**CALLISTEMON CITRINUS (CURTIS.) LEAF SHOWS ANALGESIC AND ANTIDIARRHEAL ACTIVITIES IN SWISS-ALBINO MICE MODEL**

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**ABSTRACT**

*Callistemon citrinus* (Curtis.) is an evergreen shrub that grows in Bangladesh. In order to explore the medicinal potentials, the leaves of this plant were investigated in Swiss albino mice model. The collected leaves were extracted with methanol and assayed for analgesic activity in Swiss-albino mice model by acetic acid-induced writhing and tail immersion methods. The antidiarrheal assay was also performed against castor oil-induced diarrhea. The extract when administered to the mice orally at 200 and 400 mg/kg body weight exhibited significant analgesic and antidiarrheal activities as compared to the controlled groups. This is the first report for analgesic and antidiarrheal activities of the leaves of *C. citrinus*.

**KEYWORDS:** *Callistemon citrinus*, Myrtaceae, Analgesic, Antidiarrheal, Acetic Acid, Castor Oil, Writhing, Tail Flicking.

**INTRODUCTION**

Plants are regarded as valuable resources for providing drugs. Numerous bioassay techniques have been developed for exploring the potential bioactivities of plant kingdom. Plants are sometimes specified as nutraceuticals for keeping our body healthy based on their pharmacological roles.[1-3]
Pain is associated with injury and some diseased states. Besides, diarrhea is a leading cause of malnutrition and death among children in many countries. Medicinal plants play a key role in the development of analgesic and antidiarrheal drugs. In this regard, plants can be screened to find out new safer analgesic and antidiarrheal drugs.\[4,5\] Bangladesh is in sub-tropical zone provides numerous plants and has greater capability to offer many drugs including analgesic and antidiarrheal agents.

*Callistemon citrinus* (Curtis.) is commonly known as Red bottlebrush or Lemon bottlebrush or Crimson bottlebrush. It is an evergreen shrub belonging to the Myrtaceae family. The plant is usually 1 to 3 meters tall and its leaves are generally 3 to 7 cm in length and 5 to 8 mm in width. The veins of the leaves are visible on both sides. The flowers of this shrub usually appear in late spring to early summer. *Callistemon* species are widely scattered in the wet tropics, particularly Australia, South America and tropical Asia, but now available all over the world.\[6,8\] *C. citrinus* has been reported to have antimicrobial, herbicidal and relaxant activities.\[9,10\] Previous phytochemical investigations led to the isolation of 1,8-cineole, nitisinone and α-terpineol as major compounds from the leaves and flowers of *C. citrinus*.\[10\]

As part of our continuing studies on medicinal plants of Bangladesh.\[11,12\] leaf extract of *C. citrinus* has been evaluated for its analgesic and antidiarrheal activities in mice model for the first time and we, here in, report the results of our preliminary studies.

**MATERIALS AND METHODS**

**Plant material:** The leaves of *C. citrinus* were collected, from Gazipur, Bangladesh in August, 2013 and a voucher specimen (DACB accession number-38576) has been deposited in Bangladesh National Herbarium, Mirpur, Dhaka for future reference.

**Preparation of the methanol extract:** The collected leaves were cleaned and dried for a week and then ground into a coarse powder. The powder was stored in an airtight container and kept in a cool, dark and dry place until experiment commenced. About 1 kg of powdered material was taken in a clean, flat bottomed glass container and soaked in 5 L of methanol. It was then sealed with aluminum foil and kept for 7 days accompanying occasional shaking and stirring. The whole mixture was filtered through a clean, cotton bed followed by Whatman filter paper number 1 (Bibby RE200, Sterilin Ltd., UK). The filtrate thus obtained was evaporated by using a rotary evaporator (Heidolph, Germany) to afford a gummy concentrated crude of methanol extract.
Animals: Swiss-albino mice of either sex aged 4-5 weeks, average weight 20-25 gm were used for the experiment. They were kept in standard environmental condition (at 24 ± 1°C temperature and 55-65% relative humidity and 12 hour light/12 hour dark cycle) for a week for acclimation after their purchase and fed with rodent food purchased from International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR,B) and water ad libitum. Each group contained three mice.

Drugs and chemicals: Normal saline solution (Beximco Infusion Ltd., Bangladesh), acetic acid (Merck, Germany), Tween-80 (BDH Chemicals, UK), morphine sulphate (Gonoshasthaya Pharmaceuticals Ltd., Bangladesh), diclofenac sodium (Square Pharmaceuticals Ltd., Bangladesh), loperamide (Square Pharmaceuticals Ltd., Bangladesh) and Castor oil (Lazpharma, Bangladesh) were used in this investigation.

Acetic acid-induced writhing method for peripheral analgesic assay: The peripheral analgesic activity was evaluated in mice using acetic acid induced writhing method.\cite{13,14} The percentage inhibition was calculated using the formula –

\[
\% \text{ Inhibition} = \frac{\text{Mean number of writhing (control)} - \text{Mean number of writhing (drug)}}{\text{Mean number of writhing (control)}} \times 100
\]

Tail immersion technique for central analgesic assay: The tail immersion method is a popular technique to evaluate central analgesic activity in mice.\cite{11} Here, the painful thermal stimulus in mice was generated by dipping the tip of the tail in hot water.

Antidiarrheal assay: In the antidiarrheal assay,\cite{15} mice of the test groups were orally administered with test samples. Thirty minutes later, castor oil (1ml/mice) was administered orally to each mouse to induce diarrhea. The animals were observed for number of defecation for each individual mouse.

\[
\% \text{ Inhibition} = \frac{\text{Mean defecation of control} - \text{Mean defecation of test sample}}{\text{Mean defecation of control}} \times 100
\]

Statistical analysis: Values were expressed as mean ± standard deviation. Statistical comparisons were done by using Student’s t-test, where p<0.05 was considered as statistically significant.
RESULTS

Acetic acid-induced writhing method: The number of writhings of the treated mice was used to evaluate the peripheral analgesia. The results are given in Table-1.

Table 1: Effect of C. citrinus on acetic acid-induced writhing in mice.

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Initial oral treatment</th>
<th>Consequent intraperitoneal 0.7% (v/v) acetic acid solution treatment</th>
<th>Average number of writhing</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>1% Tween 80 in normal saline</td>
<td>10 ml/kg body weight</td>
<td>10 ml/kg body weight</td>
<td>28.00 ± 2.00</td>
</tr>
<tr>
<td>Standard</td>
<td>Diclofenac sodium</td>
<td>1 mg/kg body weight</td>
<td>10 ml/kg body weight</td>
<td>7.33 ± 0.57*</td>
</tr>
<tr>
<td>Test group-1</td>
<td>Methanol extract</td>
<td>200 mg/kg body weight</td>
<td>10 ml/kg body weight</td>
<td>15.66 ± 1.15*</td>
</tr>
<tr>
<td>Test group-2</td>
<td>Methanol extract</td>
<td>400 mg/kg body weight</td>
<td>10 ml/kg body weight</td>
<td>12.33 ± 1.53*</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation; Standard and test groups were compared with the vehicle treated (control) group; *p<0.05

Tail immersion technique: The thermal stimuli mediated pain caused tail flicking in mice. The tail flicking latency period per minute was a parameter to measure the central pain relieving capacity of the plant. The results are described in Table-2.

Table 2: Effect of C. citrinus observed in tail immersion test in mice

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Treatment</th>
<th>Dose (route)</th>
<th>After 30 minutes</th>
<th>After 60 minutes</th>
<th>After 90 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>1% Tween 80 in normal saline</td>
<td>10 ml/kg body weight (oral)</td>
<td>4.35 ± 0.93</td>
<td>--</td>
<td>3.22 ± 1.56</td>
</tr>
<tr>
<td>Standard</td>
<td>Morphine sulphate</td>
<td>2 mg/kg body weight (subcutaneous)</td>
<td>8.90 ± 0.20**</td>
<td>104.59</td>
<td>19.12 ± 1.53**</td>
</tr>
<tr>
<td>Test group-1</td>
<td>Methanol extract</td>
<td>200 mg/kg body weight (oral)</td>
<td>4.62 ± 0.49</td>
<td>6.20</td>
<td>8.69 ± 1.64*</td>
</tr>
<tr>
<td>Test group-2</td>
<td>Methanol extract</td>
<td>400 mg/kg body weight (oral)</td>
<td>6.31 ± 1.27</td>
<td>45.05</td>
<td>8.86 ± 0.66*</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation; Standard and test groups were compared with the vehicle treated (control) group; *p<0.05; **p<0.005; TFLPM – Tail flick latency per minute; %I - % inhibition
Castor oil-induced antidiarrheal test: The number of defecation in mice was recorded for each individual mouse. The observations of the experimental groups were compared against that of the control to evaluate the antidiarrheal activity of the samples.

Table 3: Effects of C. citrinus on castor oil-induced diarrhea in mice

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Initial oral treatment</th>
<th>Consequent oral administration of castor oil solution treatment</th>
<th>After 1 hr.</th>
<th>After 2 hr.</th>
<th>After 3 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Material</td>
<td>Dose</td>
<td>AND</td>
<td>%I</td>
<td>AND</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>1% Tween 80 in normal saline</td>
<td>10 ml/kg body weight 1 ml/mice</td>
<td>2.67 ± 0.57</td>
<td>--</td>
<td>4.00 ± 1.00</td>
</tr>
<tr>
<td>Standard</td>
<td>Loperamide</td>
<td>50 mg/kg body weight 1 ml/mice</td>
<td>0.33 ± 0.57*</td>
<td>87.64</td>
<td>1.00 ± 0.57*</td>
</tr>
<tr>
<td>Test group-1</td>
<td>Methanol extract</td>
<td>200 mg/kg body weight 1 ml/mice</td>
<td>0**</td>
<td>100</td>
<td>0.33 ± 0.57*</td>
</tr>
<tr>
<td>Test group-2</td>
<td>Methanol extract</td>
<td>400 mg/kg body weight 1 ml/mice</td>
<td>0**</td>
<td>100</td>
<td>0**</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation; Standard and test groups were compared with the vehicle treated (control) group; *p<0.05; **p<0.005; AND= Average number of defecation.

DISCUSSION

The current experiment was designed to explore the analgesic and antidiarrheal potentials of C. citrinus growing in Bangladesh. The experimental data of the assay have been summarized in Tables 1 to 3. Both peripheral and central analgesia as well as antidiarrheal activity of the crude methanol extract were evaluated at 200- and 400- mg/kg body weight.

Peripheral analgesic action was monitored by acetic acid-induced writhing method as mentioned in materials and method section. For this assay, mice model was prepared by injecting acetic acid intraperitoneally to cause abdominal writhings. The methanol extract of the tested plant was able to reduce the writhing significantly (p<0.05) at dose 200- and 400-mg/kg body weight after oral administration (Table-1) as compared to the non-treated mice.
On the other hand, central analgesic activity was assessed by tail immersion method. It is based on counteracting pain produced in tail by thermal stimuli. Treatment with 200- and 400- mg/kg body weight of the plant extract significantly (p<0.05) protected the animals from the heat-stimulated pain (Table-2). In this method, the methanol extract showed significant increase of tail flick latency (p<0.05) after 60 and 90 minutes in both doses.

The generation of pain emerges from the prostaglandin production in human body. So the analgesic activity of the plant might be mediated by the inhibition of the prostaglandin production. Intraperitoneal injection of acetic acid usually produces high level of prostaglandin E₂ and prostaglandin F₂α within 30 minutes of injection along with many sympathetic neuronal mediators. Thus, it can be assumed that the tested materials might either inhibit the cyclooxygenase (COX) enzymes to block the biosynthetic pathway of prostaglandin production or inhibit the binding of prostanoids to their receptors. The extract might also be accountable for inhibiting the production of neuronal mediators. The standard drug diclofenac sodium, an established COX inhibitor, was effective for reducing the pain indicating the correlation of the prostaglandin inhibition mediated analgesic action in the present mice model.[16,17]

In the skin, a delta fibres and C fibres sensory neurons are associated with thermal pain generation. The action potential generated in the nerve directs message to the spinal cord and brain. The tested plant in the tail immersion assay might have the capability to modify the action potential and signal transmission to counter the pain produced by heat.[18]

In the castor oil-induced antidiarrheal experiment, the extract of C. citrinus produced a marked antidiarrheal effect in mice, as shown in table-3. Better antidiarrheal effect was observed at a dose of 400 mg/kg body weight as compared to the control group. This plant is rich in 1,8-cineole[10] and this secondary metabolite has been found to be very effective to manage diarrhea.[19] This might be a possible reason of the leaf extract to exhibit antidiarrheal activity.

CONCLUSION

The methanolic extract of C. citrinus was effective in managing acetic acid and heat induced pain in mice model. It was also found to be effective as antidiarrheal agent. Further comprehensive phytochemical investigation is required to isolate the bioactive molecules from this plant.
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