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Original article

In vitro cytotoxic and *in vivo* anxiolytic study of methanolic crude extract of *Sterculia villosa* seeds

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Summary. This study aimed to evaluate the *in vitro* cytotoxic and *in vivo* anxiolytic and sedative activities of the methanolic extract of *Sterculia villosa* roxb seeds. The dried powder of the seeds was extracted with methanol which was then tested to ascertain the neuropharmacological and cytotoxic potentials. The methanolic extract of *Sterculia villosa* roxb were subjected to Brine Shrimp lethality bioassay for possible cytotoxicity having LC_{50} of 8.672 µg/ml. However, fractions produced concentration dependent increase in percent of mortality of Brine Shrimp nauplii which indicated the presence of cytotoxic property. Study continued to investigate possible sedative and anxiolytic activity of the methanolic seed extract of *Sterculia villosa* roxb in mice. This study includes hole cross, open field, thiopental-sodium induced sleeping time and elevated-plus maze (EPM) tests at the dose of 200 mg/kg while on the peripheral and central nervous system the extract mild to moderately decreased the locomotor activity of mice in hole cross, open field and EPM test. However, the extract minimized the onset of sleep moderately and had maximized the duration of sleep slightly, when administered with thiopental sodium.

Key words: sedative properties, CNS depressant activity, locomotor activity, cytotoxicity

Introduction

Plants have different types of chemical constituents like alkaloid, tannin, flavonoid and phenolic compounds and from ancient times to recent days, most of drugs are directly or indirectly derived from these chemical classes. Approximately 30 % of present medicine either directly sourced or indirectly sourced from plants (1). Plants including, *Valeriana officinalis*, *Passiflora incarnate*, *Melissa officinalis*, *Humulus lupulus* are most common globally as sleep inducers in the treatment of insomnia and there are also some plants that possess potential tranquilization besides used in insomnia treatment (2).

Sterculia villosa Roxb is a member of Sterculiaceae family. This tropical plant is been locally reported to possess several medicinal properties. These properties includes, treatment of inflammation, seminal weakness, urinary problems, rheumatism, throat infection and also has anthelmintic, antidiabetic, antimicrobial, membrane stabilization and antithrombotic activity; cooling and aphrodisiac properties (3). This plant also gains popularity among tropical region as a refreshing and relaxing drink. Therefore, we tried to find out the methanolic extract from the seeds of *S. villosa* Roxb seeds having any sedative activity or not as the sedative effect of this plant does not investigated yet. This study work was conducted to establish the sedative effect of methanolic crude extract of *Sterculia villosa* Roxb seeds

using hole cross test, open field test, elevated plus-maze (EPM) test, thiopental sodium induced sleeping time test in Swiss albino mice for the first time.

Methods

Plant sample collection

Mature, fresh seeds of *Sterculia villosa* Roxb were collected in October, 2012 from Bandorban district, Bangladesh. Then the sample was identified and authenticated. A voucher specimen (Accession number: DP/CU/2012/PS-00812) has also been maintained in the herbarium, department of forest research institute, Chittagong. Later, the seeds were washed thoroughly, dried and crushed into powder using an electric grinder (Moulinex Blender AK-241, Moulinex, France).

Preparation of extracts

About 500 gm of the seeds powder were taken in a clean, flat bottom glass container and was filled with one litter of methanol. The container was sealed with foil paper and kept at room temperature for seven days. The contents of the glass container was shaken and stirred occasionally. After seven days, the mixture was filtered through a cotton plug and followed by Whatmenn filter paper No. 1. The filtrate was concentrated by rotary evaporator (RE 200, Sterling, UK) at 45 °C temperature. The yield of the extracts was 4 –5.5 % W/W (4).

Animals

Swiss albino mice (25–30 gm) were collected from International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, b) and housed under standard laboratory conditions (relative humidity 55–65 %, room temperature 23.0 ± 2.0°C) in a 12 h light dark cycle and acclimatized for 7 days where the experiment would take place. The animals were fed with standard rodent food and water formulated by ICDDR, b. This experiment involving animals was approved by the Ethical Review Committee at the Department of Pharmacy, International Islamic University Chittagong, Bangladesh and conducted according to their guidelines (Ref.: IIUC/DP/RC/2012/008).

Trial registration

For experimental clinical study on animal trial registration and permission was issued from departmental clinical ethical review committee, Department of Pharmacy, International Islamic University Chittagong. The trail registration reference number is IIUC/DP/RC/2012/008.

Hole cross test

In this experiment, a steel cage having a size of 30 × 20 × 14 cm was used. A partition which has a hole of 3 cm diameter at a height of 7.5 cm was fixed in the middle of the cage. At first, the animals were divided into three groups namely control, positive and test containing five animals in each group. Then, in the test group methanolic extract of *Sterculia villosa* Roxb at a dose of 200 mg/kg body weight were administered orally and followed by the control and positive control group were treated with vehicle (1 % Tween 80 in water at a dose of 10 ml/Kg per oral) and diazepam (at a dose of 1 mg/Kg intra peritoneal) respectively. After that, the animals were introduced into the cage and the number of passages from one chamber to other through the hole inside the cage was counted for 3 min on 0, 30, 60, 90 and 120 min intervals (5). Figure 1 illustrates the results, that were obtained for the hole cross test.

Open field test

In this experiment, open fields apparatus consist of 30 cm × 50 cm white box with walls of 27 cm were used to study the spontaneous locomotive and exploratory activity in mice. At first, the area of the open field were divided into a series of square blocks and colored black and white alternatively. The apparatus were kept in a light and sound-attenuated room. Then, the animals were divided into three groups namely test, control and positive control consist of five animals in each group. After that, the animals were treated with seeds extract (200 mg/kg body weight, orally), vehicle (1 % Tween 80 in water at a dose of 10 ml/Kg per oral) and diazepam (at a dose of 1 mg/Kg intra peritoneal). The mice were then kept into the apparatus and the number of square blocks visited by each mouse was calculated for 3 min on 0, 30, 60, 90 and 120 min intervals (6). Figure 2 illustrates the results that were obtained for the open field test.

Elevated plus-maze (EPM) test

This test was performed according to a previously described method (7) with minor modifications. The maze apparatus was consisting of two open arms (5 × 10 × 0.5 cm) and two closed arms (5 × 10 × 15 cm). The arms were radiated from a central platform (5 × 5 cm) and elevated to a height of 40 cm above the floor. The entire maze apparatus was made of dark opaque wood. The animals were divided into two groups (n = 5) and treated with *Sterculia villosa* seeds extract (200 mg/kg body weight, orally) and diazepam (at a dose of 1 mg/Kg intra peritoneal). After sixty minutes, each animal was placed at the center of the maze and observed for five minutes. Then, the number of open arm entries which is defining as entry of all four paws into an arm was recorded. The results obtained for the elevated plus-maze (EPM) test were displayed in Table 2.

Thiopental sodium induced sleeping time test

In this test, the effect of *Sterculia villosa* seeds extract on thiopental sodium induced sleeping time on mice was evaluated by a previously described method with a small modification. Swiss albino mice were separated into three groups of five animals each. In the test group seeds extract (200 mg/kg body weight, orally) was given, the control group receive vehicle (1 % Tween 80 in water at a dose of 10 ml/Kg per oral) and diazepam (at a dose of 1 mg/Kg intra peritoneal) was administered to the positive control group. Then, twenty minutes later each animals were treated with thiopental sodium (40 mg/kg body weight, intraperitoneally). After that, the animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) (8). Both the results for the onset of sleep and the duration of the sleeping time were shown in Figure 3.

Statistical analysis

Results were expressed as mean ± SEM. One-way ANOVA was used for analysis of data. Differences were considered significant at $P \leq 0.05$.

Results

Brine Shrimp Lethality Bioassay

The results of brine shrimp lethality bioassay are shown in the table 1. Test samples showed different mortality rate at different concentration. The mortality rate of brine shrimp napulii was found to be increased with the increase concentration of the sample. The median lethal concentration (LC_{50}) was calculated. The LC_{50} values of methanol of *S. villosa roxb* is 8.672 µg/ml. So, it is evident that the methanol extract of *S. villosa roxb* may be a good candidate as anticancer drug.

Hole cross test

In the evaluation of sedative effects of *Sterculia villosa* we have started our journey with hole cross test and open field test to record the spontaneous locomotor activity. The result was presented on Figure 1. From

Table 1: Cytotoxic effect of *S. villosa roxb* (Methanolic extract) on shrimp nauplii

Conc. µg/ml	Log C value of conc.	No. of nauplii taken	No. of nauplii dead	% mortaliy	Probit value of % mortality	LC_{50} µg/ml
3.125	**0.49**	20	4	20	**4.16**	
6.25	**0.79**	20	7	35	**4.62**	
12.5	**1.09**	20	13	65	**5.38**	8.672
25	**1.39**	20	17	85	**6.04**	
50	**1.69**	20	18	90	**6.28**	
100	**2**	20	19	95	**6.65**	
200	**2.3**	20	20	100	-	

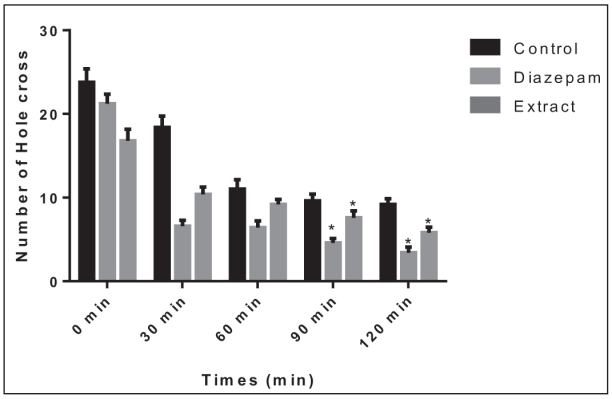

Figure 1: Hole cross test of methanolic extract of *S. villosa,* all values are expressed as mean ± SEM (n = 5), *p < 0.05; One way Analysis of Variance (ANOVA).

the result it is evident that *Sterculia villosa* caused a marked reduction in the number of hole crossed and such inhibitory effect was started at 30 min and continued up to 120 min after administration of methanolic extract of *Sterculia villosa*.

Open field test

The result was represented in Figure 2. From the result it is evident that, the plant produces sedative effect (decrease spontaneous locomotion). The sedative effect was found at 30 min and continued up to 120 min.

Elevated plus-maze (EPM) test

The results of EPM test were presented in the Table 2. The result revealed that diazepam at a very small dose (1 mg/kg) has significant percentage of open arm entry. Methanol extract of *Sterculia villosa* at a dose of 200 mg/kg also substantially increase the percentage of open arm entry.

Thiopental sodium induced sleeping time test

The sedative effect of the *Sterculia villosa* seeds extract was confirmed upon pretreatment of the animals with the plant extract lengthen the sleeping time and shorten the sleep latency (Figure 3). Effect of *Sterculia villosa* on sleep latency and sleeping time is more intense than diazepam which is a standard sedative drug (9). *Sterculia villosa* exhibits synergistic sedative and hypnotic action with thiopental sodium.

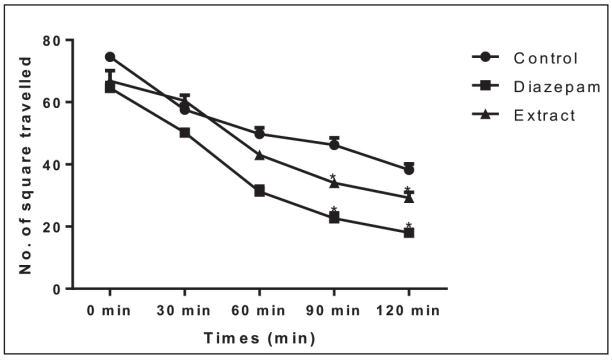

Figure 2: Open field test of methanolic extract of *S. villosa,* all values are expressed as mean ± SEM (n = 5), *p<0.05; One way Analysis of Variance (ANOVA).

Table 2: CNS depressant activity of *Sterculia villosa* extract on EPM test in mice

Groups	% Entry into open arm	% Time spent in open arm
Control	**46.84 ± 7.493**	**47.77 ± 10.869**
Diazepam	**77.14 ± 2.477***	**78.20 ± 5.369***
Extract	**52.67 ± 1.155***	**35.77 ± 8.667**

*All values are expressed as mean ± SEM (n = 5); One way Analysis of Variance (ANOVA). *p < 0.05, significant compared to control*

Discussion

Diazepam is a member of the benzodiazepine group that suppresses CNS and used in the sleeps disorders management. This drugs class interacts with GABA receptor and forms an ionophore complex (8). Diazepam has reduced onset of and increased duration of sleep time, while decreases activity, anxiety and calms the nerves; it also potentiates action of barbiturates and enhance sedation (8).

In both the Hole cross and Open field test any agents with sedative property will reduce the number of locomotion, understood as of lacking curiosity to new environment (8). Locomotor activity is an indica-

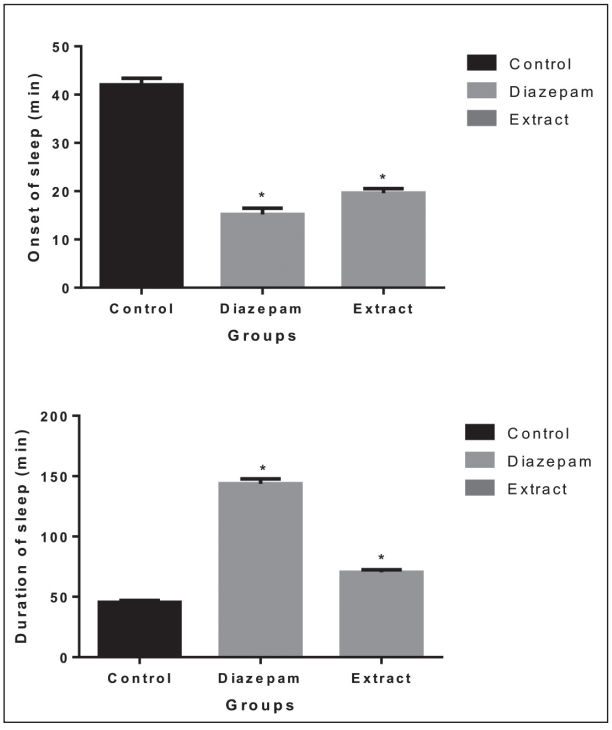

Figure 3: Thiopental induced sleeping time test of methanolic extract of *S. villosa,* all values are expressed as mean ± SEM (n = 5), *p < 0.05; One way Analysis of Variance (ANOVA).

tor of mental wakefulness or alertness and decrease locomotion which is indicative of calmness and sedation could be interpret as reduced CNS excitability (10). *Sterculia villosa* seeds extract decrease the number of hole crossed and the number of square blocks crossed in 30 min and subsequently influenced the locomotor activity in mice which indicates the sedative property.

The elevated plus-maze is considered as a popular model of animal anxiety and hence justified for used in rats and mice (7). The EPM test was performed here to evaluate the anxiolytic potential of *Sterculia villosa*. The parameter which was examined in the EPM test namely percent of open arm entry are sensitive to agents are thought to act through the GABAA receptor complex and validate the use of diazepam as a positive control. Diazepam confirms the anxiolytic effects by increasing the percentage of open arm entry (7). The results of the EPM test as displayed in Table 2 clearly disclose that methanolic extract of *Sterculia villosa* has potential anxiolytic effect, but lack of duration, this may be due to different phytochemical mixture.

Thiopental sodium belongs to the group barbiturate and induces sleep in both humans and rodents. The thiopental sodium induced sleeping time test in mice was used to investigate the sedative – hypnotic drugs (8). In our study, oral administration of *Sterculia villosa* extract 20 min before the thiopental sodium induces sleep and similar effects were observed with diazepam. Much evidence have suggested that, CNS depressant barbiturates like Thiopental sodium binds to the barbiturate binding site on the GABA receptor complex and stimulate GABA mediated hyper polarization of postsynaptic neurons (8). From our results close relationship between the sedative effect of *Sterculia villosa* and diazepam could be suggested.

The methanolic extract have been investigated for LC_{50} to ensure the cellular viability, its been found that the dose (Table 1) caused 50% death of experimented cells is moderate, thus this plant is safe to use. Phytochemical investigation of methanol extract *Sterculia villosa* under this study explored the presence of medicinally active secondary metabolites alkaloids, glycoside, steroids, tannins, terpenoids and flavonoids. The sedative and anxiolytic potential may be due to the presence of those secondary metabolites, which need further investigation to confirm and isolation.

Conclusion

The findings of our study strongly validate the rapid, long-lasting and statistically significant sedative activity of *Sterculia villosa*. Though, further extensive research is needed to isolate the active principles of the plants and to understand the underlying mechanism behind the pharmacological activity of this plant.

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Authors' contributions

We worked together as a research group in this project. SB and MB contributed in the conception and design of study; assist in drafting and revising the manuscript. SA and MH participated in the design of study; acquisition, data collection, analysis and interpretation of data; literature search. All authors read and approved the final manuscript.

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