PROTECTIVE EFFECT OF *NEOALSOMITRA CLAVIGERA* IN CHEMICAL AND STRESS INDUCED GASTRIC MUCOSAL LESIONS IN RATS

P.K Haldar[1], Sanjib Bhattacharya[2*], M. K. Zaman[3], R. Vanlalsiama[3]

[1]Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, West Bengal, India.
[2]R.R.S. College of Pharmacy, Amethi 227405, U.P., India
[3]Dept. of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004, Assam, India.

***Corresponding author:** Sanjib Bhattacharya, R.R.S. College of Pharmacy, Amethi 227407, U.P., India. Phone: +91-9919798896, E-mail: sakkwai@yahoo.com

Summary

Neoalsomitra clavigera (Wall) Hutch. (Cucurbitaceae) is a woody climber plant found in south-east Asia including north-east India and its ripe fruits are used traditionally in India for stomachache, peptic ulcer and diarrhea. In present study, the aqueous extract from the ripe fruits of *Neoalsomitra clavigera* was evaluated for its protective effects on gastric mucosal lesion in Wistar albino rats against acetylsalicylic acid (ASA), ethanol and water immersion restraint stress induced gastric mucosal damage. In each model the cold maceration aqueous fruit extract was employed at the doses of 100 and 200 mg/kg body weight, *p.o* along with the standard drug ranitidine hydrochloride at a dose of 35 mg/kg, *p.o*. The extract exhibited significant reduction of gastric mucosal lesions in chemical (ASA, and ethanol) as well as in stress-induced model.

Key words: *Neoalsomitra clavigera*, gastric mucosal damage, ASA, ethanol, water immersion stress, Ranitidine HCl.

Neoalsomitra clavigera (Wall) Hutch. (Cucurbitaceae), called '*Lalrunga-dawibur'* in *Mizo* is a woody climber plant species occurring in South-East Asian countries including North-East India, Nepal, Bangladesh, Myanmar, Malaysia and in Australia. In India it is distributed in the hilly region of Arunachal Pradesh, Assam, Meghalaya, Mizoram and Sikkim at an altitude of around 3000-4000 ft. The ripe fruits of *N. clavigera* are traditionally used by the people of Mizoram as one of the most effective medicine for stomachache, peptic ulcer, and diarrhea. For the treatment of peptic ulcer the infusion of the fruit is taken daily for a few weeks. The fruit thus can be used for three to four times before the bitterness disappears [(1, 2, 3)].

Thorough search of literature did not reveal any reports of biological investigations carried out on *N. clavigera*. The present work therefore, attempts to evaluate the anti-ulcer activity of the aqueous extract from the ripe fruits of *N. clavigera* growing in Mizoram, India to justify its folkloric uses.

Methods

The ripe fruits and aerial parts of *Neoalsomitra clavigera* (Wall.) Hutch. were collected during the month of November 2005 from Thingdawl, Mizoram state, India. The species was identified by the Botanical Survey of India, Eastern Circle, Shillong, India, and a voucher specimen (No. DUPS-05-003) was kept in Dept. of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004, India, for future reference. After collection, the fruits were shade dried at temperature 21-24°C and ground into coarse powder.

The air dried ripe fruits of *N. clavigera* were extracted with water by cold maceration. Powdered plant materials (70 g) were macerated with 400 ml of water at 21-24°C temperature for 2 days with frequent shaking. After 2 days, the extracts were filtered and to the marc part 300 ml of water 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 (extracts) were evaporated to dryness in a vacuum oven and kept in a desiccator for future use.

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 x 23 cm x 10 cm) with not more than three animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2^{o}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.

For acetyl salicylic acid (ASA) induced gastric ulcer [4] the rats were weighed and divided into four groups each consisting six rats (n = 6). All rats were fasted for 48 h with water *ad lbitum*. The first group of animals which served as control received distilled water 10 ml/kg body weight *p.o*. The second group of animals which served as standard, received ranitidine hydrochloride at the dose of 35 mg/kg body weight *p.o*.[5]. The third and fourth group of animals received aqueous extract of *N. clavigera* at the doses of 100 mg and 200 mg/kg body weight *p.o*. respectively.

30 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 body weight was given orally to each rat. After 6 h, 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 [6].

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 %.

In ethanol induced gastric ulcer [7] 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. 1 h after the administration of standard and test drugs, the animals received ethanol at a dose of 1 ml/200g body weight *p.o*. After 1 h, 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.

In stress induced gastric ulcer [8] 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 ± 2ºC. Then the animals were killed 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 above.

The results are expressed as mean ± Standard Error of Mean (SEM). Student's '*t*' test was used to verify the statistical significance at $P < 0.05$.

Table 1. Inhibition of ASA induced gastric ulceration by aqueous extract of *N. clavigera.*

Treatment	Dose (mg/kg, *p.o*)	Ulcer index ± SEM	% Inhibition
Control	Water, 10 ml/kg, *p.o*	3.77 ± 0.21	-
N. clavigera aqueous extract	100	2.24 ± 0.47	35.26
N. clavigera aqueous extract	200	1.33 ± 0.72*	64.72
Ranitidine HCL	35	1.06 ± 0.58**	71.88

Number of animals per group (n) = 6.
SEM = Standard Error of Mean
*p< 0.05, **p < 0.01, compared to control. Degree of signicavity was assessed by Student's '*t*' test.

Table 2. Inhibition of ethanol induced gastric ulceration by aqueous extract of *N. clavigera.*

Treatment	Dose (mg/kg, *p.o*)	Ulcer index ± SEM	% Inhibition
Control	Water, 10 ml/kg, *p.o*	3.83 ± 0.19	-
N. clavigera aqueous extract	100	1.57 ± 0.73*	59.00
N. clavigera aqueous extract	200	1.39 ± 0.40*	63.70
Ranitidine HCL	35	0.94 ± 0.81**	75.45

Number of animals per group (n) = 6.
SEM = Standard Error of Mean
*p< 0.05, **p < 0.01, compared to control. Degree of signicavity was assessed by Student's '*t*' test.

Table 3. Inhibition of stress induced gastric ulceration by aqueous extract of *N. clavigera.*

Treatment	Dose (mg/kg, *p.o*)	Ulcer index ± SEM	% Inhibition
Control	Water, 10 ml/kg, *p.o*	3.46 ± 0.64	-
N. clavigera aqueous extract	100	3.12 ± 0.49	9.82
N. clavigera aqueous extract	200	1.78 ± 0.51*	48.55
Ranitidine HCl	35	0.67 ± 0.33**	80.63

Number of animals per group (n) = 6.
SEM = Standard Error of Mean
*p< 0.05, **p < 0.01, compared to control. Degree of signicavity was assessed by Student's '*t*' test.

Results

The effects of aqueous extract from *N. clavigera* fruits on ASA induced gastric ulcers are shown in Table 1. The extract at the dose of 100 mg/kg body weight showed inhibition against ulcer formation but was found statistically insignificant. The extract, however at 200 mg/kg dose significantly ($p < 0.05$) reduced the ulcerogenic lesions. The standard drug ranitidine hydrochloride exhibited significant ($p < 0.05$) inhibition of ulcers.

In ethanol induced gastric ulcer model, the effects of *N. clavigera* fruit extract are shown in Table 2. The extract at the both test doses afforded significant ($p < 0.05$) protection against ulcer formation. The standard drug ranitidine hydrochloride exhibited significant ($p < 0.01$) inhibition of ulcers.

In stress induced gastric ulcer model, the effects of *N. clavigera* fruit extract are shown in Table 3. Here, the extract at lower dose (100 mg/kg) showed negligible ulcer inhibitory activity. Its higher dose (200 mg/kg) demonstrated significant ($p < 0.05$) protection. The standard drug ranitidine hydrochloride exhibited marked and significant ($p < 0.01$) protection against gastric mucosal damage.

Discussion

Ulcers are caused by an imbalance between aggressive and defensive factors of the gastric mucosa. Gastric acid and pepsin make up the offensive factors whose proteolytic effect is buffered by mucin production, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandins [(9)]. Different therapeutic agents including plant extracts have been used to inhibit gastric acid secretion or to boost the mucosal defense mechanisms [(10)]. In present investigation, the aqueous extract of *N. clavigera* ripe fruits was screened for the anti-ulcer activity in chemical (ASA, ethanol) and stress (water immersion-induced restraint stress) induced ulcers in Wister albino rats.

Acetyl salicylic acid (ASA) 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 [(11)]. The extract was found to exhibit a significant anti-ulcer property at higher dose (200 mg/kg) against ASA induced gastric ulcer.

Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity. Ethanol induces ulcers 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 [(12)]. It has been found that oxygen derived 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 [(13)]. The extract at all test doses exhibited significant anti-ulcer activity against ethanol induced ulceration. This effect may be due to its anti-oxidant activity.

Gastrointestinal erosion is one of the consistent findings in man 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 [(14)] but gastric acid secretion increases towards the pre-stress level for a few hours when the restrained animals are subjected to additional water immersion [(15)]. 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 [(16)]. The extract also exhibited significant protection against stress induced gastric mucosal lesions.

From the present investigation it can be concluded that the aqueous extract of *Neoalsomitra clavigera* fruits afforded significant anti-ulcer activity against chemical and stress induced gastric lesions thereby reducing mucosal damage. The anti-ulcer effects of fruits from *N. clavigera* can provide a scientific basis for its traditional uses for stomachache and peptic ulcer in North-East India.

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References

1. Hooker JD. The Flora of British India. Vol. II. London: William Clower and Sons Ltd., 1879: 634.
2. Rozika R, Damdawai R. Medicinal Plants (Agri. Extn. Series-26), 1st ed. New Delhi: Directorate of Agriculture and Minor Irrigation, Govt. of India, 2001: 111-112.
3. Thansanga R. Agriculture in Mizoram. Aizawl: Directorate of Agriculture, Govt of Mizoram, 2000: 102.
4. Kunle OO, Shittu A, Nasipuri, RN, et al. Gastrointestinal activity of *Ficus sur*. Fitoterapia 1999; 70: 542-547.
5. Paul RK, Jabbar A, Rashid MA. Antiulcer activity of *Mikania cordata*. Fitoterapia 2000; 71: 701-703.
6. Liu XM, Zakaria MNM, Islam MW, et al. Anti-inflammatory and anti-ulcer activity of *Calligonum comosum* in rats. Fitoterapia 2001; 72: 487-491.
7. Hollander D, Tarnawski A, Krause WJ, Gergely H. Protective effect of sucralfate against alcohol induced gastric mucosal injury in the rat: Macroscopic, histologic, ultrastructural and functional time sequence analysis. Gastroenterology 1985; 88: 366-374.
8. Bacchi EM, Sertie JAA. Antiulcer action of *Styrax camporum* and *Caesalpinia ferra* in rats. Planta Med 1994; 60: 118-120.
9. Goel RK, Bhattacharya SK. Gastrointestinal mucosal defense and mucosal protective agents. Indian J Exp Biol 1999; 29: 701.
10. Goel RK, Sairam K. Antiulcer drugs from indigenous sources with emphasis on *Musa sapientum,* Tamrabhasma, *Asparagus racemosus* and *Zingiber officinale.* Indian J Pharm 2002; 34: 100-110.
11. Vane JT. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin like drugs. Nature 1971; 231: 232-236.
12. Glavin GB, Szabo S. Experimental gastric mucosal injury, laboratory models reveal mechanism of pathogenesis and new therapeutic strategies. FASEB J1992; 6: 825-831.
13. Loguercio C, Taranto D, Beneduce F, Balance VV, Vincentis A. Glutathione prevents ethanol induced gastric mucosal damage and depletion of sulfydryl compounds in humans. Gut 1993; 34: 161-165.
14. Brodie DA, Marshall RW, Morneo M. The effect of restraint on gastric acidity in the rat. Am J Physiol 1962; 202: 812-814.
15. Hayase M, Tukeuchi K. Gastric acid secretion and lesion formation in rats under water immersion stress. Dig Dis Sci 1986; 31: 166-171.
16. Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. Indian J Pharmacol 1993; 25: 120-135.