BIOACTIVITY STUDY OF MICROCOS PANICULATA

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Abstract
The current study was considered for the assessment of the safety profile of water extract of Microcos paniculata leaves (LWE) as well as neuropharmacological activity of both LWE and methanolic extract of Microcos paniculata fruits (FME) by following OECD guidelines and open field test respectively. In acute oral toxicity study, mortality, sign of any toxicity or behavioral changes were not noticed as the doses increased up to 4000 mg/kg. In open field test, gradual decrease of movement was found by LWE 200 mg/kg. Again, LWE 400 mg/kg exhibited both depressive and anti-depressive activities. In addition to, FME 200 mg/kg and 400 mg/kg exhibited fluctuating effects of movement at various observations. The results found in the present study indicate that both LWE and FME can be possible sources of CNS depressant as well as anti-depressant agents. But further investigation is required for the confirmation of their activities.

Key Words: Acute toxicity, neuropharmacological study, Microcos paniculata.
Introduction

_Microcos 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.

Therefore, the current study was also [1, 2] designed to assess the safety profile of the water extract of _Microcos paniculata_ leaves (LWE) as well as neuropharmacological activity of both LWE and methanolic extract of _Microcos paniculata_ fruits (FME).

Materials and Methods

_Collection and Identification of the Plant materials_

Leaves and fruits of _M. paniculata_ were collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in November, 2012. Species identification was verified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium. Dried specimens were deposited in the herbarium for future references.

_Extraction_

Aqueous and methanolic extractions were performed separately on 200 g of powdered leaves and fruits of _M. paniculata_. Fresh leaves and fruits were rinsed 3–4 times successively with running water and once with sterile distilled water. Washed plant materials were then dried in the shade for a period of 7 d. The dried plant materials were then ground by using a laboratory grinding mill (MACSALAB 200 Cross Beater, Erie, Erie, Pennsylvania, U.S.A.) and passed through a 40-mesh sieve to get fine powders. These leaves and fruits powders (200 g) were extracted in 2 L of water and methanol separately, using a soxhlet apparatus and a hot extraction procedure. Whatman No.1 filter papers were used to filter the liquid extracts. The filtrates were then dried in a hot air oven at 40 °C. The extraction yields of LWE and FME were 13.15% (w/w) and 11.08% (w/w) respectively.

_Experimental Animals_

Forty 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. Proper supply of foods and water _ad libitum_ were 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 (LD50) of the experimental samples [3]. Ten mice were categorized into two groups including control group and test group (LWE) having five animals in each group. Different concentrations of the experimental sample (LWE) including 100, 250, 500, 1000, 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_

_Open Field Test_

The test was completed according to Hawiset et al [3]. Thirty mice were divided into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Diazepam, 1mg/kg, p.o.) and test groups (LWE and FME at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. Through this test, the CNS depression activity can be

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evaluated. A series of alternating white and black squares made the open field having 40 cm height. The number of movement of the test animals i.e., total number of squares that every group of animals visited was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min.

**Statistical Analysis**

All the results were expressed as mean ± S.E. (Standard Error). One-way ANOVA following Dunnet's t test (P < 0.05, vs. control) and Post hoc Tukey's HSD test (P < 0.05, vs. standard/extract) were utilized for the statistical analyses of the neuropharmacological study by using SPSS software (version 16; IBM Corporation, New York, USA).

**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 LWE (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 (LWE). This demonstrated that the test sample didn’t produce acute oral toxicity onto the animals.

**Open Field Test**

Open field test demonstrated that, the standard drug diazepam exerted CNS depressive activity with time. Moreover, from table-1 it is clear that LWE 200 mg/kg showed gradual decrease of movement (from 1st to 5th observation period). Again, similar result was noticed by LWE 400 mg/kg as like as LWE 200 mg/kg except 5th observation where it showed increase of movement. In case of FME 200 mg/kg, fluctuating result was found including both decrease and increase of movement during various observations. Again, FME 400 mg/kg showed gradual decrease of movement up to 3rd observation (0 min, 30 min and 60 min respectively) but from 4th observation it began to expose fluctuating effects of movement.

**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 LD_{50} of the plant extract. The extract was found to be safe with a wide range of therapeutic response. Therefore, two higher doses including LWE 200 mg/kg and 400 mg/kg were used for in-vivo doses.

In the present study, LWE and FME were 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 [4]. There is a close relationship between reduced locomotor activity and sedation which is derived from CNS depression. Generally CNS depressant drugs expose their action through GABA_{A} receptor [5].

CNS depressant activity can be exerted due to the higher concentration of GABA_{A} receptor in brain [6]. GABA_{A} receptor contains various subtypes and at least 17 subunits including α_{1-6}, β_{1-3}, γ_{1-3} and others (single ε, θ, π and δ) are responsible for the diverse arrangements of its subtypes. Benzodiazepines bind with GABA_{A} receptor containing α_{2} and α_{3} subunits and elicit anxiolytic and sedative, amnesic effects respectively [7, 8]. Numerous antidepressant medications including SSRIs or NMDA receptor antagonists, TCA cause overturning of immobility position and enhancement of the instance of escape-related behavior. Generation of depression is caused due to fluctuation in functions of some neurotransmitters such as serotonin, noradrenaline and dopamine. Depression is caused because of depletion of serotonin, one of the crucial etiological features. Accessibility of extracellular serotonin is boosted up by SSRIs [9]. Antidepressant action is exerted due to interruption of uptake of 5-HT and/or noradrenaline by SSRIs and TCA. Antidepressant action is also elicited due to the enhancement of some endogenous amines like serotonin, catecholamines etc. which occur as a result of downfall in the metabolism of MAO enzyme system by MAO inhibitors [10]. In the open field test, LWE 200 mg/kg showed depressive activity, which may be due to its binding with GABA_{A} receptor. However, LWE 400 mg/kg revealed both depressive and anti-depressive activity which mechanism of actions may be as like as either LWE 200 mg/kg or TCA, MAO inhibitors, SSRIs and atypical antidepressants whose
mode of actions are not understood. Again, FME 200 mg/kg as well as 400 mg/kg disclosed both depressive and anti-depressive activities which mechanism of actions may be as like as LWE 400 mg/kg.

Conclusion
The current results demonstrated that the water extract of Microcos paniculata leaves as well as the methanolic extract of Microcos paniculata fruits might possess several neuropharmacological activities including depressive, anti-depressive activities. But further studies are required to identify the 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 these extracts 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.

List of abbreviations
CNS = Central Nervous System
OECD = Organization of Economic Cooperation and Development
P.O. = Per Oral
GABA = Gamma Amino Butyric Acid
SSRIs = Selective Serotonin Reuptake Inhibitors
NMDA = N- methyl-D-aspartate
TCA = Tricyclic Antidepressant
MAO = Monoamine Oxidase.

References
Table 1. Effect of LWE and FME on open field 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>154±2.92</td>
<td>155±1.84</td>
<td>152±1.97</td>
<td>157±1.38</td>
<td>156.2±0.73</td>
</tr>
<tr>
<td>Standard</td>
<td>1 mg/kg</td>
<td>119.6±1.63*</td>
<td>114±1.41*</td>
<td>107±0.84*</td>
<td>84.4±1.81*</td>
<td>73.2±1.39*</td>
</tr>
<tr>
<td>LWE</td>
<td>200 mg/kg</td>
<td>232.8±1.46*</td>
<td>153.2±1.07*</td>
<td>124.6±0.81*</td>
<td>97.8±1.62*</td>
<td>57.2±0.86*</td>
</tr>
<tr>
<td>LWE</td>
<td>400 mg/kg</td>
<td>194.6±1.12*</td>
<td>125.2±0.73*</td>
<td>98±1.22*</td>
<td>82.6±0.93*</td>
<td>98.6±1.60*</td>
</tr>
<tr>
<td>FME</td>
<td>200 mg/kg</td>
<td>179.6±0.81*</td>
<td>122.8±1.62*</td>
<td>80.2±0.80*</td>
<td>99.8±1.36*</td>
<td>35.4±1.50*</td>
</tr>
<tr>
<td>FME</td>
<td>400 mg/kg</td>
<td>161±0.89*</td>
<td>130.4±1.72*</td>
<td>101.2±1.07*</td>
<td>120.2±0.66*</td>
<td>102.6±1.33*</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; ■P < 0.05, vs LWE 200 group; ♦P < 0.05, vs LWE 400 group; ≠P < 0.05, vs FME 200 group; (pair-wise comparison by post-hoc Tukey test).