Antinociceptive activity evaluation of leaves of *Malva verticillata* L.

Farhana Akter and Mohammed Rahmatullah

**Abstract**

In acetic acid-induced writhing tests in mice, methanol extract of leaves of *Malva verticillata* (MEMV) dose-dependently and significantly reduced the number of writhings induced by intraperitoneal administration of acetic acid. At doses of 50, 100, 200 and 400 mg per kg body weight MEMV reduced the number of writhings by 24.1, 27.6, 34.5, and 41.4%, respectively. By comparison, a standard antinociceptive drug aspirin reduced the number of writhings by 24.1 and 51.7% respectively, when administered at doses of 200 and 400 mg per kg body weight. The results suggest that MEMV possess considerable antinociceptive activity and can possibly be used as substitute for aspirin. Preliminary phytochemical analysis showed the presence of alkaloids and flavonoids in MEMV, which classes of compounds may be responsible for the observed antinociceptive effects.

**Keywords:** Antinociceptive, *Malva verticillata*, writhing, mice

**Introduction**

*Malva verticillata* L. (Malvaceae) is an annual plant found in the wild wet areas of Bangladesh and is often consumed by the poorer sections of the population in the cooked form as a vegetable. In Bengali, the plant is known as napa shak; the last word shak indicates leafy vegetable. The plant is considered to have medicinal importance by the folk medicinal practitioners of Bangladesh and elsewhere. Folk medicinal practitioners in Dhaka city use leaves and roots of the plant to strengthen heart, liver and stomach functions and for treatment of helminthiasis and piles [1]. Leaves are inhaled for febrile illnesses in parts of Western Ethiopia [2]. Water extract of the seeds reportedly inhibited osteoclastogenesis and bone resorption and so can be a therapeutic candidate for treatment of pathological bone diseases [3]. Pain is a common phenomenon throughout the world and is experienced by millions of people every day in the acute or the chronic form. Apart from opioid drugs (which can be addictive), most pains are treated with aspirin or paracetamol. These drugs have the problem of giving rise to adverse effects like gastric ulceration and hepatotoxicity, respectively. New antinociceptive drugs are necessary and plants are potential sources of such drugs [4]. For the last few years, we had been conducting screening of antidiabetic and antinociceptive plants of Bangladesh [5-15]. The objective of the present study was to evaluate the antinociceptive potential of methanolic extract of leaves of *Malva verticillata* (MEMV) through acetic acid-induced writhing (abdominal constriction) tests in mice.

**Methods**

**Plant material collection**

Leaves of *Malva verticillata* were collected from a vegetable market in Dhaka city. The leaves were identified at the University of Development Alternative by a competent botanist and voucher specimens were deposited with the Medicinal Plant Collection Wing of the University of Development Alternative.

**Preparation of methanolic extract of Malva verticillata leaves (MEMV)**

For preparation of methanol extract of leaves of *Malva verticillata* (MEMV), leaves were thoroughly cut into small pieces, dried in the shade, and pulverized into a fine powder. 54g of the powder was extracted with 270 ml methanol over 48 hours. Methanol was evaporated at 40°C and the extract was dissolved in Tween 20 prior to administration to mice by gavaging. The final weight of the extract was 2.99g. The extract was maintained in small aliquots at -20°C till use and care was taken not to freeze-thaw the extract vials repeatedly.
Chemicals and Drugs
Aspirin was obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals
Swiss albino mice, which weighed between 12-15g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The animals were acclimatized for three days prior to actual experiments. During this time, the animals were fed with mice chow (supplied by ICDDR, B) and water ad libitum. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Antinociceptive activity evaluation through abdominal writhing test
Antinociceptive activity of MEMV was examined as previously described [16]. Mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MEMV at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MEMV, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 5 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid [9], following which period, the number of abdominal constrictions (Writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

Percent inhibition = \[(1 - \frac{W_t}{W_c}) \times 100\]

Where \(W_t\) and \(W_c\) represents the number of writhings in aspirin or MEMV administered mice (Groups 2-7), and control mice (Group 1), respectively.

Statistical analysis
Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases [9].

Preliminary phytochemical analysis
Preliminary phytochemical analysis for alkaloids, flavonoids and saponins were done as described before [10].

Table 1: Antinociceptive effect of crude methanol extract of *Malva verticillata* leaves (MEMV) in acetic acid-induced pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of abdominal constrictions</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml</td>
<td>5.8 ± 0.37</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200 mg</td>
<td>4.4 ± 0.40</td>
<td>24.1*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400 mg</td>
<td>2.8 ± 0.37</td>
<td>51.7*</td>
</tr>
<tr>
<td>(MEMV)</td>
<td>50 mg</td>
<td>4.4 ± 0.40</td>
<td>24.1*</td>
</tr>
<tr>
<td>(MEMV)</td>
<td>100 mg</td>
<td>4.2 ± 0.37</td>
<td>27.6*</td>
</tr>
<tr>
<td>(MEMV)</td>
<td>200 mg</td>
<td>3.8 ± 0.20</td>
<td>34.5*</td>
</tr>
<tr>
<td>(MEMV)</td>
<td>400 mg</td>
<td>3.4 ± 0.24</td>
<td>41.4*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *P < 0.05, significant compared to control.

Results and Discussion
Preliminary phytochemical analysis of MEMV showed the presence of alkaloids and flavonoids. Acetic acid-induced writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principle like acetic acid [17]. In acetic acid-induced writhing tests in mice, MEMV dose-dependently and significantly reduced the number of writhings induced by intraperitoneal administration of acetic acid. At doses of 50, 100, 200 and 400 mg per kg body weight MEMV reduced the number of Writhings by 24.1, 27.6, 34.5, and 41.4%, respectively. By comparison, a standard antinociceptive drug aspirin reduced the number of writhings by 24.1 and 51.7% respectively, when administered at doses of 200 and 400 mg per kg body weight. The results suggest that MEMV possess considerable antinociceptive activity and can possibly be used as substitute for aspirin.

Prostaglandins are produced from arachidonic acid by cyclooxygenases and play a role in the sensitization of pain [18]. Thus MEMV may be reducing the pain effect (number of writhings) through inhibition of cyclooxygenases and concomitant decrease in synthesis of prostaglandins involved in the sensitization of pain. However, the actual mechanism for the antinociceptive action of MEMV needs to be elucidated through further studies. The same applies to the isolation and identification of the responsible bioactive components in MEMV. Both alkaloids and flavonoids have been implicated in antinociceptive effects [19-21]. Thus these two groups of compounds may play a role in the antinociceptive effects as seen in the present study.

References

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