = Methanol extract of *Thunbergia erecta* leaves. "****p* < 0.001 vs. control group (Dunnett's test)".

Table 7. The anxiolytic effect of METE on hole-board test in mice.

Treatment	Dose (mg/kg)	Number of head dips
Control	0.1 ml/mouse	48.40 ± 2.48
Diazepam	1	15.40 ± 1.16***
METE	200	27.00 ± 1.51***
METE	400	20.60 ± 1.20***

Each value is presented as the mean ± SEM (n = 10), METE = Methanol extract of *Thunbergia erecta* leaves. "***$p < 0.001$ vs. control group (Dunnett's test)".

Table 8. The anxiolytic effect of METE on marble-burying test in mice.

Treatment	Dose (mg/kg)	Number of burying
Control	0.1 ml/mouse	19.20 ± 2.28
Diazepam	1	1.60 ± 0.92***
METE	200	7.20 ± 1.49***
METE	400	4.60 ± 0.74***

Each value is presented as the mean ± SEM (n = 10), METE = Methanol extract of *Thunbergia erecta* leaves. "***$p < 0.001$ vs. control group (Dunnett's test)".
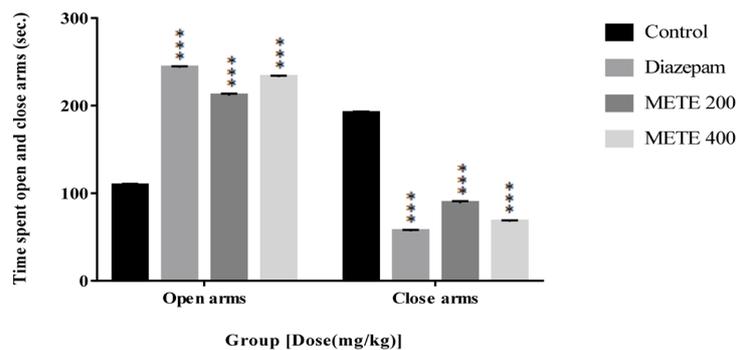

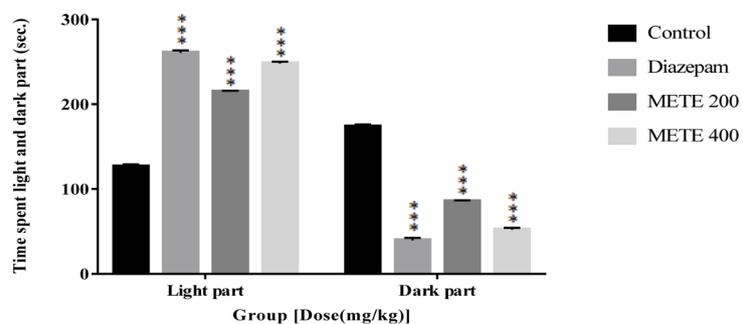Figure 4. The anxiolytic effect of METE on elevated plus-maze test (Time spent in open arms and Time spent in close arms) in mice.

Figure 5. The anxiolytic effect of METE on light-dark box test (Time spent in light part and Time spent in dark part) in mice.

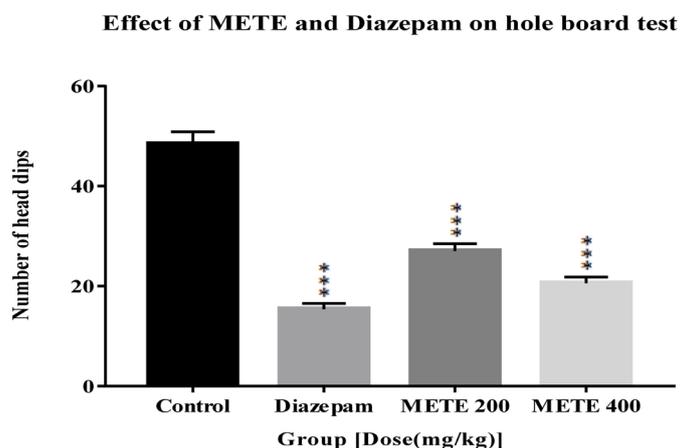**Effect of METE and Diazepam on hole board test**

Figure 6. The anxiolytic effect of METE on hole board test (number of head dips) in mice.

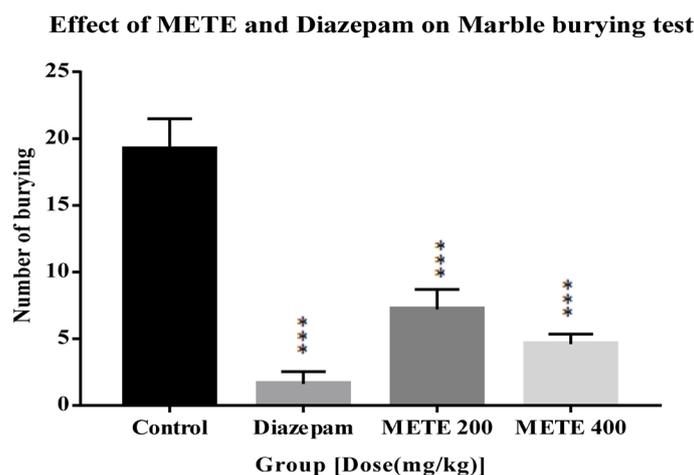**Effect of METE and Diazepam on Marble burying test**

Figure 7. The anxiolytic effect of METE on marble burying test (number of marble burying) in mice.

4. Discussion

The therapeutic benefits of medicinal plants provide us with traditional remedies from the ancient periods [22]. *Thunbergia* genus were used traditionally for managing anxiety and numerous behavior disorders. Our rudimentary screening for phytochemicals suggests the availability of alkaloids, glycosides, flavonoids, tannins which may exert neuropharmacological activity. Several phytochemical studies proved that tannins exert sedative activity. Saponins also have potent sedative activity [23]. Moreover, it is already reported that flavonoids have potent sedative and anxiolytic effects [24].

The median lethal dose (LD_{50}) of METE was found as nontoxic at 4000 mg/kg dose of body weight in mice. Our pharmacological evaluation of sedative and anxiolytic activities proposed that METE exerts as GABA-benzodiazepine receptor interaction in the brain. Several models of sedative and anxiolytic test predominately expressed that METE dose-dependently effects in recent studies.

We evaluated open field, hole cross and thiopental sodium-induce sleeping test as the sedative activity. On the contrary, we considered elevated-plus maze, light-dark box, hole-board and marble burying test as anxiolytic effects.

In open field test (Table 2 & Figure 1), the locomotion effect of METE showed that decreased number of squares crossed by mice was dose-dependent activity at second (30 min) to fifth (120 min) time point of observation. According to hole cross test (Table 3 & Figure 2), the behavioral state of mice diminished the number of hole cross (from second to fifth observation) convinced us about the sedative activity of METE. These study also demonstrated that the sedative response of METE effect almost similar to diazepam. The dose-dependent sedative effects also observed in the thiopental sodium-induce sleeping test which is reflected in chronological data in same dose and also when compared between two doses (200 and 400 mg/kg) (Table 4 & Figure 3). This validated model suggests that the activity of TS on gamma-aminobutyric acid (GABA) mediate hyperpolarization of CNS [25]. TS injection dose-dependently reduced the latency period conjointly, prolonging the duration of sleeping time. Typically, diazepam effect demonstrated similar manner as well METE.

The possible anxiolytic activity recognized in-vivo system due to interaction with the $GABA_A$-BZD receptor. Elevated-plus maze experiment is a reliable and widely most conventional method to determine the anxiolytic behavior in mice model. Recent data recommended that mice treated with METE preferred to spend the most of the time in the open arm rather than the close arm. Anxiolytic effect of METE in Elevated plus-maze test (Table 5 & Figure 4), confirmed the similar manner of diazepam. Light-dark box test (Table 6 & Figure 5), is another popular evaluation approach due to its methodological simplicity. METE treated mice selected light part to spend more time instead of dark part of the light-dark box in a dose-dependent manner. Time spent in light part express the anxiolytic-like behavior of mice. Diazepam treated mice also showed same effects when compared with control. The hole-board test (Table 7 & Figure 6), has used as measure exploratory behavior of mice. Anxiolytic activity upraised to decrease the number of head dipping activity in the hole-board test. METE remarkably diminished the head dipping activity in a dose-dependent manner, thereby suggesting the anxiolytic agent. Marble burying test (Table 8 & Figure 7), is another validated method to investigate the anxiolytic activity in mice. The number of marble burying decreased when treated with METE likewise diazepam in a dose-dependent manner, regarded as an anxiolytic action.

Gamma-amino butyric acid (GABA) is the major inhibitory amino acid neurotransmitter in the mammalian central nervous system. GABA is synthesized from glutamic acid by the enzyme glutamic acid decarboxylase (GAD) and catabolized by the enzyme GABA-transaminase (GABA-T) into succinic semi aldehyde [26]. GABA helps to regulate movement control, sight, anxiety, and many other brain functions. Benzodiazepines enhance responses to the inhibitory neurotransmitter GABA by opening GABA ($GABA_A$ receptors as the main site of action of ligands with anxiolytic activity) activated chloride channels and

allowing chloride ions to enter the neuron. This action allows the neuron to become negatively charged and resistant to excitation, which leads to the various anti-anxiety, sedative, or anti-seizure activity seen with these drugs.

However, some plant may contain GABA-T inhibitor which ultimately raise brain GABA level and reduce anxiety. In addition, GABA released into the synapse cleft can be reuptaken into both presynaptic terminals and surrounding glial cells for different purposes, relying on membrane GABA transporters (GAT), GABA taken back up into presynaptic nerve terminal. Hence, Inhibition of the re-uptake of GABA by potent and selective inhibitors of the GABA transporter enhances GABA activity thus reduce anxiety [27] [28] [29].

In current study benzodiazepine used as standard drug and the exerted neuropharmacological (sedative and anxiolytic) effect of METE convinced us to assume that the METE might follow the mechanism like diazepam on GABA$_A$ subunit.

5. Conclusion

Our experimental findings represent that several phytoconstituents present in METE demonstrate sedative and anxiolytic activity. These results support that the METE possesses sedative and anxiolytic properties like diazepam which act through binding to benzodiazepines site on GABA-BDZ receptor complex. However, the plasma GABA level measurement and the further investigation of associated physiological indexes (GAD, GABA-T) are suggested to pin-point the mechanism of sedative and anxiolytic action of METE. Additional advance studies are required to identify the active phytoconstituents associated with observed bioactivities in animal behavioral models.

Acknowledgements

Ethics Approval and Consent to Participate

All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) postulated by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/IAEC/17.02) of Stamford University Bangladesh approved all experimental rules.

Consent for Publication

Not applicable.

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Authors' Contributions

AB conceived, planned and supervised the experiment. AB contributed to data analysis and manuscript writing. AH and AAM co-ordinated experimental studies. MSAM examined the data, explained the results and drafted the manuscript. MMRB contributed to manuscript writing and AB finally edited the manuscript. Manuscript was perused and approved by all authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of Abbreviations

META = Methanolic extract of *Thunbergia erecta;*

ICDDR, B = International Center for Diarrhoeal Disease and Research, Bangladesh;

GABA = Gamma-Amino Butyric Acid;

TS = Thiopental sodium;

CNS = Central Nervous System;

BZN = Benzodiazepine;

GAD = Glutamic acid decarboxylase;

GABA-T = GABA-transaminase;

GAT = GABA transporters.