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Sedative Activity of Methanolic Extract of *Glochidion multiloculare* (Rottler ex Willd*)* Voigt Leaves

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Abstract: Bangladesh is a good repository of medicinal plants. Traditional healers utilize them for treating many pathological states. Unfortunately, very few of them have been scientifically evaluated to know about the deep inside. The current study here is designed to evaluate the *in vivo* sedative activity of the leaves of *Glochidion multiloculare* (Rottler ex Willd) Voigt. With this purpose, the plant leaves were collected and powdered for extraction with methanol. Initially, the plant extract was subjected to brine shrimp lethality bioassay to monitor the presence of bioactive molecules. Later on, different neuropharmacological studies including hole cross, open field, thiopental-sodium induced sleeping time and Elevated-Plus Maze (EPM) tests were conducted to investigate sedative action. In the brine shrimp lethality bioassay, the LC_{50} value of the extract was found 37.19 μg mL^{-1}, whereas the standard vincristine sulphate showed the LC_{50} 10.50 μg mL^{-1}. The moderate toxicity of the extract on brine shrimp indicated the existence of bioactive secondary metabolites in this extract. Besides, the extract decreased the locomotor activity of mice in hole cross, open field and EPM test indicating the CNS depression capability of the plant. Moreover, the extract was very much effective for prolonging the sleeping time (103 min) with quick onset of action (22 min) in comparison to the control group. The efficacy of the plant extract was found closer to the common sedative drug diazepam. Further investigations are required to explore the underlying mechanism of the sedative action and isolate bioactive principles.

Key words: *Glochidion multiloculare*, sedative activity, hole cross, open field, thiopental-sodium induced sleeping time, elevated-plus maze

INTRODUCTION

Plants are the natural reservoir of numerous medicinal elements which globally contribute a lot to the health management of a large number of populations globally. The World Health Organization (WHO) acknowledges the herbal medicine in health care due to their numerous benefits (Aschwanden, 2001). In the recent trend, many countries are also promoting the standardization of the plant materials for ensuring proper safety and efficacy of the drug (Huang *et al*., 2008).

Bangladesh is located in the tropical zone and blessed with a lot of herbal resources. Traditionally people of Bangladesh like to use plant derived drugs as home remedy. Besides, a lot herbal preparations have been marketed for herbal healing by many pharmaceutical companies. For exploring the multidimensional effects of the plants of Bangladesh, proper scientific investigations are required.

People sometimes suffer a lot to have a comfortable sleep due to many pathological or social factors. Sedatives are sometimes useful to provide relief. Plant sources might be a very useful for displaying the sedative effects (Cardoso-Taketa *et al*., 2008; Li *et al*., 2007; Ratnasooriya *et al*., 2006; Rolland *et al*., 1991; Schulz *et al*., 1998; Sharma *et al*., 2012a, b). In this trend, we have investigated the plant *G. multiloculare*.

G. multiloculare (Local name: Kudurpala, Family: Phyllanthaceae) indigenous to south asian subcontinent. In Bangladesh it is found in the Hills of Chittagong, Chittagong Hill Tracts, Comilla and Dinajpur. Fruits of this plant are used to treat dysentery, diarrhoea, and cough. Previous phytochemical investigations led to the isolation of glochidonol, glochidiol, glochidone, lupeol, daucosterol and stigmasterol (Hasan *et al*., 2012).

The aim of the present study was to explore the biological potentiality of this plant with special emphasis on sedative property.

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MATERIALS AND METHODS

Plant materials: The fresh plant was collected from local area of Chittagong, Bangladesh and identified by Dr. Sheikh Bokhtear Uddin, Department of Botany, University of Chittagong.

Preparation of extract: The collected fresh plant leaves were washed thoroughly with water and then air dried for a week at 35-40°C and pulverized in electric grinder. The obtained powder was successively extracted in methanol and filtered by Whatman filter paper. The filtrate so obtained was then concentrated to dryness through the evaporation of solvent using rotary evaporator under reduced pressure.

Animals: The study was conducted on Swiss albino mice purchased from International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were five to six weeks of age, weighing about 30-35 g, which were housed in colony cages (six mice per cages) at an ambient temperature of 23±2°C and relative humidity 50-60% with 12 h light and dark cycles having proper ventilation in the room. The mice were fed normal diets purchased commercially from the vendors and water *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for one week and then randomly divided into groups for experiments.

Brine shrimp lethality bioassay: This assay was performed on brine shrimp nauplii using method of Meyer *et al.*, (1982). In this experiment simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop of Chittagong, Bangladesh and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The test sample of crude extract was prepared by dissolving them in DMSO (not more than 0.01% v/v) plus sea water (3.8% NaCl in water) to attain concentrations of 12.5, 25, 50, 100, 200 and 400 μg mL^{-1}. A vial containing DMSO diluted in seawater was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From the obtained data, the percent (%) of mortality of the brine shrimp nauplii was calculated. The median lethal concentration, LC_{50} was then determined using Probit analysis.

Hole cross test: This test was performed by the method described by Takagi *et al.* (1971)for screening sedative activity in mice. The animals were divided into three groups-control, positive control and test. The test groups received methanolic extract of *G. multiloculare* leaves at the doses of 200 mg kg^{-1} body weight (b.wt.) orally whereas the control group received vehicle (1% Tween 80 in water) at dose of 10 mL kg^{-1} per oral (p.o). A steel partition was made in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The total number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after the oral treatment with test drugs. In this test diazepam was used in the positive control group as reference standard at the dose of 1 mg kg^{-1} intra peritoneal (i.p).

Open field test: The experiment was observed according to the methods described by Gupta *et al.* (1971). The dose for extract (200 mg kg^{-1} b.wt., p.o.) for vehicle (1% Tween 80 in water, 10 mL kg^{-1} p.o.) and for standard (Diazepam-1 mg kg^{-1}, p.o.) was maintained throughout the experiment. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The wall of this apparatus was 40 cm height. During the study period the total number of squares visited by the mice was counted for 3 min on 0, 30, 60, 90 and 120 min.

Elevated plus maze test: This experiment was previously performed by Lister (1987). The instrument used here consists of two open arms (5×10 cm) and two closed arms (5×10×15 cm) radiating from a platform (5×5 cm) to give the apparatus a plus sign appearance. The apparatus was situated 40 cm above the floor in which the open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. Dark opaque wood was used to make maze floor and walls. Sixty minutes after administration of the test drugs; each animal was placed at the center of the maze facing one of the enclosed arms. During the five min test period, the number of entry and duration of staying into open arms was recorded. The entry into an arm was defined as the point when the animal places all four paws onto the arm. The sound free room and observations were made from an adjacent corner was conducted. The same dose and route of hole cross test was used for this test.

Thiopental sodium induced sleeping time test: According to the experiment of Ferrini *et al.* (1974), animals were

randomly divided into three groups consisting of five mice each. The test groups were received methanolic extract of the leaves of *G. multiloculare* at dose 200 mg kg^{-1} (p.o.) body weight while the standard group was treated with diazepam (1 mg kg^{-1}, p.o.) and control group with vehicle (1% Tween 80 in water, 10 mL kg^{-1} b. wt., p.o.). Twenty minutes later, thiopental sodium (40 mg kg^{-1}, i.p.) were administered to each mouse to induce sleep. During the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep i.e., time between the loss and recovery of righting reflex the animals were observed.

Statistical analysis: All obtained data were expressed as mean±standard deviation (n = 5) and were analyzed by one way ANOVA followed by using Dunnett's test. The differences were considered significant at *p<0.05.

RESULTS

Brine shrimp lethality bioassay for bioactive compounds: The mortality rate of brine shrimp napulii was increased with the increase with the concentration of the sample. The LC_{50} value of the extract was 37.19 μg mL^{-1} where the LC_{50} of standard vincristine sulphate was 10.50 μg mL^{-1}. No mortality was found in the control group, using DMSO and sea water.

Open field test: In this test, the total number of squares traveled by the mice was suppressed significantly in the test group throughout the study period (Table 1). The sedative activity obtained for extract was statistically significant.

Hole cross test: The total numbers of hole crossed from one chamber to another by mice were counted for control, standard and test group (Table 2). In the hole cross test, the extracts showed a decrease in locomotion in the test animals during observation period as evident by the reduction in number of hole crossed by the treated mice compared to the control group. The result was comparable to the reference drug diazepam and was statistically significant (*p<0.05).

Elevated plus maze (EPM) test: The methanolic extract of *G. multiloculare* at the dose of 200 mg kg^{-1} body weight, showed the entries of mice into the open arms and the time spent in the open arms 40.11 and 30.89%, respectively. These values are significantly lower than those of control group. Result of EPM test is presented in Table 3.

Table 1: CNS depressant activity of methanol extract of *G. multiloculare* leaves on open field test in mice

Time (min)	Total No. of squares travelled		
	Control	Diazepam	Extract
0	75.67±4.42	65.00±3.21	67.67±5.22
30	72.00±2.43	54.33±4.26	56.00±4.22*
60	70.33±2.48	37.67±5.87*	38.33±6.88*
90	71.33±3.24	19.33±3.45*	27.67±4.82*
120	68.67±1.22	20.33±2.32*	22.67±3.23*

All values are expressed as mean±standard deviation (n = 5); One way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05, significant compared to control

Table 2: CNS depressant activity of methanolic extract of *G. multiloculare* on hole cross test in mice

Time (min)	Total No. of hole crossed		
	Control	Diazepam	Extract
0	18.67±0.764	15.00±1.000	16.67±1.041
30	14.67±1.258	6.00±1.323*	11.67±2.082*
60	15.33±0.764	4.67±1.041*	6.67±1.893*
90	16.67±0.764	2.67±0.289*	3.33±0.289*
120	15.00±0.500	2.00±0.500*	3.33±0.289*

Values were expressed as mean±standard deviation (n = 5); One way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05, significant compared to control

Table 3: CNS depressant activity of methanolic extract of leaves of *G. multiloculare* on elevated plus maze test in mice

Groups	Entry into open arm (%)	Time spent in open arm (%)
Control	54.61±2.522	43.22±10.165
Diazepam	37.14±2.477*	28.53±3.607*
Extract	40.11±3.293*	30.89±6.144*

Values are expressed as mean±standard deviation (n = 5); One way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05, significant compared to control

Table 4: CNS depressant activity of methanolic extract of *G. multiloculare* on thiopental sodium induced sleeping time in mice

Groups	Onset of sleep (min)	Duration of sleep (min)
Control	42.00±3.45	47.33±2.06
Diazepam	15.00±2.54*	145.00±5.12*
Extract	22.33±4.23*	102.67±1.24*

Values were expressed as mean±standard deviation (n = 5); One way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.05, significant compared to control

Thiopental sodium induced sleeping time test: In this test, the test group was treated with the extract at 200 mg kg^{-1} showed significant decrease in onset of sleep and increased duration of sleep. The extract significantly showed the onset of sleep at 22 min with 103 min duration whereas the standard diazepam displayed onset of sleep at 15 min with 145 min duration (Table 4).

DISCUSSION

Plants due to the presence of many secondary metabolites might show different types of bioactivities. Usually bioactive compounds are toxic in higher doses. So, the lethality originated from toxicity is considered as

a marker of bioactive compounds. For this purpose the brine shrimp lethality is utilized as bench top bioassay to monitor the presence of bioactive metabolites in plant (Mclaughlin *et al*., 1998). Our current study showed moderate toxicity in comparison to the vincristine sulfate which confirms the presence of bioactive principles in the *G. multiloculare* extract. It also justifies the use of this plant for treating many pathological states as used by the traditional healers.

In the modern lifestyle, neurological disorders like seizure, anxiety, panic, agitation and insomnia are very common. Various drugs like selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOIs), GABA analogues, tricyclic anitdeprresant (TCAs) etc are used for counteracting them (Llorca *et al*., 2002). These drugs are potent but also showed various side-effects. Newer types of molecules are required to develop safer and effective drugs. The γ-aminobutyric acid type A receptor ($GABA_AR$) is an important ionotropic receptor in neuropharmacological aspect. For this receptor, γ-aminobutyric acid (GABA) is the major endogenous ligand, which causes hyperpolarization of the neuron. So, the action potential is blocked and it results in the sedation (Jiang *et al*., 2007).

The current neuropharmacological study with the methanolic extract of *G. multiloculare* has been conducted. The plant extract demonstrated central nervous system depressant activity as indicated by the decrease in locomotor activity in mice in hole cross, open field and EPM test. The efficiency of the plant extract was almost similar to that of common sedative drug diazepam.

Central Nervous System (CNS) stimulating drugs accelerate motor activities such as locomotion, grooming and rearing behavior, while the CNS depressing drugs inhibit those actions (Adebiyi *et al*., 2012; Aderibigbe *et al*., 2010). Our present study showed the significant level of inhibition of the spontaneous motor activities in mice which ultimately indicates the effective induction of sedation by the studied plant extract through CNS depression.

Previously it was reported that the CNS depressants prolong the drug induced sleeping time (File and Wardill, 1975; Adebiyi *et al*., 2012). The studied plant-extract increased the thiopental sodium induced sleeping time compared to the normal saline treated group. This ability to prolong the sleeping time demonstrates the capability of the *G. multiloculare* to cause CNS depression.

However, plant extract usually contains numerous biosynthetic compounds. These might show additive or synergistic action on single or multiple target sites for displaying various types of bioactivities. Many plant extracts have already been reported to act as ligands for $GABA_A$ receptor for showing the sedative and hypnotic actions (Briskin, 2000; Jiang *et al*., 2007). From the *G. multiloculare* some triterpenes and steroids have been isolated, which might be responsible for acting as ligands for $GABA_A$ receptor and displaying sedative action (Barua *et al*., 2009).

CONCLUSION

The current investigation primarily confirmed the presence of bioactive molecules in the plant extract as indicated by the brine shrimp toxicity assay. Among the bioactive compounds, sedative components are available as proved by the different assays for sedative action. However, further studies are needed to understand the underlying mechanism of the observed activities and to isolate the bioactive molecules from this plant.

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