Sedative Activity of Methanolic Extract of *Glochidion multiloculare* (Rotller 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* (Rotller 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 LC₅₀ value of the extract was found 37.19 µg mL⁻¹, whereas the standard vincristine sulphate showed the LC₅₀ 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 glochidol, 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, LC₅₀, 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 intraperitoneal (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. for vehicle and 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 *napolii* 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

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Total No. of squares travelled</th>
<th>Control</th>
<th>Diazepam</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75.67±4.42</td>
<td>65.00±3.21</td>
<td>67.67±3.22</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>72.00±2.43</td>
<td>54.33±4.26</td>
<td>56.00±4.22*</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>70.33±2.48</td>
<td>37.67±5.87*</td>
<td>38.33±5.88*</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>71.33±3.24</td>
<td>19.33±3.45*</td>
<td>27.67±5.82*</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>68.67±1.22</td>
<td>20.33±2.52*</td>
<td>22.67±3.23*</td>
<td></td>
</tr>
</tbody>
</table>

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 methanol extract of *G. multiloculare* on hole cross test in mice

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Total No. of hole crossed</th>
<th>Control</th>
<th>Diazepam</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.67±0.764</td>
<td>15.00±1.000</td>
<td>16.67±1.041</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>14.67±1.258</td>
<td>6.00±1.325*</td>
<td>11.67±2.082*</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>15.33±0.764</td>
<td>4.67±1.041*</td>
<td>6.67±1.903*</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>16.67±0.764</td>
<td>2.67±0.200*</td>
<td>3.33±0.280*</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>15.00±0.500</td>
<td>2.00±0.500*</td>
<td>3.33±0.280*</td>
<td></td>
</tr>
</tbody>
</table>

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 methanol extract of leaves of *G. multiloculare* on elevated plus maze test in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Entry into open arm (%)</th>
<th>Time spent in open arm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.61±2.522</td>
<td>43.22±1.165</td>
</tr>
<tr>
<td>Diazepam</td>
<td>37.14±2.477*</td>
<td>28.53±3.607*</td>
</tr>
<tr>
<td>Extract</td>
<td>40.11±3.293*</td>
<td>30.89±2.144*</td>
</tr>
</tbody>
</table>

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 methanol extract of *G. multiloculare* on thiopental sodium induced sleeping time in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Onset of sleep (min)</th>
<th>Duration of sleep (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.00±3.45</td>
<td>47.33±2.06</td>
</tr>
<tr>
<td>Diazepam</td>
<td>15.00±2.54*</td>
<td>145.00±5.12*</td>
</tr>
<tr>
<td>Extract</td>
<td>22.33±4.23*</td>
<td>102.67±4.24*</td>
</tr>
</tbody>
</table>

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 (SSRIs), monoamine oxidase inhibitors (MAOIs), 
GABA analogues, tricyclic antidepressant (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<sub>A</sub> R) 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, 
Aderigbige 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<sub>A</sub> 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<sub>A</sub> 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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REFERENCES

Adebiyi, O.A., O.O. Adebiyi, O.R. Ilesanmi and Y. Raji, 
2012. Sedative effect of hydroalcoholic leaf extracts of 

Central nervous system depressant properties of 
Treculia africana Deene. Ethnobot. Leaflets, 
14: 108-112.

Aschwanden, C., 2001. Herbs for health, but how safe are 

Barna, C.C., J.D. Roy, B. Buragohain, A.G. Barna, P. Borah 
and M. Lahkar, 2009. Anxiolytic effect of 
hydroethanolic extract of Drymaria cordata L. Willd. 

Linking plant biochemistry and physiology to human 

Cardoso-Taketa, A.T., R. Pereda-Miranda, Y.H. Choi, 
profiling of the Mexican anxiolytic and sedative plant 
Galphimia glauca using nuclear magnetic resonance 
spectroscopy and multivariate data analysis. Planta 

Neuro-pharmacological studies on SB 5833, a new 
psychotherapeutic agent of the benzodiazepine 
class. Arzneimittelforschung, 24: 2029-2032.


