



Anti ulcer activity of *Naravelia zeylanica* leaves extract

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Received on: 27-03-2009; Accepted on: 29-05-2009

ABSTRACT

The objective of present study is to evaluate the anti ulcer activity of ethanol extract of leaves of *Naravelia zeylanica*. The ethanol extract of *N.zeylanica* was investigated for its anti ulcer activity against aspirin plus pylorus ligation induced gastric ulcer in rats, HCl-Ethanol induced ulcer in mice and water immersion stress induced ulcer in rats. The antiulcer activity was assessed by determining and comparing gastric volume, free acidity and ulcer inhibition in aspirin plus pylorus ligation induced gastric ulcer model. The number of lesions in HCl-Ethanol induced peptic ulcer model and mean score value of ulcer inhibition in water immersion stress induced ulcer model. A significant antiulcer activity was observed in all the models. Pylorus ligation model showed significant ($P<0.01$) reduction in gastric volume, free acidity and ulcer index as compared to control. Also extract showed 89.71% ulcer inhibition in HCl-Ethanol induced ulcer and 95.3% ulcer protection index in stress induced ulcer. This present study indicates that *Naravelia zeylanica* leaves extract have potential anti ulcer activity in the three models tested.

Keywords: Aspirin, pylorous ligation, ulceration, stress ulcer, *Naravelia zeylanica*

INTRODUCTION

Gastric ulcers one of the most widespread diseases is believed to be due to an imbalance between aggressive and protective factors. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility (1). Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF," leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide) (2).

The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently, there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources. A large number of spices and herbs have been evaluated by various researchers for their anti-ulcer effects to achieve a favorable outcome (3).

Naravelia zeylanica (Ranunculaceae) is a climbing shrub with tuberous roots. The plant is available rich all around south India. In ayurveda the plant has been extensively used by native peoples as an astringent, bitter, antipruritic and anti-inflammatory. It is also useful in pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, cephalalgia, colic inflammation, wound healing & ulcer protec-

tion. The root and stem have a strong penetrating smell and is used to relieve malarial fever and headache. Root and stem paste is applied externally for psoriasis, itches and skin allergies. The traditional medicine practitioners using the leaf and stem juices for treating intestinal worms, psoriasis & dermatitis (4).

The literature survey reveals no reports on the anti ulcer activity of the leaves extracts of *N.zeylanica*. This prompted us to investigate the anti-ulcer activity of *N.zeylanica* leaves extract.

MATERIALS AND METHODS

Plant material

The leaves of *N.zeylanica* were collected from Udupi, Karnataka, during October. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (H.S.198) was deposited in the herbarium of our institute.

Preparation of Extract

Leaves were shade dried and powdered mechanically. The powdered plant material (350 g) was repeatedly extracted in a 2000 mL round bottomed flask with 1500 mL solvents of increasing polarity starting with petroleum ether, chloroform and ethanol. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature, filtered, and evaporated to dryness under reduced pressure in a rotary evaporator.

Animals

Wistar albino rats (180 to 200 g) and Wistar mice (24-30 g) of either sex procured from Indian Institute of Sciences were used for this study. They are maintained under standard conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity $60 \pm 5\%$ and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care ac-

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Table 1: Effect of *N. zeylanica* on aspirin plus pylorus ligation induced gastric ulcer in rats

| Treatment (mg/kg) | Volume of gastric secretion (ml/100g) | Free acidity (mEq/100g) | Total acidity (mEq/100g) | pH |
|------------------------|---------------------------------------|----------------------------|---------------------------|--------------------------|
| Control (1% SCMC) | 2.633±0.042 | 225.00±6.124 | 555.00±7.50 | 2.20±0.163 |
| Ranitidine 50 | 1.317±0.172 | 148.75±13.475 [#] | 492.5±20.736 [*] | 3.167±0.166 [*] |
| <i>N.zeylanica</i> 300 | 0.983±0.083 [#] | 135.0±10.782 [#] | 552.50±10.724 | 2.333±0.210 |

Values are expressed as mean±SEM. n=6. *P<0.05, #P<0.01 as compared to control

Table 2: Effect of *N. zeylanica* on aspirin plus pylorus ligation induced gastric ulcer in rats

| Treatment | Dose (mg/kg) | Ulcer score | Ulcer inhibition (%) |
|--------------------|--------------|--------------------------|----------------------|
| Control | 1% SCMC | 3.60±0.2 | - |
| Ranitidine | 50 | 0.167±0.166 [#] | 95.37 |
| <i>N.zeylanica</i> | 300 | 1.667±0.307 [#] | 53.69 |

Values are expressed as mean±SEM. n=6. #P<0.01 as compared to control

Table 3: Effect of *N. zeylanica* on ethanol-induced histopathological lesions in gastric mucosa of mice

| Treatment | Dose (mg/kg) | Gastric lesion | Ulcer inhibition (%) |
|--------------------|--------------|---------------------------|----------------------|
| Control | 1% SCMC | 22.667±3.509 | - |
| Sucralfate | 100 | 1.167±0.5426 [#] | 94.85 |
| <i>N.zeylanica</i> | 300 | 2.333±5.57 [*] | 89.71 |

Values are expressed as mean±SEM. n=6. *P<0.05 as compared to control

Table 4: Effect of *N. zeylanica* on water immersion stress induced ulcer in rats

| Treatment | Dose (mg/kg) | Mean ulcer score | Ulcer inhibition (%) |
|--------------------|--------------|------------------------|----------------------|
| Control | 1% SCMC | 143.3±12.01 | - |
| Sucralfate | 20 | 0.0±0.0 ^s | 100 |
| <i>N.zeylanica</i> | 300 | 6.66±2.10 ^s | 95.3 |

Values are expressed as mean±SEM. n=6. ^sP<0.001 as compared to control

cording to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". All the procedures were performed in accordance with Institutional Animal ethics committee constituted as per the direction of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), under ministry of animal welfare division. Government of India, New Delhi, India.

Drugs

The reference drugs such as ranitidine, sucralfate, omeprazole and the test extract of *N. zeylanica*, were suspended in 1% sodium carboxy methyl cellulose (SCMC) and used for anti-ulcer studies. Each drug suspension was prepared freshly just before the administration. Drugs and vehicles were administered orally.

The present study followed three approaches of antiulcerogenic mechanism of the plant extract:

1. Aspirin plus pylorus ligation induced gastric ulcer in rats (antisecretory mechanism).
2. HCl - Ethanol induced ulcer in mice (cytoprotective mechanism).
3. Water immersion stress induced ulcer in rats (proton pump inhibition mechanism).

Aspirin plus pylorus ligation induced gastric ulcer in rats

The rats were divided into 3 groups (n=6). All the animals received 200 mg/kg of aspirin once daily for three days. Group I (control), received 1ml/kg, 1% SCMC. Group II (reference standard) treated with 50 mg/kg Ranitidine. Group III treated with 300 mg/kg ethanol extract of *N.zeylanica*. On the fourth day pylorus part was ligated following 36 hour fasting (5). Four hours after the pyloric ligation the animals were sacrificed by decapitation. The stomach was opened and the ulcer index was determined (6). The gastric content was titrated against

0.01 N NaOH to find out the free acidity and total acidity (7).

Ulcer lesion Index method: HCl - Ethanol induced ulcer

Swiss albino mice were divided into 3 groups (n=6). Group I (control) received 1ml/kg 1% SCMC. Group II (reference standard) received 100 mg/kg sucralfate. Group III received 300 mg/kg ethanol extract of *N.zeylanica*. After 1 hour all the animals were treated with 0.2 ml of HCl - Ethanol mixture p.o (0.3 M Hydrochloric acid and ethanol 60%) to induce gastric ulcer. After 1 hour animals were sacrificed by cervical dislocation. The stomach was excised and lesion index was determined by measuring each lesion in mm along its greater length (8).

Water immersion stress induced ulcer in rats

Stress ulcers were induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25°C for 3h (9). Rats were fasted for 24h prior to the experiment and divided in to 3 groups (n=6). Group I (control), received 1 ml/kg 1% SCMC. Group II (reference standard) treated with 20 mg/kg omeprazole. Group III received 300 mg/kg ethanol extract of *N.zeylanica*. After the drug treatment animals were allowed to swim in water for 3 hour. The stomach of each animal was removed and the extent of gastric damage was assessed (10).

Statistical analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using graph pad in stat 3 and all the results obtained in the study were compared with the vehicle control group.

Results

In aspirin plus pylorus ligation induced gastric ulcer model, the ethanol extract of *N.zeylanica* showed significant (P<0.01) reduction in gastric volume, free acidity and ulcer score (53.69% ulcer inhibition) as compared to control (Table 1, 2).

It can be observed that the number of lesions in HCl-Ethanol induced peptic ulcer group was significantly high and the ethanol extract of *N.zeylanica* pretreated group depicted marked reduction (P<0.01) in gastric lesion (89.71% ulcer inhibition) as compared to control (Table 3).

In water immersion stress induced ulcer the mean score value of ulcer inhibition was found to be very significant (P<0.001) and the % ulcer inhibition was 95.3 (Table 4).

DISCUSSION

In aspirin plus pylorus ligation model, ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In vehicle control animals aspirin plus pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in higher ulcer index. The extract of *N.zeylanica* reduced the gastric volume, free acidity, total acidity and hence ulcer index showing the anti-secretory mechanism (11). HCl-Ethanol induced gastric damage ranging from endothelial

microvascular damage to development of macroscopic gastric mucosal lesions, which is attributed mainly to the inhibition of biosynthesis of cytoprotective PG resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway (12). These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H⁺ and Na⁺ ions reducing the transmucosal potential difference and induce formation of erosions and ulcers. In this model *N.zeylanica* extract was able to produce a significant reduction of the gastric mucosal damage, indicating a probable local increase in PG synthesis(13).

Water immersion stress is one of the best model for stress induced ulcer in animals. The model provides both emotional stress as well as physiological stress to the animal. The extract showed significant (P<0.001) ulcer inhibition.

The anti ulcer effect observed in the present study might be due to a possible relationship between protection of mucosal injury, inhibition of acid secretion and the antioxidant nature of *N.zeylanica*. The *N.zeylanica* extract possess antisecretory, cytoprotective and proton pump inhibition mechanism. This study indicates that *N.zeylanica* extract has a potential anti ulcer activity. However further study is required to isolate the active molecule responsible for the activity.

ACKNOWLEDGEMENTS

The authors are grateful to management of Srinivas college of Pharmacy, Mangalore for providing necessary facilities to carry out the experiments and A.Shama Rao Foundation, Mangalore for providing financial assistance.

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Source of support: Nil, Conflict of interest: None Declared