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Antiulcer Activity of *Nerium indicum* in Rats

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Abstract: Antiulcer activity of flowers extract of *Nerium indicum* was studies in rats in which gastric ulcers were induce by oral administration of indomethacin and pylorus ligation. Flowers extract of *Nerium indicum* was administered in the dose of 500 and 1000 mg kg^{-1} orally 30 min prior to ulcer induction. The antiulcer activity was assessed by determining and comparing the ulcer index in the test group with that of the vehicle control group. Gastric total acidity and free acidity were estimated in pylorus ligated rats. Cimetidine was used as a reference drug. The results suggest that methanolic flowers extract of *Nerium indicum* possesses significant antiulcer activity.

Key words: Ulceration, cimetidine, indomethacin, pylorus-ligation, *Nerium indicum*, flowers

INTRODUCTION

Nerium indicum (Mill.) belonging to the family Apocynaceae is a wild plant known Kaner in Hindi. According to the literature survey, it is an important medicinal plant. It has been found to possess major therapeutic activities such as analgesic (Ahmed *et al*., 2006), anti-inflammatory and antinociceptive (Erdemoglu *et al*., 2003), anti-tumor (Hu *et al*., 2009), immune-stimulating (Al-Farwacchi, 2007), antibacterial (Naqvi *et al*., 1994), antiviral (Rajbhandari *et al*., 2001), antidiabetic (Tahraoui *et al*., 2007), neuroprotective (Man-Shan *et al*., 2007), molluscicidal (Zhang *et al*., 2009; Wang *et al*., 2006), Piscicidal (Sudhanshu and Ajay, 2009), immunmodulatory (Al-Farwacchi, 2007) and depressant (Zhao *et al*., 2007) activity and many more. We have studied the antiulcer potential of this plant on different models on gastric ulceration.

MATERIALS AND METHODS

Plant material: The flowers of *Nerium indicum* Mill were collected from Sagar, Madhya Pradesh, India. The sample was identified Prof. Madhuri Modak, plant taxonomist, Department of Botany, M.V.M. College, Bhopal, Madhya Pradesh and the voucher specimen has been kept in the department for future reference.

Preparation of extract: Leaves were shade dried and powdered mechanically. The powdered plant material (100 g) was extracted in a 500 mL round bottomed flask with 300 mL of methanol. The reflux time for each solvent was 30 cycles. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator (Chowdhury *et al*., 2004).

Experimental animals: Wistar rats (130-180 g) of either sex, obtained from Institute of Animal Health and Vet. and Biologicals, Rasalpura, Mhow, 453 446 (Madhya Pradesh, India) (Reg. no. 5007/SAS/2006-07) were kept in the departmental animal house at 26±2°C and relative humidity 44-56% light and dark cycles of 10 and 14 h, respectively. Animals were provided with standard rodent pellet and the food was withdrawn 18 h before the experiment through water was allowed *ad libitum*. Principles of laboratory animal care guidelines were followed and prior permission was sought from the Institute Animal Ethics Committee for conducting the study (Reg. no. - 1252/AC/09/CPCSEA).

Pylorus ligation induced ulcer: The animals were fasted for over night before pylorus ligation with water *ad libitum* (Despande and Shah, 2003; Nguelcfack *et al*., 2005; Barrelli and Izzoa, 2000). Under light ether anesthesia, the abdomen was opened by midline incision process. The pyloric portion of the stomach was slightly lifted out and ligated avoiding damage to its blood supply. *Nerium indicum* flowers extract was administered before pylorus ligation. The stomach was placed back carefully and the abdominal wall was closed with sutures. Animals were sacrificed 6 h after pylorus ligation. The stomachs were isolated and the content of the stomachs were collected and centrifuged. The volume of the gastric juice was measured and this was used for estimation of free acidity and total acidity. About 1 mL of centrifuged

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and filtered gastric secretion was titrated against 0.1 N Sodium hydroxide using Topfers reagent as indicator for determination of free acidity and 1% phenolphthalein as indicator for combined acidity. The sum of the two titrations was total acidity. The stomach was open along with the greater curvature and examine for ulcers. The ulcer index and acidity was determined following these formulas given below. Formula for calculating ulcer index:

$$U.I. = \frac{\text{Arithmetic mean of intensity in a group} + \text{No. of ulcer positive animals}}{\text{Total no. of animals}} \times 2$$

Determination of acidity:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1\ \text{N}} \times 100\ \text{mEq/L/100 g}$$

Indomethacin induced ulcer: Wistar rats either sex were used for experiment (Mahendran *et al*., 2002; Okokon and Nwafor, 2009; Maia *et al*., 2005). They are divided into four groups of six animals each. Food was withdrawn 18 h and water 1 h before the experiment. Group I (control) received only indomethacin (20 mg kg^{-1}) group II (reference or standard) received cimetidine (20 mg kg^{-1}) and group III and IV were pretreated with *Nerium indicum* flowers extract (500 and 1000 mg kg^{-1}). About 30 min later, groups III-IV were administered with indomethacin. About 4 h after indomethacin administration, animals were killed by decapitation method. The stomachs were removed and open along the grater curvature. Macroscopic examination was carried out with a hand lens and the presence of lesion was scored. Scoring of ulcer is shown in Table 1.

Table 1: Results of macoroscopic examination of ulcer

Observation	Scores
Normal stomach	0.0
Red coloration	0.5
Spot ulcer	1.0
Hemorrhagic streak	1.5
Ulcers	2.0
Perforation	3.0

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

$$\text{Protective}(\%) = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Statistical analysis: The values are represented as mean±SEM and statistical significance between treated and control groups was analyzed using of one way ANOVA followed by Dunnett's test where $p<0.001$ was considered statistically significant.

RESULTS AND DISCUSSION

The pylorus ligation induced ulcer was used to study the effect on gastric secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach that produces ulcers. Agents that reduce secretion of gastric aggressive factors such as acid and pepsin (antisecretory) and/or increase secretion mucin (cytoprotective) are effective in reducing development of gastric ulcers in this model. Flowers extract of *Nerium indicum* showed a significant reduction in ulcer index when compared to control ($p<0.001$). The both dose of flowers extract of *Nerium indicum* (500 and 1000 mg kg^{-1}, p.o.) showed a significant reduction in free acidity and total acidity ($p<0.001$) when compared to control. Results are shown in Table 2. Table 3 shows the results obtained with experimental model of indomethacin induced acute

Table 2: Effect of methanolic flowers extract of *Nerium indicum* in pylorus ligation induced ulcer

Parameters	Control	Standard	T_1 (500 mg kg^{-1})	T_2 (1000 mg kg^{-1})
Ulcer index	11.83±0.472	4.58±0.238***	5.75±0.214***	4.75±0.170***
Protection (%)	-	61.28	57.39	59.84
pH of gastric juice	2.9±0.6000	4.3±0.149***	3.9±0.881***	4.1±0.170***
Gastric vol. (mL/100 g)	8.1±0.2970	3.9±0.263***	5.3±0.288***	4.5±0.183***
Free acidity (meq/L/100 g)	78.46±0.570	37.58±0.640***	58.0±0.735***	43.45±0.476***
Total acidity (meq/L/100 g)	96.43±0.224	52.43±0.310***	69.16±0.581***	58.15±0.220***

Values are express as mean±SEM of 6 observations, statistical comparisons as follows: significant at ***$p<0.001$ compared to control group

Table 3: Effect of methanolic flowers extract of *Nerium indicum* in indomethacin induced ulcer

Groups	Treatment	Ulcer index	Protection (%)
I	Control (Indomethacin 20 mg kg^{-1})	13.91±0.427	-
II	Cimetidine (20 mg kg^{-1})	5.33±0.380***	61.68
III	*N. indicum* flowers extract (500 mg kg^{-1})	8.08±0.300***	41.91
IV	*N. indicum* flowers extract (1000 mg kg^{-1})	5.91±0.300***	57.51

Values are express as mean±SEM of 6 observations, statistical comparisons as follows: significant at ***$p<0.001$ compared to control group

gastric ulceration in rats. Methanol extract of *Nerium indicum* at dose 1000 mg kg^{-1} body weight demonstrated reduction mean ulcer score when compared to the animals not treated with extract (control). The results of experimentally induced ulceration with indomethacin showed that methanol flowers extract of *Nerium indicum* cause decrease ulcer score when compared to the control. This was a significant. This suggest that methanolic flowers extract of *Nerium indicum* antiulcer effect is likely mediated which might be similar to that of cimetidine which equally reduced the severity of gastric lesions developed by indomethacin in this study. Cimetidine is a know stable analogue of PGE_2. This drug inhibits the gastric acid secretion, both basal and that occurring in response to food and also increase the secretion of mucus and bicarbonate.

CONCLUSION

The results suggest that the flowers extract of *Nerium indicum* Mill. possesses antiulcer effect. It prevented the development of gastric ulcers induced by pylorus ligation and indomethacin.

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