Middle-East Journal of Scientific Research 12 (10): 1349-1353, 2012
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2012.12.10.1699

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

[1]Sanjib Bhattacharya and [2]Md. Kamaruz Zaman

[1]Bengal School of Technology (A College of Pharmacy),
Delhi Road, Sugandha, Hooghly 712102, West Bengal, India
[2]Department of Pharmaceutical Sciences,
Dibrugarh University, Dibrugarh 786004, Assam, India

Abstract: *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae) is a large prickly shrub occurring in North-Eastern India and its roots are used traditionally for several medicinal purposes. The present study was performed to assess gastric ulcer protective effects of its root in Wistar albino rats. Here, the aqueous extract from the roots of *Zanthoxylum nitidum* (ZNA) was evaluated for its ameliorative effects on gastric mucosal lesions in 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 body weight, prior to chemical or stress challenge, followed by determination of ulcer index. Ranitidine hydrochloride orally at the dose of 35 mg/kg 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 Wistar rats, thus confirming its antiulcer potential.

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

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.

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].

Corresponding Author: Sanjib Bhattacharya, Pharmacognosy Division, Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India, Tel: + 91 9874331777, Fax: + 91 33 26864281.

Previously the present authors have reported essential oil composition of fruits and leaves, antibacterial and pharmacognostic studies of stem bark and root, anti-nociceptiveand 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 not 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, 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 lbitum*. 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:

(Control mean - Treated mean/ Control mean) × 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 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 the 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 1: Influence of ZNA on ASA induced gastric ulceration in rats

Treatments	Ulcer index	% Protection
Control	3.77±0.21	-
ZNA (100 mg/kg)	2.60±0.39*	31.03
ZNA (200 mg/kg)	1.71±0.62**	54.64
Ranitidine HCl	1.06 ± 0.58**	71.88

Data are presented as mean ± SEM, *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)	1.53 ± 0.42**	60.05
ZNA (200 mg/kg)	1.28 ± 0.50**	66.58
Ranitidine HCl	0.94 ± 0.81**	75.45

Data are presented as mean ± SEM, **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)	3.06^{ns}	11.56
ZNA (200 mg/kg)	1.92**	44.54
Ranitidine HCl	0.67 ± 0.33**	80.63

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_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 [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.

ACKNOWLEDGEMENT

The authors are thankful to the authority of Dibrugarh University, Dibrugarh, Assam 786004, India, for providing necessary facilities the present work.

REFERENCES

1. Tripathi, K.D., 2000. Essentials of Medical Pharmacology. New Delhi: Jaypee Brothers Medical Publishers Pvt. Ltd.
2. Hu, J., W.D. Zhang, Y.H. Shen, C. Zhang, L. Xu and R.H. Liu, 2007. Alkaloids from *Zanthoxylum nitidum* (Roxb.) DC. Biochemical Systematics and Ecology; 35: 114-117.
3. Kirtikar, K.R. and B.D. Basu, 1933. Indian Medicinal Plants. Vol. I. New Delhi: Bishen Singh Mahendra Pal Singh.
4. Anonymous, 1976. The Wealth of India, Raw Materials. Vol. XI. New Delhi: Council of Scientific and Industrial Research.
5. Kanjilal, U.N., 1997. The Flora of Assam. Vol. I (Part I). New Delhi: Omsons Publications.
6. Suffnes, M. and G.A. Cordell, 1985. Antitumor alkaloids. In: Brossi A. editor. The Alkaloids, Chemistry and Pharmacology. Vol. XXV. London: Academic Press Inc.
7. Fang, S.D., L.K. Wang and S.M. Hecht, 1993. Inhibitors of DNA topoisomerase I isolated from the roots of Zanthoxylum *nitidum*. Journal of Organic Chemistry, 58: 5025-5027.
8. Hu, K., A. Dong, H. Liu, H. Feng, Q. Sun and X. Yao, 1999. Bioactivity of traditional Chinese herbal medicine against *Pyricularia oryzae*. Pharmaceutical Biology, 37: 225-230.
9. Hu, J., W.D. Zhang, R.H. Liu, C. Zhang, Y.H. Shen and X.K. Xu, 2006. Chemical constituents in root of *Zanthoxylum nitidum*. Zhonqquo Zhong Yao Za Zhi, 31: 1689-91.
10. Shyur, L.F., J.H. Tsung, J.H. Chen, C.Y. Chin and C.P. Lo, 2005. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. International Journal of Applied Science and Engineering, 3: 195-202.
11. Bhattacharya, S. and M.K. Zaman, 2009. Essential oil composition of fruits and leaves of *Zanthoxylum nitidum* grown in upper Assam region of India. Pharmacognosy Resaerch, 1: 148-151.
12. Bhattacharya, S. and M.K. Zaman, 2009. Pharmacognostical evaluation of *Zanthoxylum nitidum* bark. International Journal of Pharm Tech Research, 1: 292-298.
13. Bhattacharya, S. and M.K. Zaman, 2009. Pharmacognostical evaluation of *Zanthoxylum nitidum* root. Pharmacognosy Journal, 1: 15-21.

14. Bhattacharya, S., M.K. Zaman and P.K. Haldar, 2009. Antibacterial activity of stem bark and root of Indian *Zanthoxylum nitidum*. Asian Journal of Pharmaceutical and Clinical Research, 2: 30-34.
15. Bhattacharya, S., P.K. Haldar and M.K. Zaman, 2010. Anti-nociceptive and locomotor activity of *Zanthoxylum nitidum* stem bark extracts in experimental animal models. Journal of Complementary and Integrative Medicine, 7: 1-8.
16. Bhattacharya, S., P.K. Haldar and M.K. Zaman, 2010. Anti-inflammatory and *in vitro* antioxidant property of *Zanthoxylum nitidum* root. Current Trends in Biotechnology and Pharmacy, 4: 774-783.
17. Bhattacharya, S. and M.K. Zaman, 2011. *In vitro* antioxidative effects of stem bark from *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae). Pharmacologyonline, 7: 1216-1223.
18. Bhattacharya, S., P.K. Haldar and M.K. Zaman, 2011. Anti-inflammatory activity and antioxidant role of *Zanthoxylum nitidum* bark. Oriental Pharmacy and Experimental Medicine, 11: 271-271.
19. Bhattacharya, S. and K. Zaman, 2012. Protective effect of *Zanthoxylum nitidum* bark in chemical and stress induced gastric mucosal lesions in male albino rats. International Journal of Pharmacology, 8: 450-454.
20. Harborne, J.B., 1998. Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis. New Delhi: Springer (India) Pvt. Ltd.
21. Kunle, O.O., A. Shittu and R.N. Nasipuri, 1999. Gastrointestinal activity of *Ficus sur*. Fitoterapia, 70: 542-547.
22. Paul, R.K., A. Jabbar and M.A. Rashid, 2000. Antiulcer activity of *Mikania cordata*. Fitoterapia, 71: 701-703.
23. Liu, X.M., M.N.M. Zakaria and M.W. Islam, 2001. Anti-inflammatory and anti-ulcer activity of *Calligonum comosum* in rats. Fitoterapia, 72: 487-491.
24. Hollander, D., A. Tarnawski, W.J. Krause and H. Gergely, 1985. Protective effect of sucralfate against alcohol induced gastric mucosal injury in the rat: Macroscopic, histologic, ultrastructural and functional time sequence analysis. Gastroenterology, 88: 366-374.
25. Bacchi, E.M. and J.A.A. Sertie, 1994. Antiulcer action of *Styrax camporum* and *Caesalpinia ferra* in rats. Planta Medica, 60: 118-120.
26. Vane, J.T., 1971. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin like drugs. Nature, 231: 232-236.
27. Glavin, G.B. and S. Szabo, 1992. Experimental gastric mucosal injury, laboratory models reveal mechanism of pathogenesis and new therapeutic strategies. FASEB J., 6: 825-831.
28. Loguercio, C., D. Taranto, F. Beneduce, V.V. Balance and A. Vincentis, 1993. Glutathione prevents ethanol induced gastric mucosal damage and depletion of sulfydryl compounds in humans. Gut, 34: 161-165.
29. Brodie, D.A., R.W. Marshall and M. Morneo, 1962. The effect of restraint on gastric acidity in the rat. American Journal of Physiology, 202: 812-814.
30. Hayase, M. and K. Tukeuchi, 1986. Gastric acid secretion and lesion formation in rats under water immersion stress. Digestive Diseases and Sciences, 31: 166-171.
31. Parmar, N.S. and J.K. Desai, 1993. A review of the current methodology for the evaluation of gastric duodenal anti- ulcer agents. Indian Journal of Pharmacology, 25: 120-135.