CNS Depressant and Antinociceptive Activities of the Aerial Parts of *Mimosa pudica*

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**Abstract:** *Mimosa pudica* is used in folk medicine in the management of diabetes, diarrhea and inflammation. The present study was designed to evaluate the central nervous system (CNS) depressant and antinociceptive activity of the methanolic extract of the aerial parts of *Mimosa pudica* (MAMP). CNS depressant study of the extract (100 and 200 mg/kg, p.o.) was done by open field and hole cross test whereas acetic acid writhing test and formalin induced pain was done for antinociceptive activity. A statistically significant (*p* < 0.05) decrease in locomotor activity was observed at all doses in the open-field and hole-cross tests. The extract significantly (*p* < 0.05) and dose dependently reduced the writhing reflex in the acetic acid-induced writhing test as well as linking response in the formalin induced inflammatory pain. The finding of this study suggested that *Mimosa pudica* possesses good CNS depressant activity along with high antinociceptive activity provide in part scientific support for the use of this species in the management of neuropsychiatric disorder.

**Key words:** CNS Depressant • Antinociceptive • *Mimosa Pudica*

**INTRODUCTION**

The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases [1]. The investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs [2]. In Bangladesh thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. The beneficial medicinal effects of plant materials typically results from combinations of secondary product present in plant such as alkaloids, steroids, tannins, phenol compounds, resins, gums, flavonoids and fatty acids which are capable of producing definite physiological action on body [3].
the whole plant extract. Seeds contain mucilage composed of d-xylose and d-glucuronic acid. The plant is reported to contain tubuline and a new class phytohormone turgorines. Various flavonoids viz. 5,7,3',4'-tetrahydroxy-6-C-β-D-glucopyranosyl flavones, 7,8,3',4'-tetrahydroxy-6-C-β-D-glucopyranosyl flavone and 4-0(β-D-glucopyranosyl-6-sulphate) gallic acid were also identified from leave extract [7-9]. With a view to find the pharmacological rationale for some of the reported and traditional uses of the plant, the methanolic extract of the aerial part of Mimosa pudica Linn. (MAMP) was evaluated for central nervous system (CNS) depressant and antinociceptive activity in mice.

MATERIAL AND METHODS

Plant Materials and Extraction: The aerial parts of M. pudica plant were collected from Rajshahi, Bangladesh in the month of September 2011 and identified by DR. M.A. Razzaque Shah PhD, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh. The dried and coarsely powdered leaves (400 g) were extracted with methanol at room temperature for 72 hrs. The filtrate was evaporated to dryness under reduced pressure (45°C) to afford the crude extract (yield ca. 6%) used in pharmacological screening.

Animals: Swiss albino mice of either sex weighing about 25-35gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) were used for the evaluation of CNS depressant and antinociceptive activity. The animals were housed under standard laboratory conditions (relative humidity 55–65%, room temperature 23.0±2.0°C and 12-hrs light, 12-hrs dark cycle). The animals were fed with a standard diet and water ad libitum. In all animal experiments, the guidelines of the Animal Experimentation Ethics Committee, ICDDR, B were followed.

Drugs and Chemicals: Folin-chiocaltu phenol reagent, were purchased from E. Merck (Germany). Tween-80 was obtained from BDH Chemicals, UK. Formalin was purchased from CDH, India. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Diclofenac Na and diazepam was obtained from Square Pharmaceuticals Ltd., Bangladesh. All chemicals used were of analytical reagent grade

Preliminary Phytochemical Analysis: The crude extract of M. pudica (MAMP) was subjected to a preliminary phytochemical screening for the presence of alkaloid, flavonoids, tannins and glycoside [10].

The Amount of Phenolic Compounds and Flavonoids: The total phenolic and flavonoid content of MAMP was determined using Folin-ciocalteu reagent [11] and aluminium chloride colorimetric method [12] respectively. The content of total phenolics in MAMP was calculated from regression equation of the calibration curve (y=0.013x+0.127, r²= 0.988) and is expressed as galic acid equivalents (GAE) and the flavonoid contents of the extract was expressed in terms of quercetin equivalent (the standard curve equation: y=0.009x-0.036).

Acute Toxicity Test: Test animals were divided into groups (n = 6 per group) which were administered different doses of the crude extract (62.5, 125, 250, 500, 1000, 2000 and 4000 mg/kg p.o.), while the control group received only the vehicle (1% Tween 80 in water, p.o.). The general signs and symptoms of toxicity were observed for 24 h and mortality was recorded for each group at the end of this period [13].

CNS Depressant Activity

Hole Cross Test: The method used was done as described by Takagi et al. [14]. The animals were divided into control, standard and test groups (n = 6 per group). The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) whereas the test group received MAMP extract (at the doses of 100 and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 3 min on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

Open Field Test: This experiment was carried out as described by Gupta et al. [15]. The animals were divided into control standard and test groups (n = 6 per group). The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.). The test group received the crude extract (at the doses of 100 and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm h) divided into a
series of squares. The number of squares visited by each animal was counted for 3 min on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

**Antinociceptive Activity**

**Acetic Acid Induced Writhing Method:** The antinociceptive activity of the samples was studied using acetic acid-induced writhing model in rats. Test samples (at the doses of 100 and 200 mg/kg) and vehicle (1% tween 80 in water) were administered orally to rats (n=6) 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g). The positive control group received Diclofenac-Na at the dose of 10 mg/kg p.o. After an interval of 5 min, the rats were observed for specific contraction of body referred to as ‘writhing’ for the next 30 min [16].

**Formalin Test:** The antinociceptive activity of the drugs was determined using the formalin test described by Dubuisson and Dennis [17]. Control group received 5% formalin. 20 μl of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of MAMP (100 and 200 mg/kg, p.o.) and Diclofenac Na (10 mg/kg, p.o.). The mice were observed for 30 min after the injection of formalin and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

**Statistical Analysis:** All data were expressed as mean ± S.E.M. One-way ANOVA followed by Dunnett’s multiple comparison tests was used to analyze the data obtained from in vivo experiments. All statistical analyses were performed with Prism 4.0 (GraphPad software Inc., San Diego, CA). P<0.05 was considered to be significant.

**RESULTS**

**Preliminary Phytochemical Analysis and Acute Toxicity:** Results of the preliminary phytochemical analysis carried out on the crude methanol extract indicated the presence of alkaloid, flavonoids, tannins and glycoside. No lethal effects were observed within 24 h after the administration of the extract at any of the doses used, even at the highest dose tested (4000 mg/kg). Therefore, the lethal dose (LD₅₀) of the extract in mice could not be determined.

**Total Phenolic and Flavonoid Contents:** Table 1 represents the content of both groups in MAMP extract. The content of total phenolics in the extract of M. pudica was determined using the Folin-ciocalteu assay, calculated from regression equation of the calibration curve (y=0.013x+0.127, r²= 0.988) and is expressed as galic acid equivalents (GAE) and the flavonoid contents of the extract was expressed in terms of quercetin equivalent (the standard curve equation: y=0.009x-0.036).

**CNS Depressant Activity**

**Open-Field Test:** In the open-field test, MAMP extract exhibited a decrease in the movements of the test animals at all dose levels tested. The results were statistically significant for all doses and followed a dose-dependent response (Table 2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>*Total phenols mg/g plant extract (in GAE)</th>
<th>*Total flavonoids mg/g plant extract (in QA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPBL</td>
<td>39.92</td>
<td>96.33 ± 1.02*</td>
<td>42.16 ± 0.61*</td>
</tr>
</tbody>
</table>

The GAE and QA values are expressed as Means±SEM of triplicate experiments. *Gallic acid equivalents (GAE, mg/g of each extract) for the total phenolic content, *Quercetin equivalent (QA, mg/g of each extract) for the total flavonoid content.

**Table 2:** Effect of methanolic extract of the aerial parts of *Mimosa pudica* on hole cross test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>10ml/kg</td>
<td>118.4 ± 1.20</td>
<td>118 ± 1.30</td>
<td>115.4 ± 0.50</td>
<td>117.4 ± 1.16</td>
<td>118 ± 0.70</td>
</tr>
<tr>
<td>Group-II</td>
<td>1mg/kg</td>
<td>117.2 ± 1.15</td>
<td>64.6± 0.43*</td>
<td>38.8± 0.58*</td>
<td>15.8± 86*</td>
<td>9.6 ± 0.50*</td>
</tr>
<tr>
<td>Group-III</td>
<td>100 mg/kg</td>
<td>118.4 ± 0.81</td>
<td>68.8±1.02*</td>
<td>51.8±1.35*</td>
<td>33.8± 02*</td>
<td>22 ± 0.71*</td>
</tr>
<tr>
<td>Group-IV</td>
<td>200 mg/kg</td>
<td>117.8 ± 1.43</td>
<td>60.8± . 06*</td>
<td>40.6±. 92*</td>
<td>20.6±0.92*</td>
<td>12.6±0.60*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, (n = 6); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MAMP.
Table 3: Effect of methanolic extract of the aerial parts of *Mimosa pudica* on Open Field test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>10ml/kg</td>
<td>12.8 ± 1.15</td>
<td>13 ± 1.41</td>
<td>13.6 ± 0.92</td>
<td>14.2 ± 0.86</td>
<td>14 ± 0.54</td>
</tr>
<tr>
<td>Group-II</td>
<td>1mg/kg</td>
<td>11.2 ± 0.58</td>
<td>6 ± 0.70*</td>
<td>4.0 ± 0.83*</td>
<td>2.4±0.81*</td>
<td>1.8±0.37*</td>
</tr>
<tr>
<td>Group-III</td>
<td>250mg/kg</td>
<td>12 ± 0.70</td>
<td>7.8 ± 0.58*</td>
<td>5.4±0.50*</td>
<td>4.2±0.37*</td>
<td>3.2±0.37*</td>
</tr>
<tr>
<td>Group-IV</td>
<td>500mg/kg</td>
<td>12.2 ± 0.66</td>
<td>6.2 ± 0.37*</td>
<td>4.0 ± 0.70*</td>
<td>2.8±0.37*</td>
<td>1.4±0.40*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, (n = 6); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MAMP.

Table 4: Effect of methanolic extract of the aerial parts of *Mimosa pudica* on acetic acid induced writhing in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>No. of writhing</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>0.1 ml/10gm</td>
<td>36.33 ± 0.55</td>
<td>64.68</td>
</tr>
<tr>
<td>Group-II</td>
<td>10mg/kg</td>
<td>12.83 ± 1.22*</td>
<td>64.68</td>
</tr>
<tr>
<td>Group-III</td>
<td>100mg/kg</td>
<td>19.0 ± 1.69*</td>
<td>47.70</td>
</tr>
<tr>
<td>Group-IV</td>
<td>200mg/kg</td>
<td>13.33 ± 0.76*</td>
<td>63.30</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, (n = 5); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Na 10 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MAMP.

Table 5: Effect of methanolic extract of the aerial parts of *Mimosa pudica* in hindpaw licking in the formalin test in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Early phase (Sec)</th>
<th>% protection</th>
<th>Late phase (Sec)</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>10 ml/kg</td>
<td>35.67 ± 1.38</td>
<td>-</td>
<td>46.0 ± 1.03</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>10 mg/kg</td>
<td>13.83 ± 0.90*</td>
<td>61.22</td>
<td>17.83 ± 0.70*</td>
<td>61.21</td>
</tr>
<tr>
<td>Group-III</td>
<td>100 mg/kg</td>
<td>28.5 ± 0.76*</td>
<td>20.10</td>
<td>21.05 ± 0.95*</td>
<td>54.23</td>
</tr>
<tr>
<td>Group-IV</td>
<td>200mg/kg</td>
<td>19.17 ± 0.65*</td>
<td>46.25</td>
<td>18.0 ± 1.46*</td>
<td>60.86</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, (n = 5); * p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Na 10 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the MAMP.

Hole-Cross Test: Results of the hole-cross test followed a similar trend to the ones observed in the open-field test. They were statistically significant for all dose levels and followed a dose-dependent response. The depressing effect was most intense during the second (60 min) and third (90 min) observation periods (Table 3).

Antinociceptive Activity

Acetic Acid-Induced Writhing Test: Table 4 shows the effects of the extract of on acetic acid-induced writhing in mice. The oral administration of both doses of MAMP significantly (p<0.001) inhibited writhing response induced by acetic acid in a dose dependent manner.

Formalin Test: MAMP (100 and 200 mg/kg, p.o.) significantly (P<0.001) suppressed the licking activity in either phase of the formalin-induced pain in mice in a dose dependent manner (Table 5). MAMP, at the dose of 200 mg/kg body weight, showed the approximately similar licking activity against second phases of formalin-induced pain than that of the standard drug diclofenac Na.

DISCUSSIONS

Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect [18]. Extracts of *M. pudica* decreased locomotor activity indicates its CNS depressant activity. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through GABA, therefore it is possible that extracts of MAMP may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts [19]. Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders [20]. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the GABA_{A} receptors in the central nervous system; which led to assume that they can act as benzodiazepine like molecules...
Phytochemical investigations also showed the presence of alkaloids, flavonoids and tannins in the extract, so might be this phytoconstituents are responsible for its CNS depressant activity. These experiments suggest that Ca\(^{2+}\) mobilization through TRPA1 cation channels is concomitant with noxious chemicals and mechanical stimuli as they produce their antinociceptive action. It is likely that the inhibitory effect of MAMP to pain response is due to inhibit the increase of the intracellular Ca\(^{2+}\) through TRPA1, presumably evoked by formalin. So, the extract of *M. pudica* may contain substances that affect the metabolism of Ca\(^{2+}\). Literature survey revealed that tannins, triterpenoids and flavonoid are the major phytoconstituents of *M. pudica*. Flavonoids, for example, have been found to suppress the intracellular Ca\(^{2+}\) ion elevation in a dose dependent manner, as well as the release of proinflammatory mediators such as TNF\(\alpha\), Kempuraj et al. [30].

Our preliminary pharmacological studies on the methanol extract of the aerial part of *Mimosa pudica* provide in part scientific support for the use of this species in traditional medicine, particularly in various ailments related to CNS disorders and pain. However, further pharmacological investigations are required to understand its underlying mode of action on the CNS and mechanism of pain inhibition. In addition, future bioactivity-guided phytochemical work should be carried out to identify any active constituent(s).

**REFERENCES**


