

## ANTIULCER ACTIVITY OF METHANOLIC LEAVES EXTRACT OF *NERIUM INDICUM* MILL.

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### ABSTRACT

The objective of present study is to evaluate the anti ulcer activity of methanol extract of leaves of *Nerium indicum*. The methanol extract of *N. indicum* was investigated for its anti ulcer activity against plus pylorus ligation induced gastric ulcer and indomethacin induced ulcer in rats. The antiulcer activity was assessed by determining and comparing gastric volume, free acidity, total acidity, pH and ulcer inhibition in plus pylorus ligation induced gastric ulcer model and mean score value of ulcer inhibition in indomethacin induced ulcer model. A significant antiulcer activity was observed in all the models. Pylorus ligation model showed significant ( $P < 0.001$ ,  $P < 0.05$ ) reduction in gastric volume, free acidity and ulcer index as compared to control. Also extract showed 69.63 % ulcer inhibition in indomethacin induced ulcer. This present study indicates that *Nerium indicum* leaves extract have potential anti ulcer activity in the two models tested.

**KEY WORDS:** Indomethacin, pylorus ligation, ulceration, ranitidine, *Nerium indicum*.

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### INTRODUCTION

*Nerium indicum* Mill. (Family: Apocynaceae) it is an ever green plant with a long history of traditional medicinal uses in many countries in the world, especially tropical and subtropical countries<sup>[1]</sup>. The plant has been used for treatment of several diseases. The plant in folk remedies to include the use as cardiogenic, diaphoretic, diuretic (promotes excretion), emetic and expectorant<sup>[2]</sup>. Decoction of leaves has been applied externally in the treatment of scabies and to reduce swellings<sup>[3]</sup>. The root is a powerful resolvent (power to disperse inflammatory). The oil prepared from the root bark is used in the treatment of leprosy and skin diseases of scaly nature<sup>[4]</sup>. The whole plant is believed to have anti-cancer properties<sup>[5-6]</sup>. The

pounded leaves and bark are used as an insecticide<sup>[7]</sup>, rat poison and parasiticide<sup>[8]</sup>. The present study was undertaken to study the effect of methanolic leaves extract of *Nerium indicum* on pylorus ligation and indomethacin induced ulcer in rats.

### MATERIALS AND METHODS

#### Plant material

The fresh leaves of *Nerium indicum* Mill was 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 was deposited at Department of Botany, M.V.M. College, Bhopal.

### Preparation of extract

Leaves were shade dried and powdered mechanically. The powdered plant material (100 g) was repeatedly extracted in a 500 mL round bottomed flask with 300 mL solvents of increasing polarity starting with petroleum ether and methanol. 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.

### Experimental animals

In bread Wistar albino rats (180 to 200 g) and Wistar mice (25-30 g) of either sex obtained from Institute of Animal Health and Vety. and Biologicals, Rasalpara, Mhow, 453 446 (Madhya Pradesh, India) (Reg. No. 5007/SAS/2006-07) were used for study. The animals were maintained in polypropylene cages of standard at a temperature of  $28 \pm 1^\circ\text{C}$  and standard 12 hour: 12 hour day / night rhythm. The animals were fed with standard rodent pellet diet and water ad libitum. Prior to the experiment, the animals were acclimatization to the laboratory condition.

### Acute toxicity study

The acute toxicity for methanolic extracts of *Nerium indicum* was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 423) method of CPCSEA (Reg. No. - 1252/AC/09/CPCSEA) was adopted for toxicity studies.

### Indomethacin induced ulcer<sup>[9-11]</sup>

Wistar rats weighing 150–200 g were used for experiment. They are randomized

into four groups of six animals each. Food was withdrawn 18 h and water 1h before the experiment. Group I (control) received only indomethacin (20 mg/kg.) group II (reference or standard) received ranitidine (50 mg/kg.) and groups III & IV were pretreated with *Nerium indicum* leaves extract (250 and 500 mg/kg.). 30 minute later, groups III-IV were administered with indomethacin. Four hours after indomethacin administration, animals were killed by decapitation method. The stomachs were removed and open along the greater curvature. Macroscopic examination was carried out with a hand lens and the presence of lesion was scored. Scoring of ulcer will be made as follows:<sup>[12]</sup>

Normal stomach.....	(0)
Red coloration .....	(0.5)
Spot ulcer.....	(1)
Hemorrhagic streak.....	(1.5)
Ulcers .....	(2)
Perforation .....	(3)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows: [13]

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

### Pylorus ligation induced ulcer<sup>[14-17]</sup>

The animals were fasted for over night before pylorus ligation with water ad libitum. 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* leaves 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. 1ml of centrifuged and filtered gastric secretion was titrated against 0.1N 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 was determined as mentioned above.

$$\text{Acidity} \equiv \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1N} \times 100 \text{ mEq/l/100g.}$$

### Statistical analysis

The values are represented as mean  $\pm$  S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, Followed by Dunnett's test where  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$  was considered statistically significant.

### RESULTS & DISCUSSION

In indomethacin-induced model, pretreatments of rats with *Nerium indicum* leaves extract rendered a dose dependent protection from indomethacin induced ulceration. When compared to the ulcer control animals. The results were tabulated in Table 1.1. The *Nerium indicum* leaves extract produced

significant reduction in ulcer index and percentage of ulcer protection. The high dose of leaves extract of *Nerium indicum* (500 mg/kg) was more effective in reducing ulcer index (4.833) and percentage of ulcer protection (69.63 %) than lower dose (250 mg/kg) of leaves extract of *Nerium indicum* (5.416) and (65.97%). Ranitidine has also effective producing reduction of ulcer index and percentage of ulcer protection. In pyloric ligation induced ulcer, effect of *Nerium indicum* leaves extract on gastric volume, free acidity, total acidity and ulcer in pylorus ligated rats were studied. *N. indicum* leaves extract reduced the volume of gastric juice by the control rats by 4.1mL. and 3.6 mL. respectively. The free acidity was reduced by 60.6 and 43.81% respectively. The dose 500 mg/kg showed maximum ulcer protection of 67.45 %. The values are shown in table 1.2.

### CONCLUSION

The effect of *Nerium indicum* leaves extract on gastric lesions induced by indomethacin can be observed in (Table 1.1). The percentage of ulcer protection 65.97 and 69.63% which was observed in the doses of 250 and 500 mg/Kg. The effect of *Nerium indicum* leaves extract on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The ulcer index is determined 4 hours after pylorus ligation. The *Nerium indicum* extracts and ranitidine significantly decreased the total acidity and free acidity; this suggests that it having an antisecretory effect.

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**Table – 1.1** Effect of *Nerium indicum* leaves extract in indomethacin induced ulcer

Group	Treatment	Ulcer index	% protection
I	Control (Indomethacin 20 mg/kg.)	15.916 ± 0.624	—
II	Ranitidine (50 mg/kg.)	4.916 ± 0.351 ***	69.11 %
III	<i>N. indicum</i> leaves extract (250 mg/kg.)	5.416 ± 0.200***	65.97 %
IV	<i>N. indicum</i> leaves extract (500 mg/kg.)	4.833 ± 0.494***	69.63%

Values are express as mean ± SEM of 6 observations, statistical comparisons as follows:

significant at \*\*\*P < 0.001 compared to control group.

Formula for calculating ulcer index:-

U.I. = Arithmetic mean of intensity in a group + No. of ulcer positive animals / total No. of animals x 2

**Table – 1.2** Effect of *Nerium indicum* leaves extract in pylorus ligation induced ulcer

Parameters	Control	Standard	T <sub>1</sub> (250 mg/kg)	T <sub>2</sub> 500 mg/kg)
Ulcer index	14.083 ± 0.676	4.333 ± 0.527***	5.666 ±0.527***	4.583 ±0.860***
% protection	—	69.23 %	59.76 %	67.45 %
pH of gastric juice	2.7 ± 0.079	4.5 ± 0.130 ns	3.1 ± 0.216	4.0 ± 0.291***
Gastric vol. (mL. /100 g.)	9.8 ± 0.352	2.9 ± 0.154***	4.1 ± 0.265***	3.6 ± 0.076***
Free acidity (meq/l/100g)	80.35± 0.254	36.73± 0.194***	60.6± 0.212***	43.81± 0.224***
Total acidity (meq/l/100g)	102.43± 0.224	62.43± 0.230***	76.16± 0.291***	63.15± 0.295***

Values are express as mean ± SEM of 6 observations, statistical comparisons as follows:

significant at \*\*\*P < 0.001, ns P>0.05 compared to control group