Evaluation of Analgesic and Neuropharmacological Activity of the Bark of *Morus alba* L. (Family: Moraceae)

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**ABSTRACT**

The aim of the study is to evaluate the analgesic and neuropharmacological properties of the bark of *Morus alba* L. This plant is well known for its analgesic and antidepressant effects. Analgesic and neuropharmacological activity was conducted at the doses of 200 and 400 mg/kg body weight using *Swiss albino* mice as animal model. Analgesic activity was investigated using one chemical (acetic acid-induced writhing test) and one thermal (hot plate test) method. In acetic acid-induced writhing test, the standard drug (Diclofenac-Na, 5 mg/kg body weight) showed the inhibition of 89.3%, in which the inhibition of the extract were 57.79% and 54.78% at a doses of 200 and 400 mg/kg body weight, respectively. Overall, the extract showed significant analgesic activity. The neuropharmacological activity was observed by hole cross and open field tests. In hole cross and open field test, the sample significantly decreased the locomotors activity compared to standard drug (Diazepam, 5 mg/kg body weight). Thus, the obtained findings of our present work provide a rationale for the use of this plant for medicinal purposes and encourage us for further investigations to obtain more fruitful results.

**Keywords**: *Morus alba*, Moraceae, Analgesic activity, Neuropharmacological activity, Antidepressant activity.

**INTRODUCTION**

*Morus alba* L. (also called white mulberry) belongs to the family of Moraceae, commonly known as Tut in Bangladesh and Mulberry tree in English (1). It is a short-lived, fast-growing, small to medium sized shrub. The trees are generally deciduous in temperate regions, but trees grown in tropical regions can be evergreen. The flowers are single-sex catkins; male catkins are 2–3.5 cm long, and female catkins 1-2 cm long. Male and female flowers are usually on separate trees although they may occur on the same tree. The fruit is 1-2.5 cm long; in many cultivated plants it varies from white to pink. The plant invades old fields, urban lots, roadsides, forest edges (2). The white mulberry is widely cultivated to feed the silkworms employed in the commercial production of silk (2). It is still cultivated in Bangladesh.

The leaves contain flavonoids, artocarpin, cycloartocarpin and analogues. The root of the plant contains flavonoids like kuwanons, sangennons, mulberrosides and mulberofurans. The fruit contains carotene, vitamins A and C, thamene, riboflavin, tannin, linoleic and stearic acids (3).

The plant is used as anti-inflammatory, hypoglycemic, anthelmintic, antioxidant, neuroprotective, liver and kidney protective, hypotensive, diuretic, anti-cough, aniviral, antimicrobial, antifungal, anti-allergic, anti-ulcerogenic, anti-stress, immunomodulatory, anti-cataract and radioprotective agent (4).
MATERIALS AND METHODS

Chemicals

Chemicals and drugs used in the study include 95% ethanol (Merck, Germany), DMSO (Merck, Germany), distilled water (Laboratory prepared), Diclofenac-Na (India), acetic acid (Merck, Germany), Diazepam (Square Pharmaceuticals Limited, Bangladesh).

Plant materials and extraction

The bark of *Morus alba* plant was collected from Comilla, Bangladesh in February 18, 2017. The plant was identified from Bangladesh National Herbarium institute, Mirpur, Dhaka. An accession number was given from there and a voucher specimen (DACB: 44917) has been deposited in the herbarium for future reference. The shade dried and powdered plant material was extracted by cold extraction method. 150 mg powder was soaked in 600 mL 95% ethanol in a glass container for 10 days accompanying regular shaking and stirring. The extract was filtered through cotton and finally with Whatmann filter paper and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure. The amount of extract was 1.2 gm and the yield value was 0.8 %.

Animals

For the experiment, *Swiss albino* mice of both sex having 3-4 weeks of age, weighing between 20 to 25 gm were collected from Jahangirnagar University, Savar, Dhaka, Bangladesh. Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions: Temperature (24.0 ± 1.00°C), relative humidity (55-65% and 12 hours light /12 hours dark cycle). Husk and excreta were removed from the cages every day (5).

Preparation of test groups

The sample was prepared by dissolving the ethanol bark extract of *Morus alba* in DMSO (few drops) and distilled water at two doses (200 mg/kg and 400 mg/kg body weight). DMSO, distilled water and sample were mixed with the help of vortex apparatus. The sample was completely dissolved in ethanol. Few drops of DMSO was added to dissolve the sample completely in distilled water.

PHYTOCHEMICAL SCREENING

The crude extract was screened to detect the presence of various categories of phytochemicals such as alkaloids, reducing sugar, tannins, glycosides, flavonoids, carbohydrates, saponins etc. by standard method (6).

ANALGESIC ACTIVITY

Analgesic means a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. So, analgesic activity means capacity of a substance to neutralize the pain sensation (7).

The study of analgesic activity of the *Morus alba* was performed in animal models for both central and peripheral mechanism of pain. For the screening of analgesic activity against peripheral mechanism of pain acetic acid-induced writhing test was considered. On the other hand, to evaluate the analgesic activity against centrally mediated pain hot plate test was used.

Acetic acid-induced writhing test

The acetic acid-induced writhing method is an analgesic behavioural observation assessment method that demonstrates a noxious stimulation in mice. 5 mL test sample and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as ‘writhing’ for the next 10 min (8). The animals were divided into control, positive control and test group-I and II containing five mice each. The test group received sample at the doses of 200 and 400 mg/kg body weight intraperitoneally whereas the control group received vehicle (Distilled water) and the positive control group received Diclofenace-Na at the dose of 5 mg/kg body weight.

Hot plate test

Hot plate test was used to measure the response latencies based on the procedure described by Eddy and Leimbach, 1993 (9). In this experiment, hot plate was maintained at 50±0.05°C. The reaction time was recorded.
for animals pre-treated with DMSO (0.10 mL/kg body weight 30 min before orally) as control, ethanol extract of bark (200 and 400 mg/kg body weight, 30 min before) and Diclofenac-Na (5 mg/kg body weight intraperitoneally, 15 min before) which has used as a positive control group. Animals were placed into the hot plate chamber and the time of latency was defined as the time period between the zero point, when the animal was placed on the hot plate surface and the time when animal licked its back paw or jumped off to avoid thermal pain. The latent period of response was taken as the index of antinociception and was determined at the pre-treatment 0, 30, 60, 90 and 120 min after the administration of sample and standard in the order to minimize the damage on the animal paws. The cut off time was taken as 20 seconds.

NEUROPHARMACOLOGICAL ACTIVITY
The purpose of this study was to examine neuropharmacological effect of ethanol extract of the bark of *Morus alba* on mice in a peripheral model of CNS activity.

**Hole cross test**
The experiment was carried out as described by following the method of Shahriar *et. al.*, 2015 (10). A steel partition was fixed in the middle of the 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. Movement of the animals through the hole from one chamber to the other was counted for 3 minutes in the test. The observations were made on 0, 30, 60, 90 and 120 minutes after oral administration of the test drugs at the dose of 200 and 400 mg/kg body weight and the intraperitoneal administration of standard drug Diazepam at the dose of 1 mg/kg body weight.

**Open field test**
The Open field test is clearly the most frequently used of all behavioural tests in pharmacology and neuroscience. Despite the simplicity of the apparatus, however, open field behaviour is complex. Consequently, it has been used to study a variety of behavioural traits, including general motor function, exploratory activity and anxiety-related behaviours. Open-field behavioural assays are commonly used to test both locomotor and emotional activity in rodents.

This experiment was carried out as described by Shahriar *et. al.*, 2015 (10). The animals were divided into control, positive control and test group-I and II containing five mice each. The test group received *Morus alba* extract at the doses of 200 and 400 mg/kg body weight orally whereas the control group received vehicle (DMSO + water) and the positive control group received Diazepam at the dose of 5 mg/kg body weight intraperitoneally. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by mice in test group-I and II was counted for 3 min at 0, 30, 60, 90 and 120 min and compared with the number of squares travelled by mice in positive control group.

RESULTS AND DISCUSSION
The preliminary phytochemical screening of ethanol extract of *M. alba* revealed the presence of carbohydrates, glycosides, tannins, flavonoids, reducing sugar, gums, steroids and alkaloids (Table 1).

<table>
<thead>
<tr>
<th>Phytochemical Groups</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Gums</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. Result of Phytochemical group test

Here, ‘+’ Indicate presence, ‘-‘ Indicates absence
To evaluate the analgesic activity of *M. alba* both chemical and thermal induced nociception models were applied. In acetic acid-induced writhing test, the sample showed analgesic activity which was comparable to the
reference drug, Diclofenac-Na Table 2, Figure 1). It was found to be moderate at the dose of 200 mg/kg body weight.

**Table 2. Analgesic effect of *M. alba* by acetic acid induced writhing method**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Mean ± SEM</th>
<th>% of writhing</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1 mL/mice</td>
<td>106.6 ± 5.4</td>
<td>100</td>
<td>0.00</td>
</tr>
<tr>
<td>Positive control</td>
<td>5 mg/kg b.w.</td>
<td>11.4 ± 1.9</td>
<td>10.7</td>
<td>89.31</td>
</tr>
<tr>
<td>Group-I</td>
<td>200 mg/kg b.w.</td>
<td>45 ± 9.4</td>
<td>42.21</td>
<td>57.79</td>
</tr>
<tr>
<td>Group-II</td>
<td>400 mg/kg b.w.</td>
<td>48.2 ± 7.3</td>
<td>45.21</td>
<td>54.78</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n=5), p<0.05

**Figure 1: Analgesic effect of *M. alba* by acetic acid-induced writhing method**

The hot plate test was applied to determine central and peripheral antinociceptive effect. The ethanol extract of *Morus alba* displayed a moderate to potent analgesic activity which was confirmed from hot plate test where reflex time was notably increased (Table 3, Figure 2).

**Table 3. Analgesic effect of *Morus alba* by hot plate test**

<table>
<thead>
<tr>
<th>Test 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>Control</td>
<td>0.1 mL/mice</td>
<td>6 ± 2.1</td>
<td>12.8</td>
<td>8.4 ± 10</td>
<td>10 ± 8</td>
<td>3.6</td>
</tr>
<tr>
<td>Positive</td>
<td>diclofenac-Na</td>
<td>5 mg/kg b.w.</td>
<td>11±2.6</td>
<td>8.2</td>
<td>10.8</td>
<td>16 ± 9.6</td>
</tr>
<tr>
<td>Group-I</td>
<td>200 mg/kg b.w.</td>
<td>±0.9</td>
<td>± 0.4</td>
<td>±1.12</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Group-II</td>
<td>400 mg/kg b.w.</td>
<td>±3.4</td>
<td>0.9</td>
<td>±1.99</td>
<td>±3.1</td>
<td>±</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n=5), p<0.05

**Figure 2: Analgesic effect of *Morus alba* by hot plate test**

The phytochemical groups may exert analgesic property by inhibiting the synthesis, release, and/or antagonizing the action of pain mediators at the target sites. The identified phytochemical groups namely flavonoids, tannins, and reducing sugar in *M. alba* bark extract may be responsible for analgesic activity both centrally and peripherally (11-13).
Drugs acting on the central nervous system (CNS) were first discovered by primitive humans and are still the most widely used group of pharmacologic agents for CNS action. Both hole cross and open field test are widely used method for screening of neuropharmacological activity. The most important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the central nervous system. The sample did not decrease the locomotor activity in hole cross test as showed by the results of the hole cross test (Table 4, Figure 3).

**Table 4. Neuropharmacological activity of *Morus alba* by hole cross test**

<table>
<thead>
<tr>
<th>Group</th>
<th>Route of Administration</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>Control</td>
<td>Oral</td>
<td>5.2±0.7</td>
<td>2.8±0.4</td>
<td>4.6±0.7</td>
<td>2.4±0.4</td>
<td>2±0.3</td>
</tr>
<tr>
<td>Positive control i.p.</td>
<td>12.4±1.5</td>
<td>10.8±1.4</td>
<td>3.6±0.9</td>
<td>5±1.7</td>
<td>3±0.7</td>
<td></td>
</tr>
<tr>
<td>Group-I</td>
<td>Oral</td>
<td>5.6±1.7</td>
<td>2.6±0.7</td>
<td>4±1.05</td>
<td>2.8±0.7</td>
<td>2±0.4</td>
</tr>
<tr>
<td>Group-II</td>
<td>Oral</td>
<td>4.8±1.6</td>
<td>4.8±1.1</td>
<td>5.6±0.7</td>
<td>4.2±0.5</td>
<td>3±0.5</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n=5), p<0.05

In open field, the extract showed low locomotor activity as showed by the results of the open field test (Table 5, Figure 4). It is seen that the locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 4th observation period (90 min) at the dose of 200 mg/kg body weight while at the dose of 400 mg/kg body weight, locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 5th observation period (120 min). The identified phytochemical groups namely flavonoids, tannins, and reducing sugar in *M. alba* bark extract may be responsible for analgesic activity both centrally and peripherally (11-13). The results obtained in our present study, indicate that the extract might have depressant action on the CNS.

**Table 5. Neuropharmacological activity of *Morus alba* by open field test**

<table>
<thead>
<tr>
<th>Group</th>
<th>Route of Administration</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>Control</td>
<td>Oral</td>
<td>68.2±8.4</td>
<td>16.6±7.3</td>
<td>12.6±3.4</td>
<td>5±0.9</td>
<td>8.6±1.97</td>
</tr>
<tr>
<td>Positive control i.p.</td>
<td>89±12.9</td>
<td>49.2±6.6</td>
<td>17±9.5</td>
<td>18.2±5.1</td>
<td>24.4±6.3</td>
<td></td>
</tr>
<tr>
<td>Group-I</td>
<td>Oral</td>
<td>78.2±10.3</td>
<td>40.2±6.1</td>
<td>45.8±11.2</td>
<td>36±5.03</td>
<td>20.4±3.03</td>
</tr>
<tr>
<td>Group-II</td>
<td>Oral</td>
<td>61±6.4</td>
<td>44.6±6.3</td>
<td>24.2±3.7</td>
<td>20.8±3.2</td>
<td>26.4±5.4</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ±SEM (n=5), p<0.05

Figure 3: Neuropharmacological activity of *Morus alba* by hole cross test

Figure 4: Neuropharmacological activity of *Morus alba* by open field test
Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs elucidate their action through GABA, and hence, it is possible that ethanol extract of *M. alba* may act by potentiating GABA-ergic 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 sample (14).

In this study, locomotor activity measured by the open field test, showed that the extract decreased locomotor activity. Diazepam, which was used to induce sleep in this study, acts as specific binding sites that are closely related to γ-aminobutyric acid (GABA) receptors, the binding of benzodiazepines enhancing GABA-ergic transmission (15). It has also been reported that some flavonoids exhibit high affinity binding to the benzodiazepine site of GABA receptors. The presented phytochemical groups namely glycosides, flavonoids, and tannins in *M. alba* bark extract may be responsible for CNS depressant activity (16).

**STATISTICAL ANALYSIS**

All experiments were performed thrice and the averaged data were expressed as Mean ± Standard Error (SE).

**CONCLUSION**

Based on the result of the present study, it can be concluded that the bark of *Morus alba* possesses analgesic as well as neuropharmacological potential. Our study revealed the presence of glycosides, flavonoids, tannins and reducing sugar in the crude ethanol bark extract of *M. alba* which is responsible for these pharmacological activity. Literature review of *Morus alba* revealed the plant also have antioxidant, anti-dopaminergic and antibacterial activity in leaves and fruits, nephroprotective and antidiabetic activity in fruits. Therefore, the present study provides the evidences rationality for the traditional use of this plant as analgesic and neuropharmacological activity.

The present work indicates further studies on isolation and characterization of the active components responsible for analgesic and neuropharmacological activity.

**ACKNOWLEDGEMENT**

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تقييم النشاط المسكن والعصبي العصبي من ناحية مورس ألبا L. الأسرة: موراسي

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ملخص
كان الهدف من هذه الدراسة تقييم الخصائص المسكنة والعصبية العصبية للحاول مورس ألبا L. هذا النبات هو معروف جدا عن أثاره مسكن ومضاد للكاناس. تم إجراء النشاط المسكن والتوراراماكارولوجي بجرعات 200 و 400 ملغ/كم من وزن الجسم باستخدام الفئران البيضاء السوية كنموذج حياني. تم البحث عن النشاط المسكن باستخدام مادة كيميائية واحدة (اختبار الحمض الانتاج عن حمض الخلوك (طريقة وطريقة حرارية) اختبار الصفيحة الساخنة.) (في اختبار الخيط الناجم عن حمض الخلوك، أظهر الدواء القياسي ديكوفيتيك-د- 5 ملغ / كغم من وزن الجسم (الكثير 89.3 %، حيث كان تثبيط المستخلص 79.75 % و 54.78 % بجرعات 200 و 400 ملغ/كم من وزن الجسم على التوالي.) وعموما، أظهر استجابة نشاط مسكن كبير. وقد لوحظ النشاط المحيطي العصبي العضلي من خلال حفرة الصليب والاختبارات الميدانية المفتوحة. في حفرة الصليب اختبار الحقل المفتوح، وانخفضت العينة بشكل ملحوظ في النشاط الحركي مقارنة مع المخدرات القياسية (ديبارافين، 5 ملغ / كغم من وزن الجسم) (بالتزام، فإن النتائج التي تم الحصول عليها من عمليا الحالي توفر الأساس المنطقي لاستخدام هذا النبات للأطراف العصبية والتحفيز لمزيد من التحقيقات للحصول على نتائج مثمرة أكثر.

الكلمات الدالة: موريس ألبا L، موراسي، النشاط المسكن، النشاط العصبي، نشاط مضاد للكاناس.