ORIGINAL ARTICLE

Antinociceptive activity studies with methanol extract of *Annona reticulata* L. (Annonaceae) and *Carissa carandas* L. (Apocynaceae) leaves in Swiss albino mice


Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka-1205, Bangladesh.

ABSTRACT

The antinociceptive effect of methanolic extract of *Annona reticulata* and *Carissa carandas* leaves were studied in acetic acid-induced gastric pain model in Swiss albino mice. Extract of leaves of both plants exhibited dose-dependent and significant antinociceptive activity and decreased the number of writhings induced by intraperitoneal administration of acetic acid in mice. At doses of 50, 100, 200 and 400 mg extract per kg body weight, methanolic extract of leaves of *Carissa carandas* reduced the number of writhings by 40.9, 42.8, 59.2 and 61.2%, respectively. These results compare favorably with that of the standard antinociceptive drug, aspirin, which when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 51.0 and 73.4%, respectively. Methanolic extract of leaves of *Annona reticulata*, when administered at doses of 50, 100, 200 and 400 mg extract per kg body weight reduced the number of writhings, respectively, by 47.0, 55.1, 67.3, and 69.4%. The results suggest that leaves of both plants contain constituent(s) with strong antinociceptive activity and as such leaves of both plants merit further scientific studies for isolation of antinociceptive components leading to discovery of possibly novel and more efficacious pain-killing drugs. Pain is a common affliction of human beings and can arise from many causes. Standard pain-killing drugs like aspirin and paracetamol suffer from the problem of inducing gastric ulceration or hepatotoxicity from overdose or prolonged use. As such, newer and more efficacious painkillers can prove to be highly beneficial to human beings.

Key words: *Annona reticulata*, *Carissa carandas*, antinociceptive, Annonaceae, Apocynaceae

Introduction

*Annona reticulata* L. (Annonaceae; local name: ata) is a small deciduous tree, which is cultivated in many parts of the world including Southeast Asia, Taiwan, India, Bangladesh, Australia and West Africa. *Carissa carandas* L. (Apocynaceae; local name: koromcha) is a species of flowering shrub in the dogbane family producing berry-sized fruits used commonly in the Indian subcontinent (including Bangladesh) as a condiment or additive to spices. Both plants are cultivated in Bangladesh for their edible fruits. Additionally, various parts of these two plants like fruits, leaves, roots and barks are used in the folk medicinal system of Bangladesh for treatment of diverse ailments like pain, fever, epilepsy, gastrointestinal disorders, malaria, and diabetes.

Leaves of *Annona* species have been reported to possess antioxidant properties (Baskar et al., 2007). Various volatile compounds like -pinene, -pinene, myrcene, limonene, terpinen-4-ol, and germacrene D has been reported to be present in fruits of *Annona reticulata* (Pino et al., 2003). Annonacin, a mono-tetrahydrofuran acetylogenin, isolated from *Annona* species reportedly arrested cancer cells at the G1 phase and caused cytotoxicity (Yuan et al., 2003). Selective toxicity of squamocin, isolated from seeds of *Annona reticulata* has been reported in T24 bladder cancer cells at the S-phase mediated via a Bax-, Bad-, and caspase-3 related pathways (Yuan et al., 2006). *In vitro* anti-proliferative activity of ethanol extract of roots has been seen in A-549 (human ling carcinoma), K-562 (human chronic myelogenous leukemia bone marrow), HeLa (human cervix), and MDA-MB (human adenocarcinoma mammary gland) cancer cell lines (Suresh et al., 2011). Iso-acetogenins and acetogenins have been reported from seeds of the plant (Yu et al., 1997; Chang et al., 1998). Seeds of the plant also reportedly contain squamocin and rolliniastatin 1 (Tam et al., 1993). Cytotoxic
Acetogenins isolated from leaves of the plant include squamone, solamin, annomonicin, and rolliniastatin 2 (Chang et al., 1993). Analgesic and anti-inflammatory activity of kaur-16-en-19-oic acid, isolated from bark has been reported (Chavan et al., 2012a). A sesquiterpene fraction from the bark containing copaene, patchouline and 1H-cycloprop(e)azulene also reportedly exhibited central as well as peripheral analgesic and anti-inflammatory activities (Chavan et al., 2012b).

Histamine releasing activity has been reported for Carissa carandas roots (Joglekar and Gaitonde, 1970). Triterpenoidal constituents have been isolated from leaves (Siddiqui et al., 2003). Extract of unripe fruits of the plant has been shown to possess antidiabetic activity in alloxan-induced diabetic rats (Itankar et al., 2011). Anthelmintic activity of root bark has been shown (John et al., 2007). Hepatoprotective effect of root extract against carbon tetrachloride and paracetamol-induced hepatic oxidative stress has been reported (Hegde and Joshi, 2009). Acute hypotensive activity of extract of the plant has also been reported (Shamim and Ahmad, 2012).

The best way for obtaining knowledge, particularly traditional medicinal knowledge on medicinal plants is to conduct surveys among the traditional medicinal practitioners belonging to the mainstream or tribal populations. We had been conducting such surveys for a number of years to document the use of various plants of Bangladesh for treatment of various ailments (Rahmatullah et al., 2009a-c; Rahmatullah et al., 2010a-g; Rahmatullah et al., 2011a,b; Rahmatullah et al., 2012a-d). Furthermore, on the basis of our ethnomedicinal surveys, we had been screening medicinal plants for their anti-hyperglycemic and antinociceptive effects (Anwar et al., 2010; Jahan et al., 2010; Khan et al., 2010; Mannan et al., 2010; Rahman et al., 2010; Shoha et al., 2010; Ali et al., 2011; Barman et al., 2011; Hossan et al., 2011; Jahan et al., 2011; Rahman et al., 2011; Sutradhar et al., 2011), because diabetes and pain are two of the most common diseases affecting millions of people throughout the world. As part of this ongoing screening of medicinal plants of Bangladesh for possible antinociceptive effects, the objective of the present study was to evaluate the methanolic extract of leaves of Annona reticulata and Carissa carandas for gastric pain (as manifested by writhings) inhibitory activity in Swiss albino mice following intraperitoneal administration of acetic acid.

Materials and Methods

The leaves of Annona reticulata were collected from Dhaka district, Bangladesh during January, 2011. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 35,403). The air-dried leaves of Annona reticulata were ground into a fine powder, and 100g of the powder was extracted with methanol (1:5, w/v) for 48 hrs. The extract was evaporated to dryness. The final weight of the extract was 8.36g.

The leaves of Carissa carandas were also collected from Dhaka district, Bangladesh in January 2011. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 35,404). Air-dried leaves were ground into a fine powder, and 100g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract was 9.18g.

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

In the present study, Swiss albino male mice, which weighed between 20-25g were used. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Prior to experiments, all the animals were acclimatized for one week. During this one week period, mice were fed with standard mice feed obtained from ICDDR,B and water, given ad libitum. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternatives, Dhaka, Bangladesh.

Antinociceptive activity of the methanol extracts were examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into eleven 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 aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic leaf extract of Carissa carandas at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Groups 8-11 were administered methanolic extract of Annona reticulata leaves 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 intraperitoneal 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 bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

Student’s t-test was used to analyze any significant differences between control and experimental groups. P < 0.05, was considered significant as compared to control.
Results and Discussion

Extract of leaves of both plants exhibited dose-dependent and significant antinociceptive activity and decreased the number of writhings induced by intraperitoneal administration of acetic acid in mice. At doses of 50, 100, 200 and 400 mg extract per kg body weight, methanolic extract of leaves of *Carissa carandas* reduced the number of writhings by 40.9, 42.8, 59.2 and 61.2%, respectively. These results compare favorably with that of the standard antinociceptive drug, aspirin, which when administered at doses of 200 and 400 mg per kg body weight, reduced the number of writhings by 51.0 and 73.4%, respectively. Methanolic extract of leaves of *Annona reticulata*, when administered at doses of 50, 100, 200 and 400 mg extract per kg body weight reduced the number of writhings, respectively, by 47.0, 55.1, 67.3, and 69.4%. The results as shown in Table 1 suggest that leaves of both plants contain constituent(s) with strong antinociceptive activity and as such leaves of both plants merit further scientific studies for isolation of antinociceptive components leading to discovery of possibly novel and more efficacious pain-killing drugs.

It has been reported that both central and peripheral analgesia can be suitably detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005). Peripheral analgesia involves the peripheral nociceptive afferent neuron, while central analgesia involves an afferent input thus generating a sensation of pain. Under normal circumstances, pain is associated with electrical activity in small diameter primary afferent fibers of peripheral nerves. These fibers can be non-myelinated C-fibers or fine myelinated A fibers. The latter causes a sensation of sharp and well-localized pain and the former causes a dull burning pain (Rang *et al.*, 2003). Intraperitoneal administration of acetic acid (1%) leads to pain and inflammation mediated through production of prostaglandins [mainly prostacyclines (PGI<sub>2</sub>) and prostaglandin-E (PG-E)], which are reported to be responsible for excitation of the Aδ-nerve fibers, leading to sensation of pain (Reynolds 1982). Therefore any agent (like the extracts of leaves of *Annona reticulata* and *Carissa carandas*) that lowers the number of abdominal constrictions will demonstrate analgesia possibly by inhibition of prostaglandin synthesis.

Analgesic activity of bark of *Annona reticulata* has been previously mentioned (Chavan *et al.*, 2012 a,b) in the introductory section. The leaves of a related species, *Annona squamosa* along with the leaves of *Azadirachta indica* in a polyherbal formulation reportedly showed analgesic and anti-inflammatory activities (Sharma *et al.*, 2010). Analgesic and anti-inflammatory activity has also been reported for *Carissa carandas* fruits (Sharma *et al.*, 2007). The potential for obtaining phytochemical constituent(s) with pain-relieving activity is thus very strong from leaves of *Annona reticulata* and *Carissa carandas* considering that not only other parts of these plants has been shown to possess analgesic activity, but also the present results demonstrate strong antinociceptive activity in the leaves of both plant species.

<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>8.17 ± 0.79</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin (Group 2)</td>
<td>200 mg</td>
<td>4.00 ± 0.58</td>
<td>51.0*</td>
</tr>
<tr>
<td>Aspirin (Group 3)</td>
<td>400 mg</td>
<td>2.17 ± 0.98</td>
<td>73.4*</td>
</tr>
<tr>
<td>Carissa carandas (Group 4)</td>
<td>50 mg</td>
<td>4.83 ± 0.98</td>
<td>40.9*</td>
</tr>
<tr>
<td>Carissa carandas (Group 5)</td>
<td>100 mg</td>
<td>4.67 ± 1.02</td>
<td>42.8*</td>
</tr>
<tr>
<td>Carissa carandas (Group 6)</td>
<td>200 mg</td>
<td>3.33 ± 0.84</td>
<td>59.2*</td>
</tr>
<tr>
<td>Carissa carandas (Group 7)</td>
<td>400 mg</td>
<td>3.17 ± 0.31</td>
<td>61.2*</td>
</tr>
<tr>
<td>Annona reticulata (Group 8)</td>
<td>50 mg</td>
<td>4.33 ± 0.49</td>
<td>47.0*</td>
</tr>
<tr>
<td>Annona reticulata (Group 9)</td>
<td>100 mg</td>
<td>3.67 ± 0.56</td>
<td>55.1*</td>
</tr>
<tr>
<td>Annona reticulata (Group 10)</td>
<td>200 mg</td>
<td>2.67 ± 0.49</td>
<td>67.3*</td>
</tr>
<tr>
<td>Annona reticulata (Group 11)</td>
<td>400 mg</td>
<td>2.50 ± 1.02</td>
<td>69.4*</td>
</tr>
</tbody>
</table>

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to control.

References


