Antinociceptive Activity Evaluation of Leaves of Centella asiatica and Zizyphus mauritiana


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ABSTRACT

The antinociceptive effects of methanolic extracts of Zizyphus mauritiana and Centella asiatica leaves were studied through the intraperitoneally administered acetic acid-induced pain model in Swiss albino mice. At doses of 50, 100, 200, and 400 mg per kg body weight, MEZM caused dose-dependent and significant reductions in the number of abdominal contractions induced in mice by intraperitoneal administration of acetic acid. At the aforementioned four doses, the number of abdominal contractions (writhings) was reduced, respectively, by 31.2, 46.9, 56.2, and 62.5%. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 34.4 and 46.9%, respectively. Thus MEZM produced antinociceptive effects and caused alleviation of pain, which was greater than that of the pain-alleviating drug, aspirin, even when the latter was used at a high dose of 400 mg per kg body weight. Methanolic extract of Centella asiatica leaves (MECA), at doses of 50, 100, 200, and 400 mg per kg body weight, caused reductions in the number of writhings, respectively, by 46.9, 50.0, 53.1, and 71.9%. Dose for dose, MECA was more effective than MEZM in alleviating pain, and both extracts demonstrated stronger potency than aspirin. Since pain is a universal problem afflicting human beings of all age groups throughout the world, the extracts even in the crude form can serve as a readily available and cheaper alternative in alleviating pain. The extracts deserve further studies for isolation and identification of the responsible bioactive components responsible for the observed antinociceptive effects.

INTRODUCTION

Zizyphus mauritiana Lam., also known as Ber, Chinese Apple, Jujube, or Indian plum (local name: Boroi) is a tropical fruit tree species belonging to the family Rhamnaceae. The tree is widely distributed in Bangladesh, and the seeds obtained from fruits are used by folk medicinal practitioners for treatment of diabetes. The plant or plant parts reportedly has a number of pharmacological activities. Leaf extract has been shown to demonstrate hepatoprotective effects against chronic ethanol-induced hepatotoxicity in rat liver [9]. Aqueous ethanol extract of seed reportedly showed hypoglycemic activity in alloxan-induced diabetic mice [8]. Anticancer potential of aqueous ethanol seed extract has been shown against cancer cell lines and Ehrlich Ascites Carcinoma [23]. Antiplasmodial and antymycobacterial cyclopeptide alkaloids have been reported from roots of the plant [24]. Hypnotic effect of seed extract in mice has also been described [49].

Centella asiatica (L.) Urb. (local name: Thankuni) is a small, herbaceous, annual plant of the family Mackinlayaceae or subfamily Mackinlayoideae of family Apiaceae, and is native to Bangladesh, India, Sri Lanka, northern Australia, Indonesia, Iran, Malaysia, Melanesia, Philippines, Papua New Guinea, and other parts of Asia. Centella asiatica is an important medicinal herb that is widely used in south Asia. Triterpenoids and saponins, the primary constituents of Centella asiatica are mainly believed to be responsible for its wide therapeutic actions. Apart from wound healing, the herb is recommended for the treatment of various skin conditions such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhoea, fever, amenorrhea, and diseases of the female genitourinary tract, and also for relieving anxiety and improving cognition [14].

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Protective action of the plant against adriamycin-induced cardiomyopathy in rats has been reported [13]. The beneficial effect of aqueous extract of the plant against arsenic-induced oxidative stress has been reported [12]. Treatment with fresh leaf extract reportedly enhanced learning ability and memory retention power in rats [46]. Neuroprotective property of the plant has been shown against 3-nitropropionic acid-induced oxidative stress and mitochondrial dysfunctions in brain regions of prepubertal mice [52]. Neuroprotective effect of the plant has also been seen against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress [22], and experimentally induced Parkinsonism in aged Sprague-Dawley rats [15]. Extract of the plant has been shown to selectively decrease amyloid beta levels in hippocampus of Alzheimer’s disease animal model [11]. The anticonvulsant effect of the plant has been shown in pentylenetetrazol-induced seizures with respect to cholinergic neurotransmission [57].

Antigenotoxic effect of the plant extract has been seen against cyproterone acetate-induced genotoxic damage in cultured human lymphocytes [54]. The plant extract has been observed to induce apoptosis in human breast cancer cells [6].

The efficacy of the plant extract has been shown for wound healing in diabetic wound patients [25]. The plant has been seen to exert a protective role on lead-induced oxidative stress and suppressed reproductive health in male rats [48]. The plant has been shown to protect against UVB-induced HaCaT keratinocyte damage [3].

Studies conducted by our research group have centered on ethnomedicinal surveys [28-37,39-44], followed by screening of the plants obtained for antihyperglycemic, antinociceptive and cytotoxic activities [4,19,26,38,53,2,7,17,20,27,56,1,5,16,50]. As part of the screening process to locate plants with antinociceptive properties, this study was conducted to evaluate the antinociceptive potential of methanol extract of leaves of Centella asiatica, and Zizyphus mauritiana in intraperitoneally administered gastric pain model mice.

MATERIALS AND METHODS

Leaves of Ziziphus mauritiana were collected from Mirpur in Dhaka district, Bangladesh during June 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession Number 38,369). The sliced and air-dried leaves of Ziziphus mauritiana were ground into a fine powder and 100g of the powder was extracted with methanol (1:8.5, w/v) for 48 hours. The extract was evaporated to dryness at 40°C. The final weight of the extract was 4g.

Leaves of Centella asiatica were collected from Dhaka district during May 2013. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Accession Number 38,359). The sliced and air-dried leaves of Centella asiatica were ground into a fine powder and 57g of the powder was extracted with 300 ml methanol for 48 hours. The extract was evaporated to dryness at 40°C. The final weight of the extract was 4.35g.

Chemicals:

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh.

Animals:

In the present study, Swiss albino mice (male), which weighed between 12-15g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Antinociceptive activity:

Antinociceptive activity of the methanol extracts of Ziziphus mauritiana leaves (MEZM) and methanol extracts of Centella asiatica leaves (MECA) was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of six mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspargin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic whole plant extract of MEZM or MECA 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 extract, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of abdominal constrictions (writhings) was counted for 10 min.
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.

RESULTS AND DISCUSSION

The antinociceptive effects of methanolic extracts of Zizyphus mauritiana and Centella asiatica leaves were studied through the intraperitoneally administered acetic acid-induced pain model in Swiss albino mice. At doses of 50, 100, 200, and 400 mg methanolic extract of Zizyphus mauritiana leaves (MEZM) per kg body weight, MEZM caused dose-dependent and significant reductions in the number of abdominal constrictions induced in mice by intraperitoneal administration of acetic acid. At the afore-mentioned four doses, the number of abdominal constrictions (writhings) was reduced, respectively, by 31.2, 46.9, 56.2, and 62.5%. A standard antinociceptive drug, aspirin, when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 34.4 and 46.9%, respectively. Thus MEZM produced antinociceptive effects and caused alleviation of pain at the two higher doses of 200 and 400 mg per kg body weight, which was greater than that of the pain-alleviating drug, aspirin, even when the latter was used at a high dose of 400 mg per kg body weight. The results suggest that MEZM contains phytochemical component(s) with a high degree of pain relieving activity.

Table 1: Antinociceptive effect of crude methanol extract of Zizyphus mauritiana leaves in the acetic acid-induced gastric pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of writhings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>10 ml</td>
<td>6.40 ± 0.40</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>200 mg</td>
<td>4.20 ± 0.58</td>
<td>34.4*</td>
</tr>
<tr>
<td>Aspirin (Group 3)</td>
<td>400 mg</td>
<td>3.40 ± 0.24</td>
<td>46.9*</td>
</tr>
<tr>
<td>MEZM (Group 4)</td>
<td>50 mg</td>
<td>4.40 ± 0.51</td>
<td>31.2*</td>
</tr>
<tr>
<td>MEZM (Group 5)</td>
<td>100 mg</td>
<td>3.40 ± 0.87</td>
<td>46.9*</td>
</tr>
<tr>
<td>MEZM (Group 6)</td>
<td>200 mg</td>
<td>2.80 ± 0.58</td>
<td>56.2*</td>
</tr>
<tr>
<td>MEZM (Group 7)</td>
<td>400 mg</td>
<td>2.40 ± 0.51</td>
<td>62.5*</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.

Table 2: Antinociceptive effect of crude methanol extract of Centella asiatica leaves in the acetic acid-induced gastric pain model mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Mean number of writhings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>200 mg</td>
<td>4.20 ± 0.58</td>
<td>34.4*</td>
</tr>
<tr>
<td>Aspirin (Group 3)</td>
<td>400 mg</td>
<td>3.40 ± 0.24</td>
<td>46.9*</td>
</tr>
<tr>
<td>MECA (Group 4)</td>
<td>50 mg</td>
<td>3.40 ± 0.81</td>
<td>46.9*</td>
</tr>
<tr>
<td>MECA (Group 5)</td>
<td>100 mg</td>
<td>3.20 ± 0.37</td>
<td>50.0*</td>
</tr>
<tr>
<td>MECA (Group 6)</td>
<td>200 mg</td>
<td>3.00 ± 0.32</td>
<td>53.1*</td>
</tr>
<tr>
<td>MECA (Group 7)</td>
<td>400 mg</td>
<td>1.80 ± 0.58</td>
<td>71.9*</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.

Methanolic extract of Centella asiatica leaves (MECA), at doses of 50, 100, 200, and 400 mg per kg body weight, caused reductions in the number of writhings, respectively, by 46.9, 50.0, 53.1, and 71.9%. Dose for dose, MECA was more effective than MEZM in alleviating pain, and MECA demonstrated at the three higher doses of 100, 200 and 400 mg per kg body weight, stronger potency than aspirin. It is to be noted that asiatic acid is present in Centella asiatica leaves, and the compound reportedly showed antinociceptive and anti-inflammatory activities in mice [18]. Asiaticoside, another component present in Centella asiatica, showed antipyretic and anti-inflammatory effects in lipopolysaccharide-trested rat [58]. Thus these two compounds, asiatic acid and asiaticoside, can account for the observed antinociceptive effects of Centella asiatica leaves as observed in the present study.

Sensation of pain has been attributed to any increase in the expression of prostaglandins [mainly prostacyclines (PGI2) and prostaglandin- (PG-E)], and so causing sensation of pain [47,45]. Intraperitoneal administration of acetic acid releases prostaglandins like PGE2 and PGF2alpha and their levels are increased [10]. As such, the antinociceptive activity exhibited by crude methanolic extract of the leaves of Zizyphus mauritiana and Centella asiatica may be due to the extract’s ability to block any further expression of prostaglandins, which may be mediated through inhibition of cyclooxygenase and/or lipoxygenase activities. It is noteworthy that a similar mechanism has been proposed for antinociceptive activity of Ficus deltoidea Jack (Moraceae) aqeous extract in acetic acid-induced gastric pain model [55], and this can also be the mechanism operating in the present study.
REFERENCES


