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Protective Effect of *Zanthoxylum nitidum* Bark in Chemical and Stress Induced Gastric Mucosal Lesions in Male Albino Rats

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Abstract: *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae), called Tez-mui or Tejamool in Assamese, is a large prickly shrub occurring in North-Eastern India and its roots are used traditionally for several medicinal purposes. In the present study, the aqueous extract from the stem bark of *Zanthoxylum nitidum* (ZNA) was evaluated for its protective effects on gastric mucosal lesions in male Wistar albino rats against acetylsalicylic acid (ASA), ethanol and water immersion restraint stress induced gastric mucosal damage. In each model, ZNA was administered orally to rats at the doses of 100 and 200 mg kg^{-1} body weight, prior to chemical or stress challenge, followed by determination of ulcer index. Ranitidine hydrochloride at the dose of 35 mg kg^{-1}, p.o. served as the reference drug. The test extract exhibited dose dependent and significant amelioration of gastric mucosal lesions in chemical (ASA and ethanol) as well as in stress-induced ulcers in male Wistar albino rats, thus confirming its antiulcer potential.

Key words: *Zanthoxylum nitidum,* gastric mucosal damage, ASA, ethanol, stress

INTRODUCTION

Peptic ulcer is a disease of the part of gastrointestinal tract which is exposed to gastric acid and pepsin. It occurs due to an imbalance between certain aggressive factors like acid, pepsin and *Helicobacter pylori* and the defensive factors, namely gastric mucus and bicarbonate secretion, prostaglandins etc. Recently the involvement of *Helicobacter pylori* infection in peptic ulcer formation and recurrence has been reported. 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 (Tripathi, 2008). Current management of peptic ulcer diseases in the developing countries is generally limited to suppression of pain, with little or no strategy aimed at healing of ulcers. Herbal medicine is emerging as an alternative strategy to presently available synthetic drugs for management of peptic ulcer diseases possibly due to their perceived effectiveness, less possibilities of serious side effects and easy availability at comparatively lower costs.

Zanthoxylum nitidum (Roxb.) DC (Rutaceae), called *Tez-mui* or *Tejamool* in Assamese is a morphologically variable plant species occurring throughout the South-East Asian countries and in Australia (Hu *et al*., 2007). In India it occurs as a large prickly shrub in the North-Eastern Indian region in particular Sikkim, Assam and Nagaland states. In India, this plant is traditionally used for several medicinal purposes. The root is used in toothache, stomachache, fever, rheumatism, paresis, boils and as an insecticide and pesticide. The fruit is used in the management of stomachache, cough, colic, vomiting, diarrhea and paresis; and as an aromatic, stimulant and piscicide. Its small branches, seeds and stem bark are prescribed in fever, diarrhea and cholera (Kirtikar and Basu, 1933; Anonymous, 1976; Kanjilal, 1997). The authors have noticed that the village people of upper Assam of India use the young stems of this plant as chewing stick for oral health especially for toothache and gingivitis.

The previous workers have reported antispasmodic, anti-tumor, antifungal, antioxidant, analgesic and anti-inflammatory activities of the root of *Z. nitidum* mainly from China, Japan and Taiwan (Suffnes and Cordell, 1985; Fang *et al*., 1993; Hu *et al*., 1999, 2006; Shyur *et al*., 2005). Previously, the present authors have reported the essential oil composition of its fruits and leaves,

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antibacterial and pharmacognostic studies of stem bark and root, anti-nociceptive activity of stem bark, anti-inflammatory and antioxidant activities of stem bark and root of *Z. nitidum* grown in India (Bhattacharya and Zaman, 2009a, b, c, 2011; Bhattacharya *et al*., 2009, 2010a,b, 2011). However, there are no reports of anti-ulcer investigations carried out on *Z. nitidum*. Therefore, the present investigation attempted to report the preliminary results of studies on possible gastric ulcer protective effects of Indian *Z. nitidum* stem bark 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 2006 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. 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. Then the stem barks were shade dried at temperature 21-24°C and ground into coarse powder with a mechanical grinder and stored in air-tight container.

Preparation of extract: The powdered plant material (160 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 methods (Harborne, 1998).

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 Wistar 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 not more than three animals per cage and 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, Gwuahati, 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^{-1} b.wt. p.o. The second group of animals served as reference, received ranitidine hydrochloride at the dose of 35 mg kg^{-1} b.wt. p.o. (Kunle *et al*., 1999; Paul *et al*., 2000). The third and fourth groups of animals received ZNA at the doses of 100 mg and 200 mg kg^{-1} b.wt. p.o., respectively.

Thirty minutes after administration of distilled water, ranitidine hydrochloride and test extract to the four groups as mentioned above, an aqueous suspension of ASA at the dose of 250 mg kg^{-1} b.wt. was given orally to each rat (Kamsiah *et al*., 2005). 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 (Liu *et al*., 2001).

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:

$$\text{Inhibition (\%)} = \frac{\text{Control mean-Treated mean}}{\text{Control mean}} \times 100$$

Ethanol induced gastric ulcer: The rats were fasted for 18 h and deprived of water for 12 h before experiment. The rats were divided into four groups (n = 6) and 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 b.wt. 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 (Hollander *et al*., 1985; Al-Attar, 2011).

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 xiphoid 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. The stomach was rinsed with normal saline and examined for gastric mucosal damage and scored as described above (Bacchi and Sertie, 1994; Gill *et al*., 2011a).

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 true alkaloids, flavonoids, carbohydrates, proteins and amino acids. The effects of ZNA on ASA induced gastric ulcers are summarized in Table 1. The extract at the dose of 100 mg kg^{-1} b.wt. exerted significant ($p<0.05$) inhibition against ulcer formation. The extract, however at 200 mg kg^{-1} 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 the both test 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^{-1}) demonstrated significant ($p<0.05$) ulcer inhibitory activity. Its higher dose (200 mg kg^{-1}) offered highly 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.

Table 1: Influence of ZNA on ASA induced gastric ulceration in rats

Treatments	Ulcer index	Protection (%)
Control	3.77±0.21	-
ZNA (100 mg kg^{-1})	2.67±0.52*	29.18
ZNA (200 mg kg^{-1})	1.79±0.35**	52.52
Ranitidine HCl	1.06±0.58**	71.88

Data are presented as Mean±SEM, n = 6. *p< 0.05, **p<0.001, compared to control

Table 2: Influence of ZNA on ethanol induced gastric ulceration in rats

Treatments	Ulcer index	Protection (%)
Control	3.83±0.19	-
ZNA (100 mg kg^{-1})	1.61±0.43**	57.96
ZNA (200 mg kg^{-1})	1.33±0.37**	65.27
Ranitidine HCl	0.94±0.81**	75.45

Data are presented as Mean±SEM, n = 6. **p<0.001, compared to control

Table 3: Influence of ZNA on stress induced gastric ulceration in rats

Treatments	Ulcer index	Protection (%)
Control	3.46±0.64	-
ZNA (100 mg kg^{-1})	2.29±0.51*	33.82
ZNA (200 mg kg^{-1})	1.90±0.46**	45.09
Ranitidine HCl	0.67±0.33**	80.63

Data are presented as Mean±SEM, n = 6.*p< 0.05, **p<0.001, compared to control

DISCUSSION

In the present investigation, the aqueous extract of *Z. nitidum* stem bark (ZNA) was screened for the anti-ulcer activity in chemical (ASA, ethanol) and stress (water immersion-induced restraint stress) induced ulcers in Wistar albino rats. It was found that ZNA afforded 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_2 and PGI_2) 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 (Vane, 1971). 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 and 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 (Glavin and Szabo, 1992; Al-Rejaie, 2009). 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 (Loguercio *et al*., 1993; Gill *et al*., 2011b).

In general, redox imbalance in gastric mucosal tissues is known to participate in the formation of gastric ulcers (Kisaoglu *et al*., 2011). The ZNA at all test 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 (Bhattacharya and Zaman, 2011).

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 (Brodie *et al*., 1962), but gastric acid secretion increases towards the pre-stress level for a few hours when the restrained animals are subjected to additional water immersion (Hayase and Takeuchi, 1986). 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 (Parmar and Desai, 1993). Here, ZNA at the both test does dose dependently exhibited significant protection against stress induced gastric mucosal lesions in rats. The overall observed effects in the present study were found to be in agreement with those reported by the previous workers (Alsaif, 2004; Alhaider *et al*., 2006; Abd El-Kader *et al*., 2011).

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

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