ACUTE TOXICITY AND NEUROPHARMACOLOGICAL STUDY OF THE CHLOROFORM EXTRACT OF MICROCOS PANICULATA BARKS

Aziz, M.A.*; Sarkar, K.K.; Akter, M.I.; Kabir, A.K.L.

1Department of Pharmacy, Jessore University of Science & Technology, Bangladesh,
2Department of Pharmacy, Stamford University Bangladesh,
3Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Bangladesh.

*debusubju@gmail.com

Abstract
The current study was considered for the assessment of the safety profile as well as neuropharmacological activity of the chloroform extract of Microcos paniculata barks (BCE) by following OECD guidelines, elevated plus-maze and hole cross test respectively. Mortality, sign of any toxicity or behavioral changes were not noticed as the doses increased up to 4000 mg/kg. In elevated plus-maze test, depressive, anti-depressive and anxiolytic activities of BCE 200 mg/kg were observed. Again, BCE 400 mg/kg exhibited depressive and anti-depressive activities. In addition to, gradual decrease of movement was found by both BCE 200 mg/kg and 400 mg/kg respectively but BCE 200 mg/kg showed significant (p < 0.05, vs.control) increase of movement during 180 min through hole cross test. The results found in the present study indicate that BCE can be possible sources of CNS depressant, anti-depressant and anxiolytic agents. But further investigation is required for the confirmation of their activities.

Keywords: Acute toxicity, neuropharmacological study, Microcos paniculata.
Introduction

*Micromos paniculata* L., locally known as ‘Kathgua’ or ‘Fattashi’ in Bangladesh belongs to Tiliaceae family. It grows broadly as a shrub or small tree as well as cultivated all over Bangladesh. Traditionally the plant is used for the treatment of colds, diarrhea, dyspepsia, fever, heat stroke, hepatitis, wounds due to its activity in the digestive system and to kill insects. A review of the literature demonstrated that *M. paniculata* has been found to possess a broad range of activities, such as analgesic, antidiarrheal, anti-inflammatory, antipyretic, antimicrobial, brine shrimp lethality, cytotoxic, free radical scavenging, insecticidal, larvicidal, neuropharmacological, nicotinic receptor antagonistic activities, and α-glucosidase inhibition, as well as preventative effects for angina pectoris and coronary heart disease. Moreover, acute toxicity study of the methanolic extract of *M. paniculata* fruits were carried out also [1, 2]. Therefore, the current study was designed to assess the neuropharmacological activity of the chloroform extract of *M. paniculata* barks (BCE).

Materials and Methods

**Collection and Identification of the Plant materials**

Barks of *M. paniculata* were collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in November, 2012. Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium verified the identification of the species. In the herbarium the dried specimens were preserved for the utilization as future references.

**Extraction**

200 g of powdered barks of *M. paniculata* were used for the chloroform extraction. Plant parts were then washed with running water successively for 3–4 times. Before drying in the shade for a period of 7 d, the plant parts were also rinsed with sterile distilled water. The grinding of the dried parts was then carried out through a laboratory grinding mill (Model 2000 LAB Eriez®) and then passed through a 40-mesh sieve to obtain fine powders. Through a hot extraction procedure accompanied by a Soxhlet apparatus powdered barks of *M. paniculata* were extracted in 2 L of chloroform. The liquid extract was filtered by using Whatman No.1 filter papers. Then the filtrate was kept in a hot air oven at 40°C for drying. The chloroform extraction yield of *M. paniculata* barks was 9.2% (w/w). Extract was stored at 4°C for accessory studies.

**Experimental Animals**

Fifty Swiss albino mice of both sex having weight of about 25–30 g with 6–7 weeks of old were purchased from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Therefore, the animals were kept under optimum environmental conditions along with relative humidity 55%–65%, 12 h light/12 h dark cycle and (27.00±1.00) °C temperature. Suitable food supply together with water *ad libitum* was ensured. Adaptation of the animals to the laboratory conditions for a period of seven days was completed prior to the experiment. All the protocols used in the experiments were approved by the Institutional Animal Ethical Committee of Jessore University of science and technology, Jessore, Bangladesh and were then performed with these animals.

**Acute Oral Toxicity Study**

Acute toxicity refers to the adverse effects resulting either from a single exposure or from multiple exposures over a short period of time (normally less than 24 h). According to the OECD guidelines, acute toxicity study was performed to determine the half lethal dose (LD_{50}) of the experimental samples [3]. Ten mice were categorized into two groups including control group and test group (BCE) having five animals in each group. Different concentrations of the experimental sample (BCE) including 100, 250, 500, 1 000, 2000, 3000 and 4000 mg/kg body weight were prepared and administered orally. Therefore, the animals were kept under observation every 1 h for next 5–6 h for mortality, behavioral pattern changes such as weakness, aggressiveness, food or water refusal, discharge from eyes and ears, noisy breathing, diarrhea, salivation, coma, changes in locomotor activity, injury, convulsion, pain or any sign of toxicity in each group of animals. A final evaluation was also conducted at the end of a 2-week observation period [2].

**Neuropharmacological Study**

**Elevated Plus-Maze Test (EPM)**

The method of Lister was utilized to carry out elevated plus-maze test [3]. Twenty mice were taken and then categorized into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Diazepam, 1mg/kg, p.o.) and test groups (BCE at 200 and 400 mg/kg body weight, p.o.), having five mice in each group. The apparatus consisting of two opposing closed arms (50 x 10 x 30 cm) (length x width x height) and two opposing open arms (50 x 10 cm) (length x width) was kept at a height of 70 cm from the floor level.
Each mouse was placed in the centre of elevated plus-maze apparatus. Then the animals were kept under observation for counting the number of entry into the closed and open arms at 0, 30, 60, 120 and 180 min after respective treatment and every counting was continued for 3 min. The entry of all four paws into one arm was termed as arm entry.

**Hole Cross Test**
According to the method of Takagi et al. [4] the hole cross test was conducted. The apparatus was made of a cage with a size of 30×20×14 cm (length x width x height) where a steel partition was attached in the middle of this cage. Here grouping of the mice and sample administration were carried out as like as elevated Plus-maze Test (EPM). A hole was made at the center of the cage at a height of 7.5 cm which diameter was 3 cm. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 120 and 180 min after respective treatment.

**Statistical Analysis**
All the results were expressed as mean ± S.E. (Standard Error). One-way ANOVA following Dunnet’s test (P < 0.05, vs. control) and Post-hoc Bonferroni test (P < 0.05, vs. standard/extract) through the SPSS software (version 20; IBM Corporation, New York, USA). was utilized for statistical analyses of the neuropharmacological studies which was considered statistically significant.

**Results**
**Acute Oral Toxicity Study**
At the end of the acute toxicity study, it was noticed that no mortality as well as sign of any toxicity or behavioral changes were found as the dose increased up to 4000 mg/kg for BCE (test group). Similar result was obtained in case of control group. Moreover, the animals lived up to 2 weeks after administration of the test sample (BCE). This demonstrated that the test sample didn’t produce acute oral toxicity onto the animals.

**Neuropharmacological Study**
**Elevated Plus-Maze Test (EPM)**
The result of the table-1 exhibited that standard drug diazepam revealed depressive activity with time. In addition to, BCE 200 mg/kg showed fluctuating effects including both depressive and antidepressive activities during different observations. Again, depressive activities were exerted by the high dose (400 mg/kg) of BCE during all observations except 4th observation. From table-2, it was obvious that standard drug diazepam exhibited depressive activity with time. Moreover, BCE 200 mg/kg demonstrated anxiolytic activity at 30 min where as it showed depressive activities at 60 min, 120 min and 180 min respectively. Besides, BCE 400 mg/kg elicited depressive activities during all observations except 4th observation.

**Hole Cross Test**
Hole cross test was utilized for the evaluation of CNS depressant property of BCE. In case of BCE 200 mg/kg, gradual decrease of movement was noticed during different observations (30 min to 120 min) but it exerted significant (p < 0.05, vs. standard) increase of movement during 180 min. Besides, BCE 400 mg/kg demonstrated gradual decrease of movement during all observations. (Table-3).

**Discussion**
Plant-derived products play a significant role in traditional system of medicine. But scientifically approved toxicity studies have been carried out on few of them. Therefore, it is mandatory to know about acute oral toxicity studies for the assessment of exact range of doses for subsequent usage as well as recognition of the significant adverse effects of the materials under experiment. Besides, acute oral toxicity study has been a key factor for the investigation of therapeutic index of drugs and xenobiotics [2]. As no mortality was found with the increment of dose up to 4000 mg/kg, it was not possible to estimate LD₅₀ of the plant extract. The extract was found to be safe with a wide range of therapeutic response. Therefore, two higher doses including BCE 200 mg/kg and 400 mg/kg were used for in-vivo doses. In the present study, the effect of chloroform extract of Microcos paniculata bark was evaluated for neuropharmacological activity. The assessment of CNS activity of any drug depends on the locomotor activities of animals. The estimation of the level of excitability of the CNS refers to the locomotor activity of animal. An increase in alertness is regarded as locomotor activity and reduction in locomotor activity is an indication of sedative effect [5]. There is a close relationship between reduced locomotor activity and sedation which is derived from CNS depression [6]. The number of arm entries of Y-maze and Elevated plus-maze test apparatus was observed for the determination of locomotor activity [7]. Generally CNS depressant drugs expose their action through GABAₐ receptor [6]. CNS depressant activity can be exerted due to the higher concentration of GABAₐ receptor in brain [8].
GABA\textsubscript{A} receptor contains various subtypes and at least 17 subunits including \(\alpha_{1-6}, \beta_{1-3}, \gamma_{1-3}\) and others (single e, \(\theta, \pi\) and \(\delta\)) are responsible for the diverse arrangements of its subtypes. Benzodiazepines bind with GABA\textsubscript{A} receptor containing \(\alpha_2\) and \(\delta\) subunits and elicit anxiolytic and sedative, amnesic effects respectively. Anxiolytic effects of benzodiazepines are related to the secondary suppression of serotonergic and/or nonadrenergic and other excitatory systems [9, 10]. At present it has been reported that numerous plants elicit their anxiolytic effects by animal models of anxiety [11, 12]. EPM is one of them utilized for the evaluation of anxiolytic effect of drugs [13, 14]. Besides, anxiolytic agents enhance the frequency of entries as well as time spent in open arms of the EPM [13]. Anxiolytic compounds not only decrease the natural animal phobia to the open arms but also improve exploration in the elevated-plus maze test. Otherwise, the forced or voluntary passages of the animal into the closed arms of the EPM along with hormonal and behavioral changes are the indication of increased anxiety [15, 16]. It was reported by Montgomery (1955) that when placed in mazes consisting of both open and closed arms the rodents spend more time in closed arms. Abstaining open arm indicates an exposure of fear and anxiety. On the basis of these statements, the elevated plus maze test has been a tool for the identification of selective anxiolytic effect of drugs [17]. Handley & Mithani (1984) further reported that rodents avoid the open arms and also demonstrated that diazepam, an anxiolytic agent decreased the aversion of open arm [18]. Elevated plus maze, a well known model is utilized for the assessment of anxiety-like behaviour in rodents in which elevated and open place entry is avoided [19, 20]. Different antidepressant drugs including TCA, NMDA receptor antagonists or SSRIs enhance the occurrence of escape-related behavior and overturn the immobility position. Depression is originated due to the oscillation of the role of neurotransmitters mainly serotonin, noradrenaline and dopamine. Lack of serotonin results in depression which is one of the potential causative features. SSRIs boost up the accessibility of extracellular serotonin [21]. SSRIs and TCA elicit antidepressant action through the inhibition of uptake of 5-HT and/or noradrenaline [22]. MAO inhibitors exert their antidepressant action through the reduction of metabolism of MAO enzyme system as well as the enhancement of some endogenous amines like serotonin, catecholamines etc [23]. In case of elevated plus-maze test, BCE 200 mg/kg and 400 mg/kg exhibited depressive activity by binding with GABA\textsubscript{A} receptor. Besides, BCE 200 mg/kg exerted anti-depressive activity which mechanism of action may be as like as TCA, MAO inhibitors, SSRIs or atypical antidepressants whose mode of action is not clear (table-1). In table-2, BCE 200 mg/kg demonstrated anxiolytic activity which mechanism of action may be due to binding with \(\alpha_2\) subunit of GABA\textsubscript{A} receptor. Again, the mechanism of depressive activity of both lower and higher doses of BCE may be as like as table-1 (binding with GABA\textsubscript{A} receptor). In hole cross test, gradual decrease of movement was exhibited by both BCE 200 mg/kg and 400 mg/kg. This kind of CNS depressant activity may be due to binding of BCE with GABA\textsubscript{A} receptor.

**Conclusion**

The current results demonstrated that chloroform extract of Microcos paniculata barks might possess several neuropharmacological activities including depressive, anti-depressive and anxiolytic activities. But further studies are required to identify exact bioactive compounds responsible for the neuropharmacological effects as well as to fully elucidate the underlying mechanism of action for the development of neuropharmacological agents. Besides, genotoxicity study of this extract may be a prominent area for the researchers.

**Conflict of interests**

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

**Abbreviations**

BCE: Chloroform extract of Microcos paniculata barks
CNS: Central Nervous System
OECD: Organization of Economic Cooperation and Development
P.O.: Per Oral
EPM: Elevated Plus-Maze
GABA: Gamma Amino Butyric Acid
TCA: Tricyclic Antidepressant
NMDA: N- methyl-D-aspartate
SSRIs: Selective Serotonin Reuptake Inhibitors
MAO: Monoamine Oxidase

**References**

Table 1: Effect of BCE on elevated plus-maze apparatus after entrance into closed arms.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>1.30±0.39</td>
<td>1.36±0.19</td>
<td>1.32±0.25</td>
<td>1.36±0.36</td>
<td>1.30±0.48</td>
</tr>
<tr>
<td>Standard</td>
<td>1 mg/kg</td>
<td>0.60±0.19</td>
<td>1.20±0.54</td>
<td>1.80±0.83</td>
<td>2.30±0.97</td>
<td>3.00±0.61*</td>
</tr>
<tr>
<td>BCE</td>
<td>200 mg/kg</td>
<td>1.20±0.26</td>
<td>0.60±0.20</td>
<td>2.00±0.36</td>
<td>0.20±0.05</td>
<td>0.40±0.11*</td>
</tr>
<tr>
<td>BCE</td>
<td>400 mg/kg</td>
<td>0.40±0.18</td>
<td>0.60±0.27</td>
<td>0.80±0.41</td>
<td>1.00±0.41</td>
<td>0.20±0.06*</td>
</tr>
</tbody>
</table>

Number of movement in closed arms is presented as mean ± standard error. *P<0.05, vs control (Dunnett’s t test); □P < 0.05, vs standard (pair-wise comparison by post-hoc Bonferroni test).

Table 2: Effect of BCE on elevated plus-maze apparatus after entrance into open arms.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>1.60±0.30</td>
<td>1.80±0.34</td>
<td>1.80±0.53</td>
<td>1.70±0.40</td>
<td>1.58±0.44</td>
</tr>
<tr>
<td>Standard</td>
<td>1 mg/kg</td>
<td>3.00±0.40</td>
<td>2.40±0.54</td>
<td>1.00±0.30</td>
<td>0.40±0.14*</td>
<td>0.20±0.05*</td>
</tr>
<tr>
<td>BCE</td>
<td>200 mg/kg</td>
<td>8.00±0.56*</td>
<td>8.20±0.66*</td>
<td>5.80±0.35*</td>
<td>1.80±0.24*</td>
<td>1.40±0.19</td>
</tr>
<tr>
<td>BCE</td>
<td>400 mg/kg</td>
<td>8.00±0.58*</td>
<td>5.00±0.53*</td>
<td>1.80±0.24*</td>
<td>1.20±0.20</td>
<td>1.24±0.31</td>
</tr>
</tbody>
</table>

Number of movement in open arms is presented as mean ± standard error. *P<0.05, vs control (Dunnett’s t test); □P < 0.05, vs standard; ■P < 0.05, vs BCE 200 mg/kg (pair-wise comparison by post-hoc Bonferroni test).

Table 3: Effect of BCE on Hole cross test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>3.20±0.35</td>
<td>3.24±0.31</td>
<td>3.22±0.49</td>
<td>3.22±0.25</td>
<td>3.26±0.27</td>
</tr>
<tr>
<td>Standard</td>
<td>1 mg/kg</td>
<td>3.00±0.32</td>
<td>1.00±0.29*</td>
<td>0.80±0.20*</td>
<td>0.20±0.03*</td>
<td>0.10±0.03*</td>
</tr>
<tr>
<td>BCE</td>
<td>200 mg/kg</td>
<td>3.40±0.28</td>
<td>1.60±0.37*</td>
<td>1.40±0.44*</td>
<td>1.00±0.24*</td>
<td>2.40±0.47*</td>
</tr>
<tr>
<td>BCE</td>
<td>400 mg/kg</td>
<td>3.40±0.37</td>
<td>2.60±0.32*</td>
<td>2.00±0.41</td>
<td>1.80±0.40*</td>
<td>1.60±0.28*</td>
</tr>
</tbody>
</table>

Number of movement is presented as mean ± standard error. *P<0.05, vs control (Dunnett’s t test); ■P < 0.05, vs standard (pair-wise comparison by post-hoc Bonferroni test).