ANTI-INFLAMMATORY EVALUATION OF ZANTHOXYLUM NITIDUM IN A MURINE MODEL OF ACUTE INFLAMMATION

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Summary

Zanthoxylum nitidum (Roxb.) DC (Rutaceae), is a morphologically variable plant occurring in South-East Asian countries and Australia. In North-East India the plant has traditionally been used for various medicinal purposes. Present study was undertaken to evaluate the acute anti-inflammatory activity of the aqueous and ethanol extracts of Z. nitidum stem bark and root by carrageenan induced hind paw oedema in Swiss albino mice. Present anti-inflammatory screening demonstrated very significant acute anti-inflammatory effects of aqueous extracts from both stem bark and root, in reduction of carrageenan induced mouse paw oedema, while the ethanol extracts showed only insignificant effects. The aqueous extract of root was found to be the most potent. Present investigation therefore demonstrates that the stem bark and root of Z. nitidum exhibited potential anti-inflammatory effects in Swiss mice thereby validating its traditional uses in North-East India.

Key words: Zanthoxylum nitidum, anti-inflammatory, carrageenan, mice.

Introduction

Zanthoxylum nitidum (Roxb.) DC (Rutaceae), commonly called Tez-mui or Tejamool in Assamese, is a morphologically variable plant species occurring in South-East Asian countries and in Northern Australia (1). In India it grows as a large prickly shrub mainly in North-East India (Sikkim, Assam and Nagaland states). In India the plant has traditionally been used for various medicinal purposes. The root is used in toothache, stomachache, fever, rheumatism, paresis, boils and as an insecticide and piscicide. The fruit is used in the treatment of stomachache, cough, colic, vomiting, diarrhoea, and paresis and as an aromatic, stimulant and piscicide.
The small branches, seeds and stem bark are used in fever, diarrhoea and cholera (2-4). It has come to the author’s notice that the rural people of upper Assam, India use the young stems of this plant as chewing stick in treatment of toothache and gingivitis. Previously the author have reported essential oil composition of fruits and leaves, antibacterial effects of stem bark and root, anti-nociceptive activity of stem bark of *Z. nitidum* from India (5-7). There are no reports of anti-inflammatory investigations carried out on *Z. nitidum* of Indian habitat. The present work therefore, attempts to evaluate the acute anti-inflammatory activity of the aqueous and ethanol extracts from the stem bark and root of *Z. nitidum* in Swiss albino mice.

**Materials and methods**

**Plant Material:** The mature entire plants of *Z. nitidum* were collected during the month of November 2006 from the outskirts of Dibrugarh University campus, in Dibrugarh district of Assam, India. The species was identified by Dr. S. J. Phukan, taxonomist, from Botanical Survey of India, Eastern Circle, Shillong, India, and a voucher specimen (No. DUPS-06-003) was deposited in Department of Pharmaceutical Sciences, Dibrugarh University, for future reference. Immediately after collection, the underground root parts were separated from the shoots. All the prickles were removed from the stems and branches carefully by using a sharp knife, without harming the bark. Then the barks were peeled off from the shoots. The plant materials were shade dried at temperature 21-24°C and ground mechanically into coarse powders.

**Drugs and chemicals:** The following drugs and chemicals were obtained from the sources specified: Rectified Sprit (BP): Bengal Chemicals and Pharmaceuticals Ltd., Kolkata; Tween 80: Ranbaxy Fine Chemicals Ltd., New Delhi; Ibuprofen: Perk Indus Pharmaceuticals Pvt. Ltd., Faridabad, India; λ-Carrageenan (Type IV): S. D. Fine Chemicals Ltd., Bombay. Double-distilled water from all glass still was employed throughout the study.

**Preparation of extracts:** The air-dried stem bark and root of *Z. nitidum* were extracted with water (double-distilled water) and ethanol (90 % v/v) by maceration. Powdered plant materials (175 g) were macerated with 400 ml of solvent at 21-24°C temperature for 2 days with frequent shaking. After 2 days, the extracts were filtered by using Whatman filter paper (No. 1) and to the marc part 300 ml of the solvent was added and allowed to stand for next two days at same temperature for second time maceration (re-maceration) and after two days, again filtered similarly. The combined filtrates (macerates) were evaporated to dryness *in vacuo* (at 35°C and 0.8 Mpa) in a Buchi evaporator, R-114 and stored in a vacuum desiccator for future use. The extracts were denoted as RE (Root Ethanol), RA (Root Aqueous), BE (Bark Ethanol) and BA (Bark Aqueous). The phytochemical studies on these extracts were reported elsewhere (8, 9).

**Animals:** Adult male Swiss albino mice weighing 20 to 22 g were maintained under standard laboratory conditions (temperature 25 ± 2°C with dark and light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were fasted for 10 h with water *ad libitum* before commencement of treatments.
Carrageenan induced paw oedema in mice (10, 11): The mice were divided into ten groups \((n = 6)\). The first group of animals (which served as control) received normal saline (10 ml/kg body weight) intraperitonially. The second group of animals (which served as reference) received ibuprofen at a dose of 50 mg/kg body weight intraperitonially. The remaining eight groups received four test extracts (RE, RA, BE, BA) at the doses of 75 mg and 150 mg/kg body weight intraperitonially. 30 minutes after administration of normal saline, ibuprofen and test extracts to the animal groups as mentioned above; 0.1 ml of freshly prepared 1 % w/v suspension of carrageenan (in double-distilled water) was injected subcutaneously to the plantar surface of the left hind paw, to all groups of mice. Mice paw diameters were measured by using a slide calipers before and after 4 h of carrageenan challenge. Increase in paw thickness as a measure of inflammatory oedema was calculated using the formula \(P_t - P_o\), where \(P_o\) is the initial paw thickness and \(P_t\) is the thickness at time t (4 h). Percentage inhibition of inflammation was calculated by the following formula,

\[
(1 - \frac{P_t}{P_c}) \times 100\%.
\]

Where \(P_t\) is the increase in paw thickness of the treated and \(P_c\) is that of control.

Statistical analysis: The results were expressed as mean ± standard error of mean (SEM). The results were analyzed for statistical significance by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s post hoc test for significance.

Results

The acute anti-inflammatory activity of \(Z. \) nitidum extracts was evaluated by carrageenan induced mouse paw oedema. The results are summarized in Table 1. Among the root extracts the ethanol extract of root exhibited feeble dose dependent inhibition in both doses (75 and 150 mg/kg) but was found statistically insignificant. The aqueous extract of root on the other hand at both test doses showed pronounced, very significant \((p < 0.05 \text{ and } p < 0.001 \text{ respectively})\) and dose dependent inhibition of paw oedema formation.

Among the stem bark extracts the ethanol extract showed very weak (insignificant) but surprisingly dose dependent inhibition of oedema at both test doses (75 and 150 mg/kg). The aqueous extract at a dose of 75 mg/kg showed some inhibitory activity but was insignificant. Its higher dose (150 mg/kg) exhibited marked and significant \((p < 0.001)\) inhibition of mouse paw oedema.

The reference nonsteroidal anti-inflammatory drug ibuprofen demonstrated significant \((p < 0.01)\) suppression of mouse paw oedema. However, it showed lesser protective effect than the aqueous extracts of stem bark and root both at higher doses.
Table 1. Influence of *Z. nitidum* extracts on carrageenan induced paw oedema in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Initial paw thickness ± SEM (cm)</th>
<th>Paw thickness after 4 h ± SEM (cm)</th>
<th>Increase in thickness ± SEM (cm)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.19 ± 0.023</td>
<td>0.34 ± 0.024</td>
<td>0.15 ± 0.021</td>
<td>-</td>
</tr>
<tr>
<td>RE</td>
<td>75</td>
<td>0.18 ± 0.044</td>
<td>0.31 ± 0.018</td>
<td>0.13 ± 0.035</td>
<td>13.34</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.20 ± 0.012</td>
<td>0.31 ± 0.011</td>
<td>0.11 ± 0.029</td>
<td>26.66</td>
</tr>
<tr>
<td>RA</td>
<td>75</td>
<td>0.18 ± 0.032</td>
<td>0.27 ± 0.009*</td>
<td>0.09 ± 0.017*</td>
<td>40.00</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.19 ± 0.039</td>
<td>0.24 ± 0.008***</td>
<td>0.05 ± 0.027***</td>
<td>66.67</td>
</tr>
<tr>
<td>BE</td>
<td>75</td>
<td>0.21 ± 0.018</td>
<td>0.35 ± 0.016</td>
<td>0.14 ± 0.031</td>
<td>6.67</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.18 ± 0.033</td>
<td>0.31 ± 0.019</td>
<td>0.13 ± 0.037</td>
<td>13.34</td>
</tr>
<tr>
<td>BA</td>
<td>75</td>
<td>0.20 ± 0.020</td>
<td>0.32 ± 0.028</td>
<td>0.12 ± 0.023</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.19 ± 0.014</td>
<td>0.25 ± 0.015***</td>
<td>0.06 ± 0.042***</td>
<td>60.00</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>50</td>
<td>0.18 ± 0.032</td>
<td>0.25 ± 0.007**</td>
<td>0.07 ± 0.030**</td>
<td>53.34</td>
</tr>
</tbody>
</table>

Numbers of animals per group (n) = 6. SEM: Standard Error of Mean.
*\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \) compared to control.

Discussion

Carrageenan induced hind paw oedema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory agents as it is not known to be antigenic and it is devoid of apparent systemic side effects (12). Carrageenan induced rat paw oedema is a widely used test to determine anti-inflammatory activity and it has been fully characterized. Carrageenan induced mouse paw oedema, although less widely used, has been increasingly used to test new anti-inflammatory drugs as well as plant extracts (11).

Levy (1969) first described that injection of carrageenan (1 %) in the mouse hind paw causes an oedema similar, as time course, to the rat, but less powerful in proportion (13). Henriques *et al* (1987) showed that carrageenan injection into mouse paw induces a biphasic oedema. The first phase is characterized by an oedema of little intensity and unrelated to the dose of carrageenan used, while the second phase develops after 24 hrs displaying a more pronounced oedema with a maximum effect between 48-72 h (14). Consistent with these observations, Posadas *et al* (2004) reported that injection of 1 % carrageenan in mouse hind paw causes a biphasic response: an early inflammatory response (acute phase) that lasts 6 h and a second late response (chronic phase) that peaks at 72 h and declining at 96 h (11). These findings confirm that carrageenan induced mouse hind paw oedema is biphasic, involving both acute and chronic phases, as opposite to the rat hind paw oedema that shows an acute phase only.
In present study, acute anti-inflammatory effects of *Z. nitidum* extracts were evaluated by using aforesaid method. Carrageenan induced acute mouse hind paw oedema involves several mediators i.e. histamine, serotonin, bradykinin, prostaglandins (PGs) and nitric oxide, responsible for increasing vascular permeability and cellular infiltration leading to formation of oedema (11). Several nonsteroidal anti-inflammatory drugs have been found to be active in the murine model at the same extent as the rat model (15).

In present investigation, the mouse paw thickness was measured by using sliding calipers instead of conventional plethysmometer or volumetric mercury displacement method. In fact, common mercury plethysmometers are not suitable for measurement of such small volume differences (11). Hence, Posadas *et al* (2004) recommended using specially designed hydroplethysmometer for small volumes, for measurement of mouse paw volumes (11). Alternatively, the slide calipers method can be applied for this purpose. Sharma *et al* (2004) reported that the slide calipers method is more sensitive to detect lowest inflammatory pedal response when compared with plethysmometric method (16).

The overall results indicate differential acute anti-inflammatory effects of *Z. nitidum* extracts in Swiss mice. The aqueous extract of root was found to be the most potent. This can thus offer a scientific basis for traditional use of *Z. nitidum* root in rheumatism. The aqueous extract of stem bark also showed significant activity. Ethanol extracts of both stem bark and root however showed weaker actions. Therefore, the activities were chiefly found in the aqueous extracts. The outcome of present study can substantiate the traditional and folkloric uses of *Z. nitidum* in North-East India. Purification of the extracts and further studies can reveal the exact mechanisms and constituents behind the anti-inflammatory activity of *Z. nitidum*.

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**References**


