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# Methanol extract of *Spathodea campanulata* P. (Beauv.) leaves demonstrate sedative and anxiolytic like actions on swiss albino mice

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## Abstract

**Background:** *Spathodea campanulata* P. Beauv. (Bignoniaceae) is a very common plant in Bangladesh which is locally called "Rudrapalash". In Nigeria leaves extract of *S. campanulata* has a reputation of being used as an anticonvulsant. In this connection, the aim of this study was to investigate other neuropharmacological effects like sedative and anxiolytic activities of methanol extract of *S. campanulata* (MESC) leaves in different mice tests.

**Methods:** To assay sedative activity mice were subjected to open field and hole-cross test, whereas anxiolytic activity was checked by the elevated-plus maze, light-dark box, and hole-board test. For each test, mice were divided into control group (deionized water, 0.1 ml/mouse, p.o.), standard group (diazepam, 1 mg/kg, i.p) and three test groups (200, 400 and 600 mg/kg MESC, p.o.). The acute toxicity test and phytochemical screening of MESC were performed before the pharmacological study.

**Results:** The result demonstrated strong sedative and anxiolytic activity of MESC in a dose-dependent manner. All doses of MESC (200, 400 & 600 mg/kg) reduced the number of (square & hole) crossed by mice in both open field and hole cross tests ( $p < 0.001$ ). On the other hand, in elevated plus-maze and light-dark box test mice opted to stay more in open arms and light box instead of close arms and dark box ( $p < 0.001$ ). In hole-board test MESC (200, 400 & 600 mg/kg) elevated the number of head dipping ( $p < 0.001$ ) dose-dependently. Phytochemical investigation indicated the presence of alkaloid, saponin, glycoside, carbohydrate, flavonoid, and tannin in MESC.

**Conclusion:** The experimental results explicit that *S. campanulata* leaves possess sedative and anxiolytic activities, hence suggest further chemical investigation to identify specific phytoconstituents responsible for sedative and anxiolytic effects.

**Keywords:** *Spathodea campanulata*, Sedative, Anxiolytic, Open-field, Hole-cross, Elevated plus-maze, Light-dark box, Hole-board

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## Introduction

Globally, anxiety is the most frequently happening mental disorder and the lifetime prevalence of anxiety have ranged from 10% to 25%. Epidemiological studies have ensured the high figure of sleep complaints too. More than 30% among the adult people has been facing the sleeping problem and sleep ailment is considered as the second most usual manifest of mental suffering [1–3].

WHO EML declared ‘diazepam’ (diazepam has been chosen as a reference drug substance in the current study) the essential medicine for anxiety and sleep disturbances. Diazepam represents benzodiazepines which have the most reliable record for both potency and safety. In spite of having outstanding effectiveness, diazepam demonstrates serious untoward effects like headaches, drowsiness, muscle weakness, confusion, and depression. Serious adverse effects also include dysarthria, vertigo, visual disturbances, gastrointestinal upset, urinary retention or incontinence, and amnesia. Additionally, due to paradoxical excitation, some patients may feel like aggression, hostility, and disinhibition. The rare intervention of hypersensitivity reactions, Jaundice, and blood disorders. Respiratory depression and hypotension can occur occasionally at high doses (parenteral administration). Even, Diazepam can deteriorate driving performance in a healthy person [4].

Therefore new synthetic drugs having fewer side effects and higher potency are most expected for the treatment and management of insomnia and anxiety; the plant could be a great source for this purpose.

*Spathodea campanulata* P. Beauv. (Bignoniaceae), is one of the world’s most spectacular flowering trees which is commonly known as “African tulip tree” or “Tulipier du Gabon”. It is extensively distributed throughout Africa and is cultivated as an ornamental tree in tropical and sub-tropical countries, including American countries. The African tulip tree is a large upright tree with glossy deep green pinnate leaves and glorious orange-scarlet flowers [5, 6].

In Bangladesh, *S. campanulata* is locally known as “Rudrapalash” which has been used as a folk medicine for the treatment of different diseases. The stem-bark is considered anti-hyperglycemic, anti-malaria, used in treating various skin diseases, stomachaches and diarrhea. Moreover, stem- bark decoction has also been displayed anti-HIV activity. The flowers are used as a diuretic and anti-inflammatory agent whereas the leaves are utilized in curing kidney diseases, urethral inflammations and as an antidote against animal poisons. Plant leaves also been reported to have antiplasmodial, analgesic, anti-inflammatory and anti-larvicidal activity [7–9]. *S. campanulata* leaf extract is used by the people of South-Eastern Nigeria for its anticonvulsant, analgesic and anti-inflammatory effects [10] and antiplasmodial effect [11].

Researchers have isolated several phytochemicals from different parts of the plant. The leaves reported to comprise Spathosides A, B, and C, Verminoside, 6'-O-trans-caffeoyl-loganic acid, Catalpol and Ajugol [12], Spathodol, Caffeic acid, Phenolic acid and Flavonoids [13]. Flowers have 1,1-diethoxy-3-methyl-butane, N-hexadecanoic acid, 1,2 benzene dicarboxylic acid diisooctyl ester, and oleic acid [14]. Phytol,  $\alpha$ -methyl Cinnamaldehyde,  $\beta$ -sitosterol-3-acetate, naringenin, catechin-3-O- $\alpha$ -rhamnopyranoside and 5, 6, 4' trihydroxy flavonol-7-O- $\alpha$ -rhamnopyranoside, anthocyanins [15] while its floral nectar contains a complex mixture of triterpenoids and steroids [16]. The constituents isolated from stem bark were n-alcohols (35%), octacosanol and triacontanol [17]. Spathoside, n-alkanes, linear aliphatic alcohols, sitosterol and their esters, beta-sitosterol-3-O-beta-D glucopyranoside, oleanolic acid, pomolic acid, p-hydroxybenzoic acid, phenylethanol ester, 13 $\beta$ -acetoxyoleanolic acid, siaresinolic acid, 3 $\beta$ -acetoxy-12-hydroxyoleanan-28, 13-olide and oleanolic acid have been isolated from the stem bark of *S. campanulata* [18, 19]. Moreover, Root peels contain Methyl p-hydroxybenzoate and p-hydroxybenzoic acid whereas fruits have various types of Polyphenols, Tannins, Saponins and Glucosides [19].

Recently, a glycoside (Pentacyclic triterpenoid compound) named urs-12-en-27 $\alpha$ , 30 di-oic acid 3-0- $\alpha$ -L-rhamnopyranosyl (1  $\rightarrow$  2)- $\alpha$ -L-arabinopyranoside has been isolated from leaves which has been proven to have extensive anticonvulsant activity [20].

Available research articles reported that drug substances having an anticonvulsant effect can also exert sedative and anxiolytic-like actions [15]. In this connection, Diazepam (used as a reference drug in this study) is standard one whose actions are mostly attributed to enhance the action of gamma-aminobutyric acid (GABA) [21]. It is noteworthy that, plants reported as anticonvulsants like *Verbena officinalis*, *Swertia corymbosa*, *Securidaca longepedunculata*, *Nauclea latifolia* has simultaneously shown sedative and anxiolytic activity [22–25]. Therefore, this research work has been conducted to investigate the speculated sedative and anxiolytic activities of leaves of *S. campanulata* and hence to find out the scientific ground for its traditional use in the management of central nervous system ailments.

## Materials and methods

### Plant collection and identification

Fresh leaves of *Spathodea campanulata* were collected randomly from Gazipur, Bangladesh in April, 2018. The collected plant samples were then identified by Mr. Sardar Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium (Mirpur, Dhaka, Bangladesh, where voucher specimen has been deposited with a number DACB: 46540 for further references. The extraneous, undesired substances were removed by hand and plant materials were

washed by using freshwater. Washed leaves were then dried at considerably low (not exceeding 50 °C) temperature and grounded to coarse powder by high capacity grinding machine.

#### Preparation of plant extract

Approximately 400 g dried powder leaves were soaked in 2.5 L of methanol upon occasional shaking for 7 days and then obtained extract was filtered by using clean cotton cloth followed by Whatman filter paper No.1. The filtrate was concentrated with a Heidolph rotary evaporator at low temperature (40–45 °C) and pressure and finally 70 g (yield 17%) semisolid mass of methanol extract of leaves of *Spathodea campanulata* (MESC) was obtained.

#### Animals

To evaluate sedative and anxiolytic activity 150 Swiss Albino mice of either sex, 3–4 weeks of age, weighing between 20 and 25 g, were collected from the Animal House of Jahangirnagar University, Savar. Animals were housed under controlled environmental conditions (temperature: 22.0 ± 2.00C, relative humidity: 55–65%) with 12 h light/12 h dark cycle and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for minimum 3 days before the experiment and were fasted overnight just before the experiment. The animals were assigned to the various experimental groups ( $n = 6$  per group) arbitrarily.

#### Drugs and administration

The drugs and chemicals used in the current study: Diazepam, Methanol, and DMSO (Merck, Germany). Diazepam was purchased from Monoara Hospital, manufactured by Aristo Pharma Ltd., Bangladesh. For each test, mice were divided into five groups including control, positive control and three experimental groups. The doses and treatment schedules were selected on the basis of scientific literature [26].

Each mouse of the positive control group received 0.1 ml diazepam [dose 1 mg/kg body weight (b.w.), intraperitoneally (i.p.)] (DZP, Aristo Pharma Ltd., Bangladesh) as a sedative and anxiolytic drug. Each mouse in the three experimental groups received 0.1 ml MESC (200, 400 and 600 mg/kg b.w., per os (p.o.)). Animals in the control group received normal saline (0.9% NaCl solution, 0.1 ml/mice) orally. To minimize the solvent effect, 0.9% NaCl solution was used as a vehicle in all experimental groups.

In sample group, mice were administered with MESC (at 200, 400 and 600 mg/kg b.w., p.o.) 30 mins prior the experimental observation whereas, in positive control group, mice were treated with diazepam (1 mg/kg, i.p.) 15 mins prior the observation.

However, 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.

#### Phytochemical screening

The freshly prepared MESC was qualitatively screened for the presence of various phytochemicals following standard procedures [27].

#### Acute toxicity study

For acute oral toxicity of MESC, OCD guideline no.425 was followed accordingly. Mice (6 per treatment) were treated orally with MESC at the doses of 500, 2000 and 4000 mg/kg BW. After gavages, the animals were kept in separate cages and supplied with food and water ad libitum. The mice were then observed for any abnormal behaviors, allergic symptoms, and mortality during 72 h study period [28].

#### Sedative activity test on mice

##### Open field test (OFT)

To assess the sedative activity of methanol extract of leaves of *Spathodea campanulata* (MESC) Swiss Albino mice were subjected to the Open field method. To investigate the emotional behavior of the animals the open field method is most frequently used. The method was carried out on mice as described by Gupta et al. (1971) with slight modification [29]. The open field apparatus consisted of a wooden field of half-square MESC, divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 50 cm height and was placed in a murky lit room. The animals from control, positive control, and test groups were placed in the middle of the open field to explore freely and the number of squares visited by them was counted for 3 min at 0, 30, 60, 90, and 120 min after the treatments. The open field instrument was cleaned after each test session to prevent the next mouse from being influenced by the odors deposited in the urine and feces of the previous mouse.

##### Hole cross test (HCT)

The method was carried out as described by Takagi et al., 1971 [30]. A wood partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm was made at a height of 7.5 cm in the center of the cage. The animals from each group were placed in one side of the chamber and the number of passage of a mouse through the hole from one chamber to other was counted for 3 min at 0, 30, 60, 90 and 120 min after the administration of the saline, standard drug and MESC.

**Table 1** Results of different chemical group test of the MESC

Metabolites	Tests	Result
Alkaloids	Mayer's test	+
	Dragendorff's test	+
	Wagner's test	+
	Hager's test	+
Glycosides	Modified borntrager's test	+
Carbohydrates	Molisch's test	+
	Fehling's test	+
Flavonoids	Lead acetate test	+
Saponins	Frothing test	+
	Foam test	+
Tannins	Gelatin test	+
Steroid	Libermann burchard's test	+

**Anxiolytic activity test on mice**

**Elevated plus - maze test (EPMT)**

The elevated plus-maze test (EPMT) is a frequently used method to investigate the anxiolytic effects. The apparatus consisted of two open arms (length 15 cm × width 5 cm), two closed arms (length 15 cm × width 5 cm × height 5 cm) and a central platform (5 cm × 5 cm). The maze was lifted 50 cm above the floor level. According to the Drugs and Administration, mice from respective groups were treated with vehicle, diazepam, and MESC. After the desired time (30 min after the treatment with vehicle or MESC and 15 min after diazepam) mice from each group was placed individually into the center of the maze, facing its head to one of the open arms and allowed to explore the maze for 5 min. The time spent in the open and closed arms, the number of entries in open and closed arms were recorded for marked 5 mins using a stopwatch. Increased activity in the open arms was indicative of less anxiety. Entry into an arm was determined if mice placed all four feet into the arm. The maze was cleaned with 10% ethanol solution after each test [31, 32].

**Light - Dark box test (LDBT)**

The light-dark box test (LDBT) method is another popular method to study anxiolytic action in mice. The

apparatus rectangular box (46 × 27 × 30 cm<sup>3</sup>) having open-top, divided into a small (18 × 27 cm<sup>2</sup>) and a large (27 × 27 cm<sup>2</sup>) compartment with a fixed partition. A small hole of 3 cm is located in the middle of the partition at the floor level. The small compartment was covered with a black painted lid and illuminated with a dim light. On the other hand, the large compartment was painted white and brightly illuminated by a 60 W electric light. After 30 min of successful oral gavages of vehicle, MESC or diazepam, mice were put in the center of the open compartment, facing away from the dark one, and was allowed to explore the novel environment for 5 mins. During this period, the animals were observed and the time spent in the bright compartment and the total number of transitions in between the compartments were recorded. This experiment exploited the conflict between the animal's tendency to explore a new environment and its fear of bright light [33, 34].

**Hole - board test (HBT)**

The test was conducted as described by Takeda et al., 1998 [35]. The equipment consists of a wooden box (40 cm × 40 cm × 25 cm) with 16 equidistant holes 3 cm on the floor. The center of each hole was located 10 cm closest from the wall of the box. The box was placed 15 cm above the earth and divided into squares (10 cm × 10 cm) with a waterproof marker. Each animal was placed singly in the center of the board and the number of head dipping into the hole was recorded over a 5-min exploration period on the board. Head dipping was counted only when both eyes disappeared into the hole.

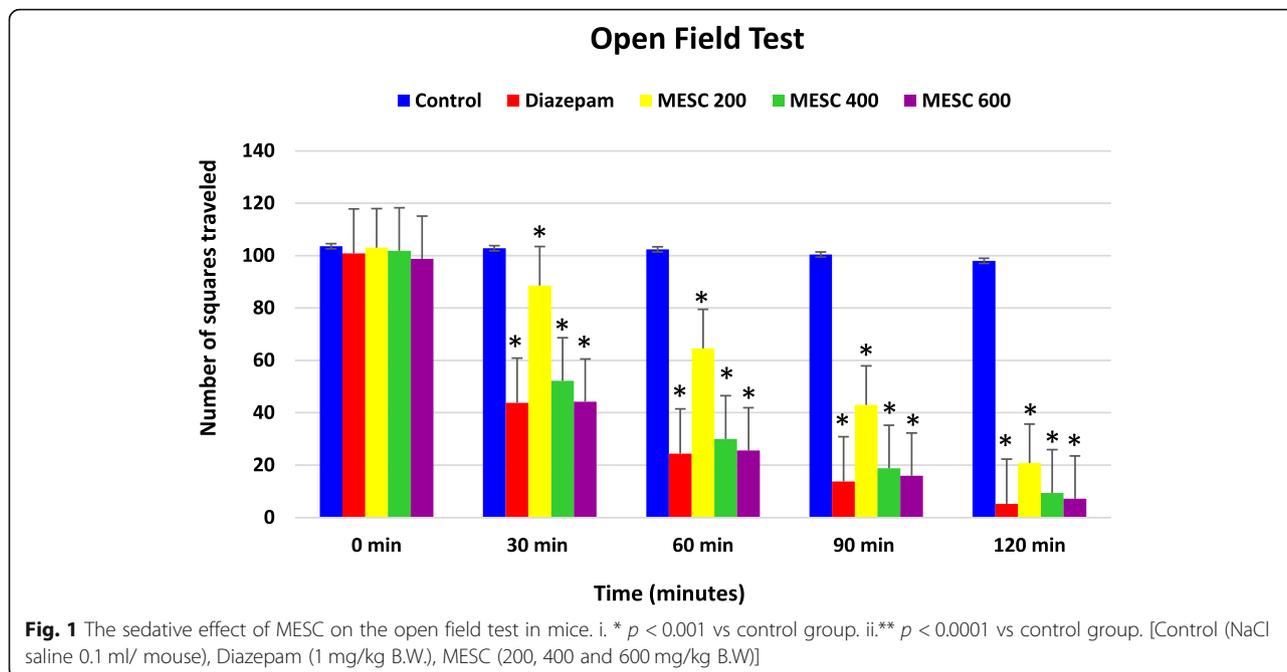
**Statistical analysis**

The statistical analysis of the results was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test in SPSS 16.0 software. The results are expressed as the mean ± standard error mean (S.E.M.) and Differences between groups were considered significant at a level of  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ .

**Table 2** The sedative effect of MESC 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 ± 0.93	106.60 ± 0.66	102.40 ± 0.97	100.40 ± 1.02	98.00 ± 1.14
Diazepam	1	100.80 ± 0.60	43.80 ± 1.07*	24.40 ± 0.81*	13.80 ± 0.80*	5.20 ± 0.37*
MESC	200	103.00 ± 0.70	88.60 ± 0.51*	64.60 ± 0.95*	43.00 ± 1.52*	20.80 ± 1.57*
MESC	400	101.80 ± 0.86	52.20 ± 1.06*	30.00 ± 0.70*	18.80 ± 0.58*	9.40 ± 0.51*
MESC	600	98.80 ± 0.58	44.20 ± 0.91*	25.60 ± 1.02*	16.00 ± 0.70*	7.20 ± 0.48*

i.\*  $p < 0.001$  vs control group  
 ii.\*\*  $p < 0.0001$  vs control group



**Results**

**Phytochemical screening**

The phytochemical screening revealed presence of alkaloid, glycoside, flavonoid, saponin, tannin, and steroid in methanol extract of *Spathodea campanulata* leaves (Table 1).

**Acute toxicity study**

No hypersensitive symptoms or mortality in mice were seen over the 72 h observation period (after oral administration of 500-4000 mg/kg MESC). This nontoxic profile of MESC convinced us to select the dose (200, 400 and 600 mg/kg) for this study.

**Sedative activity study**

**Open field and hole cross test**

Both of the open field and hole cross method exhibited CNS depressant activity. In Open field study (Table 2, Fig. 1) the number of squares crossed and in hole cross-

study (Table 3, Fig. 2) the number of holes crossed dropped significantly ( $p < 0.001$ ) at doses of 200, 400 and 600 mg/kg b.w of MESC as well as of 1 mg/kg diazepam. For open field test, one-way ANOVA pointed at a statistically significant differences between the tested groups [ANOVA:  $F (2.57,2630.91), p = 0.000$ ]. For hole-cross test, one-way ANOVA pointed at a statistically significant differences between the tested groups [ANOVA:  $F (4.82,308.35), p = 0.000$ ].

**Anxiolytic activity study**

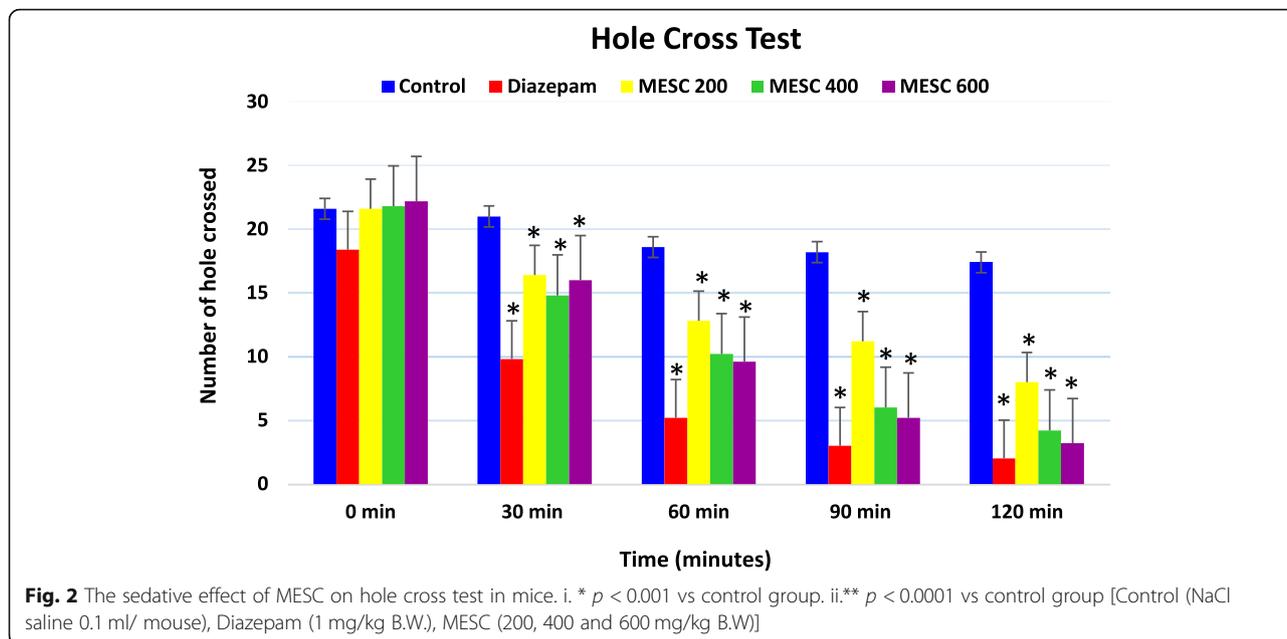
**Elevated plus maze test (EPMT)**

In EPMT the total time spent in open arms increased significantly ( $p < 0.001$ ) at all doses (200, 400 and 600 mg/kg) of MESC when compared with the control group. The data of MESC tend to similar to that of the positive control group (Table 4, Fig. 3). One-way ANOVA pointed at a statistically significant differences between the tested groups [ANOVA:  $F (164.02, 2074.70), p = 0.000$ ].

**Table 3** The sedative effect of MESC 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/mouse	21.60 ± 0.52	21.00 ± 0.84	18.60 ± 0.24	18.20 ± 0.73	17.40 ± 0.51
Diazepam	1	18.40 ± 0.51	9.80 ± 0.58*	5.20 ± 0.58*	3.00 ± 0.31*	2.00 ± 0.31*
MESC	200	21.60 ± 0.10	16.40 ± 0.51*	12.80 ± 0.37*	11.20 ± 0.37*	8.00 ± 0.31*
MESC	400	21.80 ± 2.34	14.80 ± 1.24*	10.20 ± 1.98*	6.00 ± 2.67*	4.20 ± 2.96*
MESC	600	22.20 ± 1.06	16.00 ± 0.70*	9.60 ± 0.24*	5.20 ± 0.20*	3.20 ± 0.20*

i. \*  $p < 0.001$  vs control group  
 ii.\*\*  $p < 0.0001$  vs control group.



**Light dark box test (LDBT)**

In the case of LDBT, mice treated with MESC preferred to stay more in lightbox than dark one which is similar to standard (diazepam 1 mg/kg) group and statistically significant ( $p < 0.001$ ) (Table 5, Fig. 4). One-way ANOVA pointed at a statistically significant differences between the tested groups [ANOVA: F (227.52,2389.28),  $p = 0.000$ ].

**Hole board test**

In hole board test, the number of head dipping was diminished significantly ( $p < 0.001$ ) in mice treated with MESC (at 200 and 400 mg/kg) which is very much similar to the group treated with diazepam (Table 6, Fig. 5). One-way ANOVA pointed at a statistically significant differences between the tested groups [ANOVA: F (227.52),  $p = 0.000$ ].

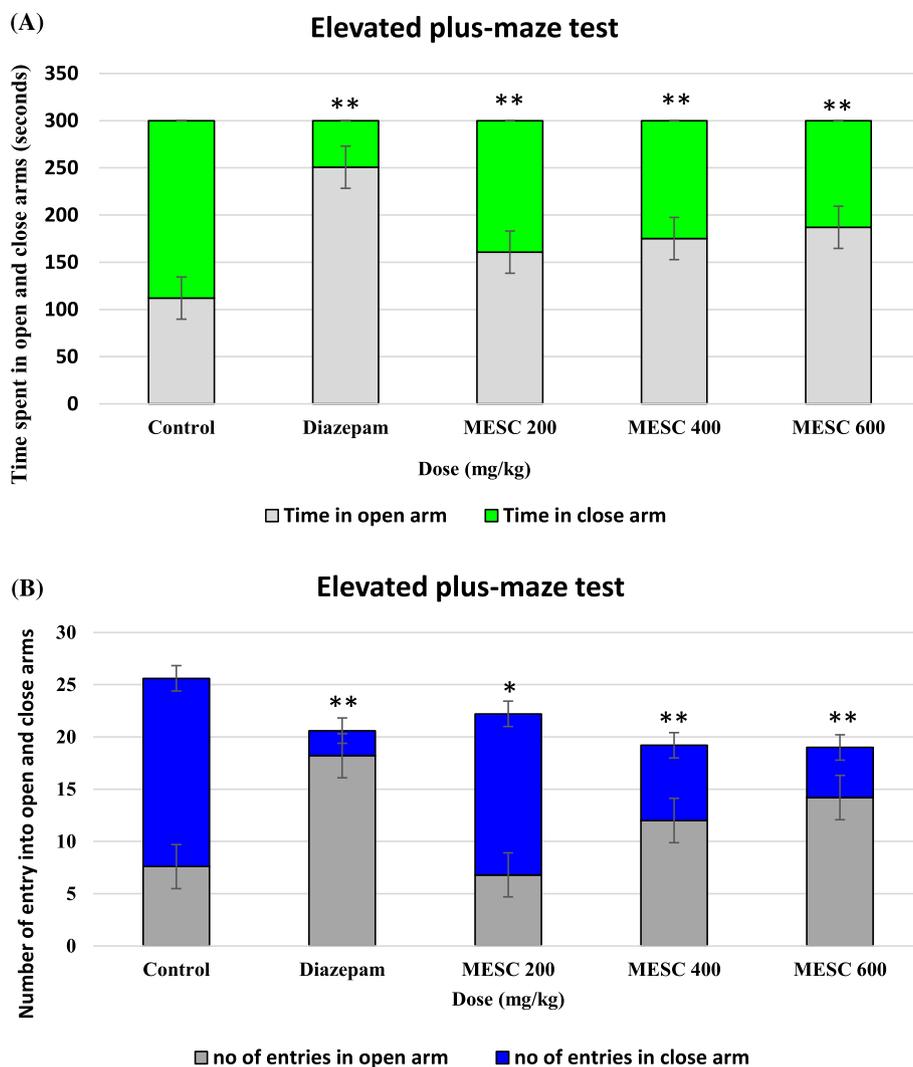
**Discussion**

Our present study was designed to investigate the CNS effect of MESC on mice and the outcome manifested that MESC has a sedative and anxiolytic effect. Our preliminary phytochemical screening suggested the presence of alkaloids, flavonoids, glycosides, carbohydrates, tannins, steroids and saponins in the MESC (Table 1). Several experimental research works demonstrated that the plant extracts rich in alkaloids and flavonoids dominate sedative and anxiolytic actions mediated through their chemical attraction (in vitro) with the benzodiazepine site of the GABA ergic complex system. Direct or indirect modulation of this receptor outcomes to this effect [36]. In addition, tannin and saponin are responsible for the non-specific depressive effect on CNS [30]. Hence, pointing to the presence of phytochemicals in the MESC, it may exhibit the sedative and anxiolytic effects on the CNS.

**Table 4** The anxiolytic effect of MESC on elevated plus-maze test in mice

Treatment	Dose (mg/kg)	Time spent in open arms (s)	Time spent in close arms (s)	Entries in open arms (s)	Entries in closed arms (s)	Total no. of entries
Control	0.1 ml/mouse	113 ± 0.83 (37.33)	187 ± 0.24 (29.67)	8 ± 0.83 (62.67)	16 ± 0.70 (70.31)	26
Diazepam	1	250.60 ± 0.92** (85.53)	49.40 ± 0.93** (16.47)	18.20 ± 0.37** (88.35)	2.40 ± 0.40** (11.65)	21
MESC	200	160.60 ± 1.88** (53.53)	139.40 ± 0.54** (30.63)	6.80 ± 1.88* (46.47)	15.40 ± 0.34* (69.37)	22
MESC	400	175.20 ± 0.86** (58.40)	124.80 ± 0.86** (62.50)	12.00 ± 0.37** (41.60)	7.20 ± 0.25** (37.50)	19
MESC	600	187.00 ± 2.02** (62.33)	113.00 ± 2.02** (74.74)	14.20 ± 0.58** (37.67)	4.80 ± 0.37** (25.26)	19

i. \*  $p < 0.001$  vs control group  
 ii.\*\*  $p < 0.0001$  vs control group  
 # within bracket result expressed in percentage (%)



**Fig. 3** The anxiolytic effect of MESC on elevated plus-maze test in mice. **a** Time spent in open and close arms; **b** Number of entry into open and close arms. i. \*  $p < 0.001$  vs control group. ii.\*\*  $p < 0.0001$  vs control group. [Control (NaCl saline 0.1 ml/ mouse), Diazepam (1 mg/kg B.W.), MESC (200, 400 and 600 mg/kg B.W.)]

To investigate the explorative behavior of the animals, most frequently followed methods are an open field and hole cross methods. It is substantially demonstrated that benzodiazepine-like drugs decrease the locomotor activity of animals by oppressing the curiosity of the animals

about a new environment [37]. In the open field test, sedative action of diazepam decreased the number of squares crossed by mice and thus suppressed their expanding in the new environment. The experimental data of MESC reported in open field tests showed

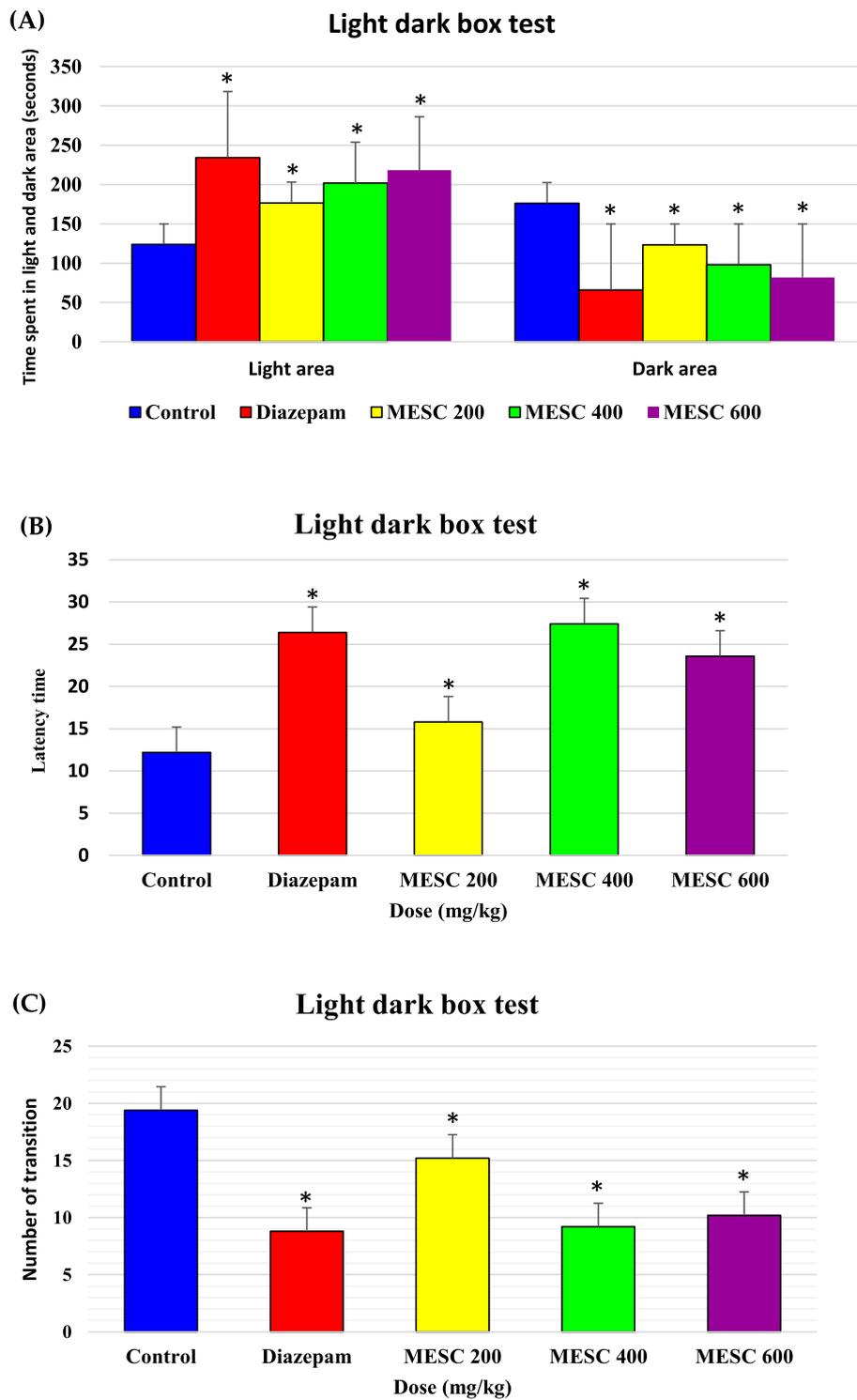
**Table 5** The anxiolytic effect of MESC on light-dark box test in mice

Treatment	Dose (mg/kg)	Latency time (s)	Transitions	Time spent in the light box (s)	Time spent in the dark Box (s)
Control	0.1 ml/mouse	12.20 ± 0.37	19.40 ± 0.50	123.80 ± 0.73 ( <b>41.27</b> )	176.20 ± 0.73 ( <b>58.73</b> )
Diazepam	1	26.40 ± 0.51*	8.80 ± 0.37*	234.20 ± 1.01* ( <b>78.07</b> )	65.80 ± 1.01* ( <b>21.93</b> )
MESC	200	15.80 ± 0.37*	15.20 ± 0.37*	176.60 ± 2.61* ( <b>48.53</b> )	123.40 ± 2.61* ( <b>51.47</b> )
MESC	400	21.20 ± 0.24*	11.80 ± 0.37*	202.00 ± 1.0* ( <b>67.33</b> )	98.00 ± 1.0* ( <b>32.67</b> )
MESC	600	23.60 ± 0.74*	10.20 ± 0.37*	218.20 ± 2.14* ( <b>72.73</b> )	81.80 ± 2.14* ( <b>27.27</b> )

i. \*  $p < 0.001$  vs control group

ii.\*\*  $p < 0.0001$  vs control group.

# within bracket result expressed in percentage (%).



**Fig. 4** The anxiolytic effect of MESC on light-dark box test in mice. **a** Time spent in light and dark area; **b** Latency time; and **c** Number of transition. i. \*  $p < 0.001$  vs control group. ii.\*\*  $p < 0.0001$  vs control group. [Control (NaCl saline 0.1 ml/ mouse), Diazepam (1 mg/kg B.W.), MESC (200, 400 and 600 mg/kg B.W.)]

**Table 6** The anxiolytic effect of MESC on hole-board test in mice

Treatment	Dose (mg/kg)	Number of head dips
Control	0.1 ml/mouse	20.00 ± 1.24
Diazepam	1	56.80 ± 1.58**
MESC	200	28.20 ± 1.28**
MESC	400	36.00 ± 2.41**
MESC	600	48.20 ± 0.59**

i. \*  $p < 0.001$  vs control group  
 ii.\*\*  $p < 0.0001$  vs control group.

satisfactory behavioral changes similar to diazepam which conserves the sedative effect (Table 2, Fig. 1).

In the hole cross test, MESC diminished the count of hole crossed by mice which were quite similar to responses of that found in mice treated with diazepam. This observation reflects the characteristics of the sedative activity of MESC which is comparable to benzodiazepine (BDZ) compounds (Table 3, Fig. 2).

The anxiolytic action of MESC was evaluated by elevated plus-maze, light-dark box, and hole-board test. The open arm behaviors of mice in EPM reflect a conflict between the animal’s instinctive manner to keep itself in a protected domain (e.g. closed arms) and motive to explore in a new environment, where the anxiolytic components stimulate their exploratory activities in the open arm [31, 38].

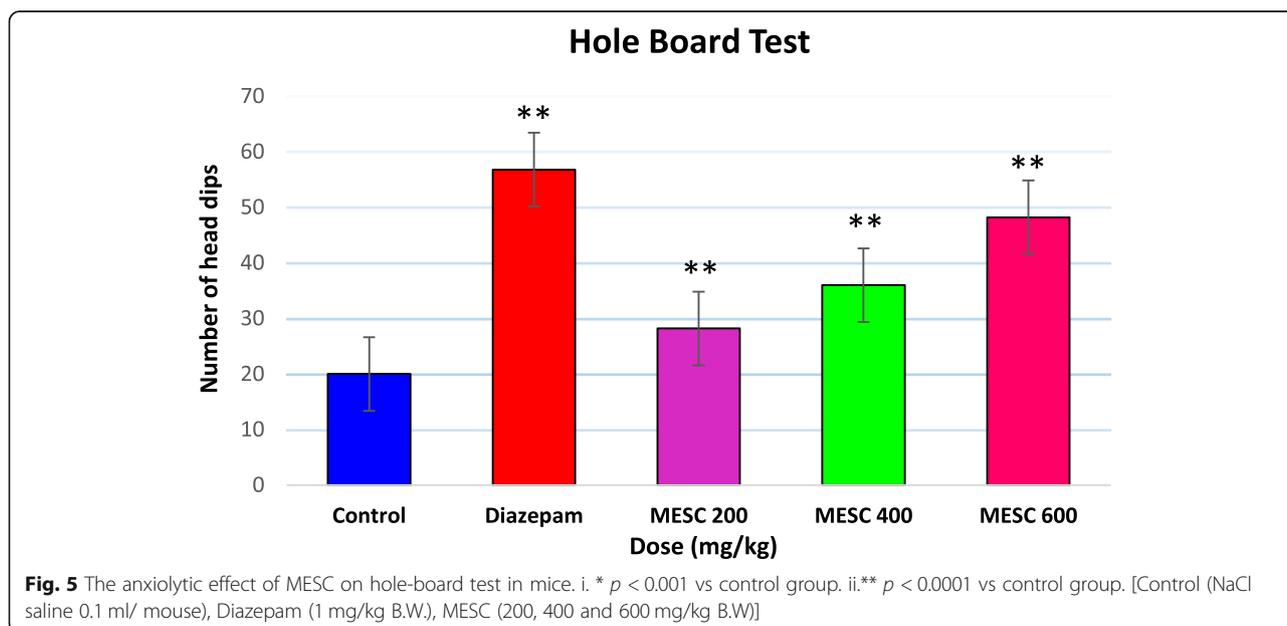
Here in EPMT, standard anxiolytic agent (diazepam) significantly increased both the number of open arm entries and the total time spent in open arms. Similar observations noticed in mice treated with MESC where both of the number of entries and the proportion of

time spent in open arms of the maze were increased significantly (Table 4, Fig. 3).

Besides, the anxiolytic effect testified via the light-dark box test which is a popular scientific tool to study anxiolytic or anxiogenic agents [39]. It has been presumed that the time spent in bright compartment of the box is most useful and consistent factor to connote anxiety. This experiment express anxiolytic properties of MESC as it enhanced the time spent in the light compartment (Table 5, Fig. 4).

Furthermore, to observe anxiolytic effects the hole-board test (Table 6, Fig. 5) renders an easy technique to assess the pattern of the response of rodents to a foreign environment. In this method it is easy to observe and quantify the behavioral response, however, the head-dipping behavior of the animal is directly related to their emotional feeling [40]. The frequency and period of head-dipping are accepted to measure neophilia (or directed exploration), that are independent from the general locomotor activity of mice [41, 42]. Typically, high levels of head-dipping are translated as an index of neophilia, while low levels indicate lack of neophilia or reflect a high anxiety-like state in the animal [43, 44].

GABA assist to modulate motion control, vision, anxiety, and many other functions of the central nervous system. The anxiolytic, and sedative activities of benzodiazepines like diazepam are particularly attributed to enhancing the action of gamma-aminobutyric acid (GABA) which is a major inhibitory neurotransmitter in the central nervous system. Diazepam largely mediates its effects via the GABA<sub>A</sub> receptor [45]. GABA<sub>A</sub> receptors are fast-acting neurotransmitter receptors with an integral Cl<sup>-</sup> channel and several allosteric binding sites.



Benzodiazepines bind at the alpha ( $\alpha$ ) subunit of GABA<sub>A</sub> receptor and prolong the associated chloride channel opening which ultimately prolong the inhibitory state of action potential (prolonging the hyperpolarization state) leading to sedation or anxiolytic effect [46, 47]. Hence, in present investigation benzodiazepine used as a standard drug, the demonstrated sedative and anxiolytic effect of MESC convinced us to speculate that the MESC might act via interaction on GABA<sub>A</sub> subunit.

GABA is synthesized by the enzymatic [glutamic acid decarboxylase (GAD)] catalysis of glutamic acid and GABA-transaminase (GABA-T) enzyme catabolize GABA into succinic semialdehyde [48]. Nevertheless, some plant might contain GABA-T inhibitor, which can raise brain GABA level, thus reduce anxiety. Additionally, by membrane GABA transporters (GAT), released GABA is reuptaken into both presynaptic neurons and circumventing glial cells from the synaptic cleft. Thus, Selective GABA transporter Inhibition can suppress the re-uptake of GABA which ultimately reduces anxiety by enhancing GABA activity [49].

In CNS, L-glycine acts as another major inhibitory neurotransmitter which has profound sedative and anti-anxiety effect, while also being capable of improving mood and cognition. Thus, the sedative and anxiolytic effects of MESC might be due to the direct activation of postsynaptic glycine receptors [50].

However, Suppression of action of anxiogenic hormone-like glutamate, serotonin, cholecystokinin, corticotrophin, and acetylcholine may reduce anxiety level [51].

In this connection, alkaloids, glycosides, and flavonoids in plant extract exhibit sedative and anxiolytic-like actions through the interaction with GABA<sub>A</sub> receptors [52–54]. Based on the result of the current investigation and previous reports we can say that the phytochemicals as mentioned earlier present in *S. campanulata* are sedative and anxiolytic.

## Conclusion

In conclusion, the present findings in our study indicate that MESC possesses prominent sedative and anxiolytic activity. The effect is rapid, long-lasting, and statistically significant at all the experimental doses tested. Therefore, these results provide the scientific validation for the use of this plant in traditional medicine in the treatment of various ailments related to CNS disorders. However, further studies are needed to isolate the bioactive compound(s) and elucidate the precise molecular mechanisms responsible for the pharmacological activities of the plant. These bioactivity-guided phytopharmacological works will allow us to identify pharmaceutical lead(s) with better tolerability and lesser side effects in new drug development.

## Abbreviations

MESC: Methanol extract of *Spathodea ampanulata* leaves; b.w: Body weight; i.p: Intraperitoneal; p.o.: Per oral; 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

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## Authors' contributions

AB designed, conceived, coordinated and supervised the research work. PB performed experimental work. AB drafted the manuscript, did statistical analysis and interpreted results. MSAM have drawn the graph. AB critically revised the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

All the tested mice were treated according to the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) developed by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The experimental design was authorized by the Institutional Animal Ethical Committee (SUB/IAEC/18) of Stamford University Bangladesh (consent no: 01/2018, date of project approval 10/04/2018).

## Consent for publication

Not applicable.

## Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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