Phytochemical Screening and Pharmacological Evaluation of the Methanolic Extract of Cissus Elongata Roxb. Leaves

Emran Hossain1*, Abdullah Aziz1, 2, 3, Nusrat Jahan Vabna4, Irin Akter5, Sukria Hossain6, Shahin Sarker1, Kishor Mazumder 1, 2, 7

1 Department of Pharmacy, Jashore University of Science and Technology, Jashore, Bangladesh.
2 School of Optometry and Vision Science, University of New South Wales, Sydney, Australia.
3 Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh.
4 Department of Pharmacy, University of Asia Pacific, Bangladesh.
5 Department of Pharmacy, Stamford University Bangladesh, Bangladesh.
6 Department of Pharmaceutical Sciences, North South University, Bangladesh.
7 School of Biomedical Sciences, Charles Sturt University, Australia.

ABSTRACT

This study evaluates the pharmacological activity of the methanolic extract of Cissus elongata Roxb. leaves. Dried leaves of C. elongata were extracted with methanol. The crude extract was then tested to identify the presence of different phytoconstituents. To evaluate the pharmacological effects of this plant—analgesic, anti-diarrheal, anti-inflammatory and antipyretic, tests were conducted using animal models. Analgesic activity was evaluated by an acetic acid-induced writhing test, formalin-induced paw-licking test paw-licking immersion test. Castor oil-induced anti-diarrheal test was performed to evaluate anti-diarrheal activity. Xylene-induced ear edema and brewer’s yeast-induced pyrexia test were performed to investigate anti-inflammatory and antipyretic activity respectively. Phytochemical screening revealed the presence of carbohydrates, tannins, fat, and fixed oil. In writhing test, the extract showed 45.68±3.99 % and 52.28±1.67 % inhibition of writhing at a dose of 200 mg/kg and 400 mg/kg respectively whereas it was 73.09±1.01 % for the standard drug compared to the control group. In the formalin-induced paw-licking test, the percentage inhibition of licking by both doses of the extract was more in the delayed phase than in the acute phase. In the tail immersion test, 200 mg/kg dose didn’t show a significant effect but 400 mg/kg showed significant (*P<0.05 vs. control) analgesic activity at 30 min, 60 min and 120 min time periods. In an anti-diarrheal study, 400 mg/kg dose showed (68.42±0.87 % inhibition of diarrhea) almost similar anti-diarrheal effect as the standard drug loperamide HCl (71.05±0.58 %). In xylene induced ear edema test, both doses of the extract showed little anti-inflammatory effect compared to the standard drug but both doses showed significant (*P<0.05 vs. control) reduction in body temperature in the antipyretic study.

Keywords: Analgesic, anti-diarrheal, anti-inflammatory, antipyretic, Cissus elongata.

1. Introduction

From primitive times various segment of plants have been used to treat many diseases. The World Health Organization (WHO) has enumerated that around 80% people of in developing and underdeveloped countries depend on conventional medicines to satisfy their basic health needs1. New sources of biologically active compounds are being introduced from plant origin which are considered as one of the richest sources of drugs in the traditional medicinal system2. Nowadays drugs of natural
origin are becoming more popular. There are about 5000 different types of plant species growing in Bangladesh and around 250 species are used as medicinal plants.

*Cissus elongata* Roxb. belongs to Vitaceae family, traditionally known as ‘Dhemna’ or ‘Chemna’ in local areas of Bengal. This plant is primarily distributed in East and South-East Asia including China and Taiwan. In Bangladesh, it is usually found in hilly areas like Chittagong, Banandarban. It is a very large, glabrous climbing shrubs, having profoundly lobed elliptic-lanceolate, serrate leaves (12-17.5 cm) with digitately 3–5 foliolates. Some phytochemicals like alkaloids, flavonoids, sterols, tannins, terpenoids and saponins were found in *C. elongata*. Moreover, this plant has antioxidant, antibacterial, and, antifungal properties. Ethnic people use *C. elongata* to be cured of various malady such as cardiac ailments, infections, anti-poisonous, gastrointestinal abnormalities. Till now, we have not noticed any study regarding the analgesic, antipyretic, anti-diarrheal and anti-inflammatory activity of the methanolic extract of *Cissus elongata* leaves. Therefore, the current study aimed to investigate the analgesic, anti-diarrheal, anti-inflammatory, and antipyretic activity of the methanolic extract of *C. elongata* Roxb. leaves.

2. Materials and Methods

2.1 Collection of Plant

The mature leaves of *Cissus elongata* were procured from Monirampur, Jashore, Bangladesh in October, 2020. Collected plant was identified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium. A dried specimen was deposited in the national herbarium for future concern.

2.2 Preparation of Extract

*Cissus elongata* leaves were extracted using methanol as solvent. After collection, Leaves were thoroughly rinsed and dried under sunlight for seven days. The leaves were then crushed to make fine powder by a grinder. 1000 gm of grinded powder was soaked in an adequate amount of methanol one week days at ambient temperature with occasional stirring and shaking. The mixture was then filtered and the filtrate was concentrated using a rotary evaporator to obtain the viscous extract of *C. elongata*. The obtained thick mixture was dried at ambient temperature.

2.3 Drugs and Chemicals

Diclofenac sodium, tramadol HCl, loperamide HCl, dexamethasone, and paracetamol were purchased from local medicine shops manufactured by Square Pharmaceuticals Ltd., Bangladesh. Other reagents and chemicals were obtained from Merk, Germany. Analytical-grade chemicals and reagents were used for this study.

2.4 Experimental Animals

*Swiss albino* mice (Mus musculus) of both sex, 5-6 weeks of age, collected from the Pharmacology lab, Jahangirnagar University, Bangladesh, were selected for the investigation. These animals were apparently healthy and weighed 20-30 g and were kept at a controlled temperature (25 ± 1°C). The standard guideline for treating and handling animals adopted by the Animal Ethical Committee of the Jashore University of Science and Technology was pursued for *in vivo* tests and all the experiments were carried out following the internationally accepted principles for laboratory animal handling.

2.5 Evaluation of Phytochemical Screening of Methanolic Extract of *C. Elongtata*

Phytochemical screening was conducted to identify the presence of phytochemicals such as carbohydrates, alkaloids, tannins, flavonoids, fat and essential oils. Freshly prepared methanolic extract of *C. elongata* (MECE) were subjected to various identification tests to identify the presence of different phytochemicals.

2.5.1 Molisch’s Test for Carbohydrates

500 mg of methanolic extract of *C. elongata* was accurately weighted. The weighted extract was dissolved in 5 mL of distilled water. The prepared solution was filtered through a membrane filter. The filtrate was collected and a few drops of Molisch’s reagent was introduced to it. About 1 mL of concentrated H₂SO₄ was
added to it with care. The mixture was kept at ambient temperature for 2 minutes and 5 mL of deionized water was added to it. A reddish color band was formed in the middle of the two solvents which confirms the presence of carbohydrates.

2.5.2 Test for Fat & Fixed Oils

Approximately 5 drops of a solution prepared for the previous test were mixed with 1% CuSO₄ solution and stirred for two minutes. After that, a few drops of instantly prepared 10% (w/v) sodium hydroxide solution was added slowly. The presence of fat & fixed oil was confirmed with the formation of a clean blue solution.

2.5.3 Mayer’s Test for Alkaloids

About 50 mg of C. elongata extract was taken. 5 mL of 1% HCl was added to it and gently stirred to dissolve. Then the solution was filtered through a membrane filter. Around two mL of the filtrate was taken in a glass test tube. A few drops of Mayer’s reagent were added to it. A white creamy precipitate is formed if an alkaloid is present.

2.5.4 FeCl₃ Test for Tannins

5 mL distilled water was used to dissolve the crude extract (50 mg) in which a few drops of 5% FeCl₃ were added. The presence of tannin was confirmed when a bluish black color solution was formed.

2.5.5 Alkali Test for Flavonoids

About 100 mg of plant extract was dissolved in 10 mL of methanol. The prepared solution was filtered using a membrane filter and filtrate was collected. 1 mL of filtrate was taken into a test tube and a few drops of freshly prepared 10% sodium hydroxide solution was added to it. A deep yellow color was formed. A few drops of concentrated hydrochloric acid were added to it. The presence of flavonoid is confirmed if the color is lost upon the addition of acid.

2.6 In Vivo Experiments of Methanolic Extract of C. Elongata

2.6.1 Acute Toxicity Study:

Acute toxicity study was performed to determine whether the test samples show any toxicity or adverse effects on the tested animals. Acute toxicity usually occurs within a very short period usually within 24 hours of exposure upon administering a large single dose or multiple doses. This study was performed according to the Organization of Economic Cooperation and Development (OECD) guideline. Ten animals were taken to perform this study. They were divided into two groups and each group consisted of five animals. Between these two groups, one group was given MECE at different doses, while the other group served as control. Extract was administered to the animals of test groups at a dose of 100, 200, 600, 1200, 1800, 2500 and 3500 mg/kg of body weight. After administration, the animals were closely observed for five to six hours to identify whether any symptoms of toxicity such as mortality, difficulty in breathing, change in behavior, irritation, flow of saliva, seizure, pupil enlargement or constriction, changes in food habit, locomotion or any other symptoms take place. The animals were further observed for two weeks to identify any toxicity.

2.6.2 Evaluation of Analgesic Activity of Methanolic Extract of C. elongata

2.6.2.1 Acetic Acid-Induced Writhing Test in Mice

This experiment was performed following the method of Koster et al. Swiss albino mice of both sexes were taken and divided into four groups, each group having five animals. Group I (Control group) received 1% tween 80 in 0.9% normal saline intraperitoneally at a dose of 10 ml/kg body weight. Group II (positive control group) was administered diclofenac sodium intraperitoneally at the dose of 10mg/kg body weight. Groups III and Group IV were given a methanolic extract of C. elongata at a dose of 200 mg/kg and 400 mg/kg respectively through the intraperitoneal route. After 45 minutes of administering the drugs, 0.7% acetic acid (10 ml/kg) was injected through an intraperitoneal route into each mouse. After 15 minutes of injecting acetic acid, the mice were observed closely for 5 minutes and the number of abdominal constrictions was counted in this period. Percent inhibition of writhing was calculated using the following formula.


% inhibition = 1 - \[
\frac{\text{No.of writhing (extract or standard drug)}}{\text{No.of writhing (normal control)}} \times 100\% \]

2.6.2.2 Formalin-Induced Paw-Licking Test in Mice

For the formalin-induced paw-licking test, the animals were divided into four groups each group having five mice. One group received the standard drug diclofenac sodium at a dose of 10 mg/kg and was considered as the standard group, another group received 1% tween 80 in 0.9% normal saline and was considered the control group. The other two groups received extracts at a dose of 200 mg/kg and 400 mg/kg respectively. 1 h later respective treatment mice of every group were injected 20 µL of 2.7% formalin into the sub-plantar space of the left hind paw. Paw licking time was recorded at the early phase (0–5 min) and late phase (20–25 min) after the administration of formalin.

2.6.2.3 Tail Immersion Test

This test was carried out according to the method of Aydin et al., which was utilized to assess the central mechanism of pain-relieving action. Mice were assembled and treated as depicted already. Tramadol (100 mg/kg) was utilized as the reference medicine. The animals fasted for 16 h with free access to water. The basal response time of the mouse was measured by inundating the tail tips of the mouse (final 1–2 cm) in hot water of (55 ± 1) °C. The time taken to withdraw the tail from the water was recorded and compared with the control group. The latent period of the tail flick reaction was recorded 30 min before and after 30, 60, 120 and 180 min of the particular treatment of each bunch.

2.6.3 Evaluation of Anti-diarrheal Activity of Methanolic Extract of Cissus elongata

2.6.3.1 Castor Oil Induced Anti-diarrheal Test

This test was executed by the adjusted strategy of Shoba and Thomas. Mice were chosen on the basis of defecating diarrheal feces by orally applying 0.5 ml castor oil orally. The mice fasted for 16 h with water ad libitum. Mice of the control group received 1% tween 80 in 0.9% normal saline (10 mL/kg), and the standard group was administered 3 mg/kg loperamide HCl. The other two groups received plant extract at a dose of 200 mg/kg and 400 mg/kg body weight. 30 minutes later, each mouse received 0.5 ml of castor oil. Blotting paper was set for each mouse and was changed every hour. The number of diarrheal feces was recorded for a period of 4 h and the rate of the hindrance of defecation was calculated for each group of mice.

2.6.4 Determination of In-Vivo Anti-inflammatory Activity

2.6.4.1 Xylene-Induced Ear Edema Test

This experiment was conducted by the method of Tang et al. with slight modification. Each animal was divided into four groups of five. They were given plant extract (200 and 400 mg/kg), dexamethasone (100 mg/kg), and 1% tween 80 in 0.9% normal saline (10 mL/kg) orally. One hour after the respective treatment of mice, 20 µL xylene was added to the interior and posterior surface of the right ear lobe to cause edema. The left ear was freed from xylene treatment and was regarded as a control. They were anesthetized for 30 minutes and both ears were separated and cut circularly with a 7 mm diameter cork borer. The left and right parts of the ear were weighted, and the percentage inhibition of ear edema was determined by comparing it with the xylene-free left ear.

2.6.5 Evaluation of Antipyretic Activity of C. elongata

2.6.5.1 Brewer’s Yeast-Induced Pyrexia Test

This experiment was carried out by Srinivasan et al. with slight modification. Rectal temperature of each mouse was measured carefully before inducing pyrexia by employing a digital thermometer that was inserted 2 cm into the rectum. 15% (w/v) suspension of brewer’s yeast was injected subcutaneously at a dose of 10 mL/kg within the back underneath the scruff of the neck and rubbed completely. The mice that showed an increment in temperature of at least 0.6°C after 18 hours of infusion were regarded as pyretic mice and chosen for this test.
Mice were fasted overnight with water ad libitum before giving respective treatment. Distilled water (control), paracetamol (100 mg/kg, standard) and plant extract (200 mg/kg and 400 mg/kg, test sample) were given orally to the pyretic mice for examining their antipyretic action. The rectal temperature of each pyretic mouse was observed at 1, 2, 3, and 4 h by a digital thermometer.

2.6.6 Statistical Analysis
All results are presented as mean ± standard error (SE). All tests were analyzed statistically by one-way ANOVA followed by Dunnett’s t-test. *P < 0.05 was regarded as statistically significant. All data were analyzed using SPSS software (version 16; IBM Corporation, New York, USA).

3. Results

3.1 Phytochemical Screening
The Phytochemical screening of C. elongtata revealed the presence of carbohydrates, tannins, fat and fixed oils. The result of the phytochemical screening has been summarized in table 1.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Test name</th>
<th>Observation (MECE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test, Fehling’s test</td>
<td>++ +</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>_ _ _</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl₃ test</td>
<td>+ + +</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alkali test</td>
<td>_ _ _</td>
</tr>
<tr>
<td>Fat and fixed oils</td>
<td>CuSO₄ test</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

3.2 Acute Toxicity Study
No sign of toxicity was observed during the observation period of two weeks. Both the experimental and test groups showed similar results which indicate that the methanolic extract of C. elongata leaves does not cause any acute toxicity to the test animals.

3.3 Evaluation of Analgesic Activity

3.3.1 Acetic Acid-Induced Writhing Test Result
Table 2 presents the percent inhibition of writhing by different groups of mice. Among the plant extracts, the maximum percent inhibition (52.28±1.67%) was obtained by MECE 400 mg/kg.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>No. of writhing</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10ml/kg</td>
<td>39.40±3.07</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>100mg/kg</td>
<td>10.60±1.07*</td>
<td>73.09±1.01*</td>
</tr>
<tr>
<td>MECE</td>
<td>200mg/kg</td>
<td>21.40±4.05*</td>
<td>45.68±3.99*</td>
</tr>
<tr>
<td>MECE</td>
<td>400mg/kg</td>
<td>18.80±1.88*</td>
<td>52.28±1.67*</td>
</tr>
</tbody>
</table>

All experimental values are denoted as mean ± standard error of mean (SEM). n= 5 mice in each group. *P<0.05, vs. control (Dunnett’s t test).

3.3.2 Formalin-induced paw licking
The percent inhibition of formalin-induced paw licking is exhibited in table 3. Both MECE 200 mg/kg and 400 mg/kg showed increased inhibition of formalin-induced paw licking from acute to delayed phase (40.46±7.87% to 76.70±3.04% and 72.75±8.03% to 84.09±3.01% respectively).
Table 3: Effect of methanolic extract of *C. elongata* leaves (MECE) in formalin-induced paw-licking test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Licking in acute phase (s)</th>
<th>Inhibition in acute phase (%)</th>
<th>Licking in delayed phase (s)</th>
<th>Inhibition in delayed phase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10ml/kg</td>
<td>129.0±5.73</td>
<td>0.00±0.00</td>
<td>53.57±5.69</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>100mg/kg</td>
<td>66.71±6.40*</td>
<td>48.28±6.3.99*</td>
<td>6.24±1.81*</td>
<td>88.35±1.76*</td>
</tr>
<tr>
<td>MECE</td>
<td>200mg/kg</td>
<td>76.80±8.07*</td>
<td>40.46±7.87*</td>
<td>12.48±3.62*</td>
<td>76.70±3.04*</td>
</tr>
<tr>
<td>MECE</td>
<td>400mg/kg</td>
<td>35.14±8.36*</td>
<td>72.75±8.03*</td>
<td>8.52±3.33*</td>
<td>84.09±3.01*</td>
</tr>
</tbody>
</table>

All experimental values are denoted as mean ± SEM. n= 5 mice in each group. *P<0.05, vs. control (Dunnett’s t-test).

3.3.3 Tail immersion test

Both the standard group and MECE had effectively increased the latency up to 60 min after their respective treatment. The maximum effect (4.88±0.58 s) of the extract, MECE 400 mg/kg, was obtained at 60 min which was significant (P<0.05) in comparison to the control (Table 4).

Table 4: Effect of methanolic extract of *C. elongata* leaves (MECE) in tail immersion test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10ml/kg</td>
<td>1.94±0.15</td>
<td>1.98±0.12</td>
<td>1.97±0.05</td>
<td>1.88±0.06</td>
<td>1.87±0.12</td>
</tr>
<tr>
<td>Tramadol HCl</td>
<td>100/kg</td>
<td>2.10±0.19</td>
<td>5.00±0.43*</td>
<td>5.59±0.39*</td>
<td>4.30±0.27*</td>
<td>2.51±0.15</td>
</tr>
<tr>
<td>MECE</td>
<td>200ml/kg</td>
<td>1.69±0.29</td>
<td>2.04±0.15</td>
<td>2.18±0.34</td>
<td>2.07±0.30</td>
<td>1.99±0.39</td>
</tr>
<tr>
<td>MECE</td>
<td>400ml/kg</td>
<td>1.89±0.14</td>
<td>3.56±0.75*</td>
<td>4.88±0.58*</td>
<td>2.60±0.67*</td>
<td>2.27±0.23</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard error of mean. n= 5 mice in each group. *P<0.05, vs. control (Dunnett’s t test).

3.4 Evaluation of Anti-diarrheal Activity

3.4.1 Cast the or Oil the induced oil-induced test

In case of castor oil-induced anti-diarrheal test, loperamide HCl, MECE 200 and 400mg/kg inhibited diarrhea in mice. Both doses of the extract, 200 and 400mg/kg significantly reduced the total number of diarrheal feces. Highest and significant percentage inhibition of diarrhea (68.42±0.87%) was revealed by MECE 400 mg/kg (Table 5).

Table 5: Effect of methanolic extract of *C. elongata* leaves (MECE) in castor oil-induced anti-diarrheal test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Number of diarrheal feces</th>
<th>% inhibition of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10ml/kg</td>
<td>7.60±0.60</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Loperamide HCl</td>
<td>3mg/kg</td>
<td>2.20±0.58*</td>
<td>71.05±0.58*</td>
</tr>
<tr>
<td>MECE</td>
<td>200mg/kg</td>
<td>5.60±0.67*</td>
<td>26.31±0.67*</td>
</tr>
<tr>
<td>MECE</td>
<td>400mg/kg</td>
<td>2.40±0.87*</td>
<td>68.42±0.87*</td>
</tr>
</tbody>
</table>

All experimental values are denoted as mean ± SEM. n= 5 mice in each group. *P<0.05, vs. control (Dunnett’s t test).

3.5 Evaluation of Anti-inflammatory Activity

3.5.1 Xylene-Induced Ear Edema Test

Table 6 shows the results of various groups’ percent inhibition of xylene-induced ear edema are presented. All groups had significant ear weight variations and inhibition of ear edema in comparison to the control group. Dexamethasone (DM) showed the highest inhibition (48.08±1.13%). However, MECE at 400 mg/kg exhibited the highest inhibition (35.85±0.40%) between the two doses of the plant extract.
Table 6: Effects of methanolic extract of *C. elongata* leaves (MECE) in xylene induced ear edema test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Ear weight difference (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>13.00±1.58</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>100 mg/kg</td>
<td>6.75±1.03*</td>
<td>48.08±1.13*</td>
</tr>
<tr>
<td>MECE</td>
<td>200 mg/kg</td>
<td>9.96±1.19*</td>
<td>23.38±1.59*</td>
</tr>
<tr>
<td>MECE</td>
<td>400 mg/kg</td>
<td>8.34±0.87*</td>
<td>35.85±0.40*</td>
</tr>
</tbody>
</table>

All experimental values are denoted as mean ± SEM. n= 5 mice in each group. *P<0.05, vs. control (Dunnett’s t test).

3.6 Evaluation of Antipyretic Activity

3.6.1 Brewer’s Yeast-Induced Pyrexia Test

The antipyretic effect of the different doses of the groups (control, standard and extract) are shown in table 7. The highest antipyretic effect of the plant extract was observed one hour after its administration. There was significant post-treatment antipyretic activity of MECE (200 and 400 mg/kg) when compared to the control.

Table 7: Effects of methanolic extract of *C. elongata* leaves (MECE) in Brewer’s yeast-induced pyrexia test

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Initial rectal temperature (°C)</th>
<th>Rectal temperature in °C after 18hrs of yeast injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>36.85±0.17</td>
<td>36.97±0.11</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100 mg/kg</td>
<td>37.70±0.31*</td>
<td>38.01±0.35*</td>
</tr>
<tr>
<td>MECE</td>
<td>200 mg/kg</td>
<td>36.80±0.62*</td>
<td>37.10±0.40*</td>
</tr>
<tr>
<td>MECE</td>
<td>400 mg/kg</td>
<td>37.05±0.67*</td>
<td>37.33±0.21*</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM. n= 5 mice in each group. *P<0.05, vs. control (Dunnett’s t test).

4. Discussion

In this experiment, the suppositional analgesic, anti-diarrheal, anti-inflammatory and antipyretic activities of *C. elongata* were evaluated to observe its medicinal effects in vivo.

Pain can be defined as the sensational unpleasant experience to the body due to actual or potential tissue damage 20. Various biochemical mediators such as prostaglandins, bradykinins, substance P etc. work on the pain receptors which cause the release of the sensation by tissue damage and is thought to be the primary reason for painful sensation 21. Analgesics such as NSAIDs, steroids, opioids etc. are used to suppress pain all over the world 22, 23. The methanolic extract of *C. elongata* was used to observe its analgesic activity by undertaking a writhing test, paw-licking test and tail immersion test.

A writhing test is a chemical procedure in which pain is induced at a circumferential area by an irritant concept via nociceptors 24. The frequency of writhing is reduced with the use of analgesic compounds 25. It is employed for the assessment of circumferentially acting analgesics through prostaglandin (PG) pathways, acid-sensing ion channels, and peritoneal mast cells 26, 27. Acetic acid is used as the inciter of writhing response by which various endogenous noxious mediators like serotonin, histamine, substance P, etc. are released and nociceptive neurons are being stimulated 28 which accommodates the localized inflammatory response for the perception of pain 11. The secretion of arachidonic acid from tissue phospholipids and prostaglandins biosynthesis results in the provocation of pain 29. In this study, methanolic extract of *C. elongata* (MECE) at 400 mg/kg dose has shown significant antinociceptive activity by reducing acetic acid-induced writhing response. This effect is considered to be achieved.
by inhibiting the arachidonic acid metabolites synthesis.\textsuperscript{30} Formalin-induced paw-licking test is a well-established test method to evaluate the analgesic effect exerted by any test substance. In the in vivo model, formalin is used to produce an explicit biphasic response indicating early phase and late phase.\textsuperscript{31} Different types of responses are attained in these phases with the use of various types of analgesics. Hence, the possible mechanism of antinociceptive activity of the proposed analgesics can be elucidated by effectuating this test.\textsuperscript{32} Opioids which are usually centrally acting analgesics generally inhibit not only the initial but also the delayed phase of pain equally.\textsuperscript{33} However, dexamethasone, a peripherally acting drug only inhibits the pain of delayed phase.\textsuperscript{34} In this formalin test, the predominant inhibition of nociception at the late phase suggests that the peripheral action might be the reason for its antinociceptive effect. However, elaborative studies are required to confirm the accurate mechanism of action of this plant extract.

Tail immersion test is a well-established experimental model to measure the centrally acting analgesic effect of a drug or any test substance by producing acute pain.\textsuperscript{26} The result of this test exhibited a significant increase in tail withdrawal reflex time in response to heat stimuli after the administration of MECE to the experimental animal which appraises the central analgesic property of MECE. From these three experimental results, we can infer that the MECE may inhibit the release of endogenous pain mediators and can exert both central and peripheral analgesic effects.

Inflammation is the body's response against any harmful stimulus like allergen and damage of tissue, but massive inflammation can cause different disorders such as allergy, metabolic abnormalities etc.\textsuperscript{35} The process which is involved in arachidonic acid metabolism is associated with the onset of inflammatory action. The pathway follows either of two ways: generation of leukotrienes (LTs) by 5-lipoxygenase (5-LO) enzyme or synthesis of prostaglandin (PGs) by cyclooxygenase (COX). COX-2 significantly simulates the inflammatory factors.\textsuperscript{33} Many drugs are available for inhibiting or minimizing inflammatory responses such as steroidal drugs, NSAIDs, immunosuppressants etc.\textsuperscript{36} However, the significant level of adverse effects of these agents has led to the exploration of natural pharmacological agents to avoid or reduce adverse effects. The xylene-induced ear edema test is commonly used as an acute inflammatory model. Xylene releases different substances like bradykinin, serotonin, histamine etc. which can mediate inflammation process and ear edema through the enhancement of vascular permeability and promotion of vasodilation.\textsuperscript{37} It causes fluid accumulation on the treatment site. The inhibition of this fluid accumulation also called the anti-inflammatory effect of proposed substances is ascertained by the xylene-induced ear edema model.\textsuperscript{25} MECE markedly inhibited the increase in vascular permeability which resulted in the generation of an anti-inflammatory effect. However, for confirming the exact anti-inflammatory mechanism of the extract, extensive study is required.

The increased gastrointestinal motility and secretion and a decrease in the absorption of fluid and electrolytes is characterized as diarrhea.\textsuperscript{38} Castor oil is popular as a diarrheal agent that prevents the reabsorption of NaCl and H2O by altering the transport of H2O and different ions through the gastrointestinal mucosal membrane. A hyper-secretory response (decrease of Na\textsuperscript{+} and K\textsuperscript{+} absorption) is obtained due to the rapid flow of luminal content through small and large intestines. Consequently, the stimulation of peristaltic activity causes diarrhea.\textsuperscript{39} Ricinoleic acid, the active component of castor oil, is responsible for induction of diarrhea and can irritate the intestinal mucosa and release inflammatory mediators.\textsuperscript{38} Therefore, the anti-diarrheal activity of MECE may be due to the inhibition of peristaltic activity of GI tract.

Fever is a complex physiologic response triggered by infectious or aseptic stimuli which is also commonly found...
in the critically ill patients 40. The initiation of fever is mediated by the release of pyrogenic cytokines (tumor necrosis factor α, interleukin 1, interleukin 6, and interferons) 41. The temperature of the body increases, when the concentrations of endogenous pyrogenic substances like prostaglandin E (2) (PGE (2)) and cyclooxygenase (COX) enzymes rise within certain areas of the brain (hypothalamus) 42. Antipyretic agents are widely consumed which function by inhibiting COX enzyme and PGE 2 within the hypothalamus 42.

Brewer’s yeast-induced pyrexia test is widely used to observe the antipyretic activity of test agents. Moreover, Brewer’s yeast acts as a fever-inducing factor which activates PGE 2 and causes the elevation of body temperature of experimental animals. MECE is thought to interfere with the PGE 2 synthesis in the hypothalamus and exhibit antipyretic action 43.

5. Conclusion
From the results of multiple in vivo tests, it can be proposed that the methanolic extract of C. elongata leaves might possess analgesic, anti-inflammatory, anti-diarrheal and antipyretic activities. However, further extensive studies are necessary not only to determine and isolate the exact bioactive compounds of C. elongata leaves that are responsible for the aforementioned pharmacological activities but also to find out the accurate mechanism of action of these compounds.

Conflict of Interest
The authors declare that they have no conflict of interest.

REFERENCES
7. Tsai, W.H. and Wu, S.C. Cissus elongata Roxb. (Vitaceae), a new addition to the flora of Taiwan. Taiwania. 2000; 45(3); 235-238.
11. Aziz, M.A. Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of Microcos paniculata barks and


31. Shibata, M., Ohkubo, T., Takahashi, H., Inoki, R.
Phytochemical Screening


الفحص الكيميائي النباتي والتقييم الدوائي لمستخلص الميثانول من ورقِ
Cissus elongata Roxb

عمران حسي نُ1، عبد الله عزيز 2، نصرت جهان فابينا 3، م. إيرين أكثر 4، سكريا حسين 6،
شاهين ساركر 1، كيشور مازومدار 1، 2، 7

قسم الصيدلة، جامعة جاشور للعلوم والتكنولوجيا، بنغلاديش 1
كلية البصريات وعلوم الرؤية، جامعة نيو ساوث ويلز، أستراليا 2
قسم الصيدلة، جامعة جاهانجيرناغار، بنغلاديش 3
قسم الصيدلة، جامعة آسيا والمحيط الهادئ، بنغلاديش 4
قسم الصيدلة، جامعة ستامفورد بنغلاديش، بنغلاديش 5
قسم العلوم الصيدلانية، جامعة شمال الجنوب، بنغلاديش 6
كلية العلوم الطبية الحيوية، جامعة تشارلز ستورت، أستراليا 7

ملخص
أجريت هذه الدراسة لتقييم النشاط الدوائي لمستخلص الميثانول من أوراق Cissus elongata المجففة من C. elongata مع الميثانول. تم اختيار المستخلص الخام لتحديد وجود مكونات نباتية مختلفة لتقاسم الآثار الدوائية لهذا النبات - تم إجراء اختبارات مسكنة ومضادة للإسهال ومضادة للالتهابات ومضادة للحرارة، باستخدام نماذج حيوانية. تم تقاسم النشاط المسكین من خلال اختبار التلويح الناجم عن حمض الخليك، واختبار لعق المخلب الناجم عن الفورمالين واختبار غمر الذيل.

في اختبار التلويح، أظهر المستخلص 45.68 ± 3.99٪ و 52.28 ± 1.67٪ تثبيط التلويح بجرعتين 200 مجم / كجم و 400 مجم / كجم على التوالي بينما كان 73.09٪ ± 1.01٪ تثبيط التلويح بجرعة 200 مجم / كجم كمعدل قياسي مقابل المجموعة الضابطة. في اختبار عصبة الأذن، وجدت النجاح في المحاولة 45.68 ± 3.99٪ و 52.28 ± 1.67٪ تثبيط التلويح بجرعتين 200 مجم / كجم و 400 مجم / كجم على التوالي بينما كان 73.09٪ ± 1.01٪ تثبيط الإسهال بجرعة 400 مجم / كجم كمعدل قياسي مقابل المجموعة الضابطة. في اختبار لسعال الجزء العلوي، وجدت النجاح في المحاولة 68.42 ± 0.87٪ و 71.05 ± 0.58٪ تثبيط الإسهال بجرعتين 400 مجم / كجم و 400 مجم / كجم على التوالي بينما كان 73.09٪ ± 1.01٪ تثبيط الإسهال بجرعة 400 مجم / كجم كمعدل قياسي مقابل المجموعة الضابطة. في اختبار سبيكة، وجدت النجاح في المحاولة 68.42 ± 0.87٪ و 71.05 ± 0.58٪ تثبيط الإسهال بجرعتين 400 مجم / كجم و 400 مجم / كجم على التوالي بينما كان 73.09٪ ± 1.01٪ تثبيط الإسهال بجرعة 400 مجم / كجم كمعدل قياسي مقابل المجموعة الضابطة.

كلمات الدالة: مسكن، مضاد للإسهال، مضاد للالتهابات، خافض للحرارة، سبيكة إيلونغاتا.

المؤلف المراسل: عمران حسي

me.hossain@just.edu.bd

تاريخ استلام البحث: 19/9/2021 وتاريخ قبوله للنشر: 14/2/2022.