Ameliorative Effect of Zanthoxylum nitidum Root in Chemical and Stress Induced Gastric Mucosal Lesions in Rats

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INTRODUCTION

Peptic ulcer is a disease of the part of gastrointestinal tract which is exposed to gastric acid and pepsin. It results due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins etc) factors. Recently the involvement of Helicobacter pylori infection in peptic ulcer formation and recurrence has been reported (….Ref ??). There are several drugs like H$_2$ receptor antagonists, proton pump inhibitors and cytoprotectants are available for management for peptic ulcer conditions but all these drugs have adverse effects and limitations [1]. Current treatment of ulcers in developing countries has been largely suppression of pain, with little or no strategy aimed at a cure. Herbal medicine is emerging as an alternative treatment to available synthetic drugs for treatment of peptic ulcer possibly due to their perceived effectiveness, fewer possibilities of serious adverse effects and easy availability at lower costs.

Key words: Zanthoxylum nitidum • Gastric mucosal damage • ASA • Ethanol • Stress • Root

Zanthoxylum nitidum (Roxb.) DC (Rutaceae), called Tez-mui or Tejamool in Assamese is a morphologically variable plant species occurring in South-East Asian countries and in Australia [2]. In India it grows as a large prickly shrub particularly in the North-Eastern India (Sikkim, Assam and Nagaland states). In India, the plant is used traditionally 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, diarrhea and paresis; and as an aromatic, stimulant and piscicide. The small branches, seeds and stem bark are prescribed in fever, diarrhea and cholera [3-5]. It has come to the author’s notice that the rural people of upper Assam of North- East India use the young stems of this plant as chewing stick in treatment of toothache and gingivitis. Previous researchers have reported antispasmodic, anti-tumor, antifungal, antioxidant, analgesic and anti-inflammatory activities of the root of non-Indian Z. nitidum mainly from China, Japan and Taiwan [6-10].
Previously the present authors have reported essential oil composition of fruits and leaves, antibacterial and pharmacognostic studies of stem bark and root, anti-nociceptive and anti-ulcer activity of stem bark, anti-inflammatory and antioxidant activities of stem bark and root of Z. nitidum from India [11-19]. There are no reports of anti-ulcer investigations carried out on Z. nitidum root. The present investigation therefore, attempts to report the preliminary results of gastric ulcer protective effects of Indian Z. nitidum root on experimentally induced gastric lesions in Wistar albino rats.

MATERIALS AND METHODS

Plant Material: The fully matured entire plants of Z. nitidum were collected during the month of November 2007 from the outskirts of Dibrugarh University campus, in Dibrugarh district of Assam state, India. The species was identified by Dr. S. J. Phukan, taxonomist, from the Botanical Survey of India, Eastern Circle, Shillong, India and a voucher specimen (No. BSI/EC/Tech./2007/143) was deposited in Department of Pharmaceutical Sciences, Dibrugarh University for future reference. Immediately after collection, the roots were separated from the aerial parts washed thoroughly with running tap water and cut into small pieces. Then the plant material was shade dried at temperature 21-24°C and ground mechanically into a coarse powder and stored in an airtight container.

Preparation of Extract: Powdered plant material (150 g) was macerated with 400 ml of distilled water at 21-24°C temperature for 3 days with frequent shaking. After 3 days, the extracts were filtered and to the marc part 300 ml of the solvent was added and allowed to stand for next 2 days at same temperature for second time maceration (re-maceration) and after two days, again filtered similarly. The combined filtrates (macerates) were evaporated in vacuo at 40°C and the dry extract obtained (ZNA, yield: 13.48% w/w) was stored in a vacuum desiccator for future use. Preliminary phytochemical studies were performed on ZNA as per reported method [20].

Drugs and Chemicals: Acetyl salicylic acid (ASA) and ranitidine hydrochloride were from Mepro Pharmaceuticals Pvt. Ltd., Surendranagar, Gujarat, India. All the regents and chemicals used were of analytical grade obtained commercially. Doubled distilled water from all glass still was employed throughout the present study.

Experimental Animals: Adult male Wister albino rats weighing 180-200 g were obtained from the animal house of Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, India. The animals were grouped in polyacrylic cages (38 cm × 23 cm × 10 cm) with no more than three animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark and light circle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Gwahati, India) and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. All animal experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee, Dibrugarh University (No. DUPS/IAEC/SB-07002).

Evaluation of Gastric Mucosal Protection

Acetyl Salicylic Acid (ASA) Induced Gastric Ulcer: The rats were weighed and divided into four groups each consisting six rats (n = 6). All rats were fasted for 36 h with water ad libitum. The first group of animals which served as control received distilled water 5 ml/kg body weight p.o. The second group of animals, which served as reference, received ranitidine hydrochloride at the dose of 35 mg/kg body weight p.o. The third and fourth groups of animals received ZNA at the doses of 100 mg and 200 mg/kg body weight p.o., respectively [21, 22].

Thirty minutes after administration of distilled water, ranitidine hydrochloride and test extract were given to the four groups as mentioned above, an aqueous suspension of ASA at the dose of 250 mg/kg body weight was given orally to each rat. After 6 h, all the animals were sacrificed by cervical dislocation; the stomachs were removed and opened along the greater curvature. The stomach was rinsed with normal saline and examined grossly. The ulcer index was evaluated according to number and severity of lesions formed and scored using the following scale [23].

0 = no visible ulcers; 1 = petechial hemorrhage or minute pin point ulcers; 2 = one or two small ulcers; 3 = more than two ulcers, mainly with few large ulcers; 4 = more than two ulcers, with mainly large ulcers.

The mean ulcer indices in each group were calculated and expressed the percentage of inhibition using the following formula:

\[(\text{Control mean} - \text{Treated mean/ Control mean}) \times 100\%\]
received the drug interventions as described above in the ASA experiment. One hour after the treatments, the animals received ethanol at a dose of 1 ml/200 g body weight p.o. After 1 h, all the animals were sacrificed by cervical dislocation; the stomachs were removed and opened along the greater curvature, rinsed with normal saline. Then the gastric mucosa was observed and scored as mentioned above [24].

**Stress Induced Gastric Ulcer:** The rats were fasted for 24 h with water *ad libitum*. The rats were divided into four groups (*n* = 6) and received the drug interventions as described above in the ASA experiment. Immediately after administration, each rat was immobilized in a cylindrical cage and immersed vertically to the level of xyphoid process in a water bath for 17 h, maintained at 25±2°C. Then the animals were sacrificed by cervical dislocation; the stomachs were removed and opened along the small curvature [25]. The stomach was rinsed with normal saline and examined for gastric mucosal damage and scored as described above.

**Statistical Analysis:** All data were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett’s *post hoc* test of significance. *P* < 0.001 was considered as statistically significant.

**RESULTS**

Preliminary phytochemical studies on ZNA demonstrated the presence of flavonoids, carbohydrates, reducing sugars and amino acids in ZNA. The effects of ZNA on ASA induced gastric ulcers are summarized in Table 1. The extract at the dose of 100 mg/kg body weight exerted significant (*p* < 0.05) inhibition against ulcer formation. The extract, however at 200 mg/kg dose, more significantly (*p* < 0.001) reduced the ulcerogenic lesions. The reference drug ranitidine hydrochloride exhibited significant (*p* < 0.001) inhibition of ulcers. In ethanol induced gastric ulcer model, the effects of ZNA are shown in Table 2. In this case the extract at both tested doses afforded significant (*p* < 0.001) protection from gastric mucosal damage. The reference drug ranitidine exhibited significant (*p* < 0.001) inhibition of ulcers. In stress induced gastric ulcer model, the effects of ZNA are presented in Table 3. Here, the extract at lower dose (100 mg/kg) demonstrated negligible ulcer inhibitory activity which was found statistically insignificant.

<table>
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<tr>
<th>Treatments</th>
<th>Ulcer index</th>
<th>% Protection</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.77±0.21</td>
<td>-</td>
</tr>
<tr>
<td>ZNA (100 mg/kg)</td>
<td>2.60±0.39*</td>
<td>31.03</td>
</tr>
<tr>
<td>ZNA (200 mg/kg)</td>
<td>1.71±0.62**</td>
<td>54.64</td>
</tr>
<tr>
<td>Ranitidine HCl</td>
<td>1.06±0.58**</td>
<td>71.88</td>
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Data are presented as mean ± SEM, *p* < 0.05, **p** < 0.001, compared to control.

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<tr>
<td>Control</td>
<td>3.83±0.19</td>
<td>-</td>
</tr>
<tr>
<td>ZNA (100 mg/kg)</td>
<td>1.53±0.42**</td>
<td>60.05</td>
</tr>
<tr>
<td>ZNA (200 mg/kg)</td>
<td>1.28±0.50**</td>
<td>66.58</td>
</tr>
<tr>
<td>Ranitidine HCl</td>
<td>0.94±0.81**</td>
<td>75.45</td>
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Data are presented as mean ± SEM, **p** < 0.001, compared to control.

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<tr>
<td>Control</td>
<td>3.46±0.64</td>
<td>-</td>
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<tr>
<td>ZNA (100 mg/kg)</td>
<td>3.06**</td>
<td>11.56</td>
</tr>
<tr>
<td>ZNA (200 mg/kg)</td>
<td>1.92**</td>
<td>44.54</td>
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<tr>
<td>Ranitidine HCl</td>
<td>0.67±0.33**</td>
<td>80.63</td>
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Data are presented as mean ± SEM, **p** < 0.001, compared to control, ns: not significant.

Its higher dose (200 mg/kg) demonstrated significant (*p* < 0.001) protection. The reference drug ranitidine here also exhibited marked and significant (*p* < 0.001) protection against gastric mucosal lesions in rats.

**DISCUSSION**

In the present investigation, the aqueous extract of *Z. ntidum* root (ZNA) was screened for the anti-ulcer activity in chemical (ASA, ethanol) and stress (water immersion- induced restraint stress) induced ulcers in Wister albino rats. It was found that ZNA offered significant amelioration form ulcerative gastric lesions in albino rats in a dose dependent manner.

Acetyl salicylic acid (ASA), also known as aspirin is an analgesic drug known to cause gastric ulcer. It is a potent irreversible prostaglandin biosynthesis inhibitor and causes a dose dependent reduction in mucosal prostaglandins (PGE, and PGI) biosynthesis accompanied by an increase in the areas of gastric mucosal damage. The observed gastric mucosal lesions induced by ASA are due to the deficiency of mucosal prostaglandins [26]. The ZNA was found to exhibit a significant anti-ulcer property at the both test doses against ASA induced gastric ulcer in a dose related way.
Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity of drugs and chemicals. Ethanol induces gastric mucosal damage by reduction of gastric mucosal blood flow, mucus production, endogenous glutathione and prostaglandin levels. At the same time ethanol increases ischaemia, gastric vascular permeability and back diffusion, histamine release, generation of free radicals and production of leukotrienes [27]. It has been found that oxygen derived reactive free radicals are implicated in the mechanism of acute and chronic ulceration by ethanol and scavenging these free radicals can play an appreciable role in healing of these ulcers [28]. The ZNA at the two tested doses exhibited dose dependent and significant ameliorative activity against ethanol induced gastric ulceration. This effect may be attributed to the antioxidant activity of Z. nitidum root reported elsewhere [16].

Gastrointestinal erosion is one of the consistent findings in humans and experimental animals subjected to different types of stress. It has been shown that exposure of rats to restraint stress significantly decreases gastric acid secretion, but gastric acid secretion increases towards the pre-stress level for a few hours when the restrained animals are subjected to additional water immersion [29, 30]. Since the development gastric lesions during stress enhances significantly by exposure to water immersion, the rise in acid secretion may be important in the aggravating process of lesions during water immersion [31]. The ZNA only at the higher dose exhibited significant protection against stress induced gastric mucosal lesions in rats (so what is the reason ??).

From the present preliminary investigation, it can be concluded that the aqueous extract from the roots of Zanthoxylum nitidum grown in India afforded remarkable ameliorative effect against chemical and stress induced gastric mucosal lesions thereby confirming anti-ulcer activity in Wistar rats. Purification of the extract and further studies on Z. nitidum root may lead to development of newer safe and effective antiulcer drugs.

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REFERENCES