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ORIGINAL RESEARCH PAPER

# Evaluation of Antiulcer Activity of Leaf Extracts of *Laurus nobilis Linn*

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**Key words**
*Laurus nobilis Linn* (Lauraceae), Antiulcer activity, Indomethacin

**Abstract**
An ulcer is a craterlike lesion in a membrane while peptic ulcer is an excoriated area of stomach or intestinal mucosa. It usually occurs at site where the mucosal epithelium is exposed to aggressive factors. It is one of the major gastrointestinal disorders which occur due to an imbalance between aggressive factors (acid, pepsin and H. pylori) and the defensive factors (gastric mucus and bicarbonate secretion, prostaglandins). The present investigation was designed to determine the antiulcer effect of methanolic extract of leaves of *Laurus nobilis* Linn in indomethacin induced ulcer model. The leaf extract [methanol (70%) and acetone (30%)] *Laurus nobilis* Linn was prepared and subjected to acute toxicity as per CPCSEA guideline no.420. Two doses, i.e., 150 and 300 mg/kg, were selected for further study. In indomethacin induced ulcer model, the parameters studied were ulcer score, gastric wall mucus, hexosamine, catalase and GSH. The control group showed significant induction as compared to vehicle treated group. ($P<0.05$). At the dose of 300 mg/kg, the extract showed significant protection as compared to the control group. This antiulcer activity of *Laurus nobilis* Linn may be due to individual effect of alkaloids, flavonoids, glycosides, tannins and phenolic compounds or may be synergistic effect.

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## INTRODUCTION

The word peptic means that the cause of the problem is due to acid. An ulcer is a craterlike lesion in a membrane while peptic ulcer is an excoriated area of stomach or intestinal mucosa. It is one of the major gastrointestinal disorders which occur due to an imbalance between aggressive factors (acid, pepsin and H.pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins) factor.[1,2] Number of drugs including, proton pump inhibitors, prostaglandins analogs, $H_2$ receptors antagonist and cytoprotective drugs.[3] Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy. Hence, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules, which afford better protection and decrease the incidence of relapses.[4] Several natural drugs have been reported to poses antiulcer activity. The present study is to demonstrate safety and efficacy of the leaf extract of *Laurus nobilis Linn* as antiulcer agent. *Laurus nobilis Linn* (Family: Lauraceae) is an evergreen shrub, or more rarely a tree attaining a height of 15-20 m. The smooth bark may be olive green or reddish evergreen tree originated in Asia and spread to the Mediterranean and other countries with suitable climate. The luxurious, evergreen leaves are simple, alternate with short stalks elliptical lanceolated with slightly wavy edges. The leaf size ranges from 2.5 to 7.5 cm in length and 1.6 to 2.5 cm in breadth. The leaves give aroma on rubbing. The flowers are small, yellow in color, unisexual and appear in clusters. The fruits (berries) are cherry-like, succulent, purple to black in color, ovoid, coarsely wrinkled and contain a single seed with loose kernel.[5,6] On the literature review it was found that the leaves are used in the antioxidant, wound healing, anticonvulsant, antibacterial astringent, aphrodisiac, antispasmodic (relieves spasm and cramps) carminative, decongestant.[7,8]

## MATERIALS AND METHODS

### Collection of Plant Material

The leaves of *Laurus nobilis Linn. (Lauraceace)* was collected locally from Bhopal, India, in the month of October, 2009. The leaves were identified and authenticated by Dr Zia Ul Hasan, Assistant Professor, Department of Botany, Safia College of Science, Bhopal, (Voucher specimen No. 146/Bot/Safia/2010).

### Preparation of Extract

The leaves were dried in shade and powdered at Pinnacle Biomedical Research Institute (PBRI), Bhopal. The powdered plant material was weighed and subjected for extraction. Cleaned leaves were coarsely powdered in mixer grinder. The powered leaves were weighed (400 g), defatted with petroleum ether by cold maceration at room temp for 7 days in a jar. The defatted material was subjected to extraction using acetone and methanol (30:70) for 7 days. The filtrate was concentrated on water bath at 50 °C.

### Phytochemical Investigation

Phytochemical investigation of the leaf extract of *Laurus nobilis Linn*. showed the presence of alkaloids, glycosides, phytosterols, tannins, phenolic compounds and flavonoids.[9,10]

### Animals

Albino rats of Wistar strain of either sex weighing between 150-200 g were used. They were housed in standard cages at room temperature (25±2 °C) and provided with food and water *ad libitum*. The experiment was conducted as per the permission of Institutional animal ethical committee (IAEC) of PBRI (No. 1283/C/09/CPCSEA). All conditions were maintained according to CPCSEA norms.

### Drugs and Chemicals

Ulcer inducing drug indomethacin (Brand name: Indocap, Manufacturer: Jagsonpal pharmaceutical Ltd) and ulcer protective drug Ranitidine (Brand name: Aciloc-150, Manufacturer: Cadila) were purchased from retail medical store. All other chemicals used in this study were obtained commercially and were of analytical (AR) grade.

### Acute Toxicity Studies

The acute toxicity study was carried out according OECD (Organization for Economic Cooperation and Development) 420 guideline which is based on a stepwise procedure with the use of a minimum number

of animals per step. Wistar rats of either sex (150-200 g) were used for this study. Group of 4 rats were fasted for 24 h, after which, the test drug was administered to them by oral route in the range of 100-3000 mg/kg body weight. The animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h for mortality and general behavior.

## Selection of Dose

As the extract was found to be safe up to the dose of 3000 mg/kg, the 1/10$^{th}$ and 1/20$^{th}$ of NOAEL, i.e., 3000 mg/kg, were selected as doses for present experimental work.

## Antiulcer Activity by Indomethacin Induced Ulcer Model

Albino rats of either sex weighing 150-200 g were divided into 5 groups containing six animals in each group (Table 1) and fasted for 36 h with water ad libitum. Group I served as Vehicle treated and Group V served as standard antiulcer drug (Ranitidine 50 mg/kg b.w, p.o.). Groups III, IV received extract dose. Indomethacin (30 mg/kg b.w, p.o.) was administered to the animal groups II to V, 60 min after the respective treatments. Animals were sacrificed by cervical dislocation after 5 h. The stomach was opened along greater curvature and observed for ulcer and other biochemical parameters like gastric wall mucus, hexosamine, catalase, GSH (glutathione).[10,11]

Table 1 Animal groups used for antiulcer activity of *Laurus nobilis Linn*

| Group No | Treatment | No. of Animals |
|---|---|---|
| **I** | Vehicle treated (DMSO) | 6 |
| **II** | Vehicle + Indomethacin | 6 |
| **III** | Extract (150 mg/kg) + Indomethacin | 6 |
| **IV** | Extract (300 mg/kg) + Indomethacin | 6 |
| **V** | Standard drug + Indomethacin | 6 |

## Histopathological Evaluation

Gastric tissue samples were immersed in a 10% formalin solution for histopathological examination following the assessment of ulcer score. The processed tissue was embedded in paraffin block and sections about 5 µm thick were cut using an optical rotary microtome. These sections were stained with haematoxylin-eosin and mounted with Canada balsam. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema, and erosions using an arbitrary scale for severity assessment of these changes.[12]

## Ulcer Score

The following arbitrary scoring system (Table 2) was used to grade the incidence and severity of lesion.[13,14]

Table 2 Scale for grading the severity of ulceration

| Ulcer Score | Inference |
|---|---|
| **0** | Normal |
| **1** | Red coloration |
| **2** | Spot ulcers |
| **3** | Haemorrhagic streaks |
| **4** | Ulcers > 3 but < 5 |
| **5** | Ulcers > 5 |

The Mean Ulcer Index UI was calculated using following formula:

$$UI = U_N + U_S + U_P \times 10^{-1}$$

Where, $U_N$ is the average of number of ulcer per animal, $U_S$ is the average of severity score, and $U_P$ is the percentage of animals with ulcers.

## Evaluation of Biochemical Parameters

### *Estimation of Gastric Wall Mucus (Mucin)*

The glandular segment from stomach was transferred immediately into 10 mL of 0.1% w/v Alcian blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate pH 5). Dye complexed with the gastric wall mucus was extracted with 10 mL of 0.5 M magnesium chloride by shaking intermittently for 1 min at 30 min intervals for 2 h. Blue extracts (4 mL) were shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 4000 rpm for 10 min and the absorbance of the aqueous layer was recorded at 580 nm.[15]

### *Hexosamine Assay*

The gastric tissue weighed and prepared homogenate in 3 mL HCl (3 N). This homogenate was neutralized with 3 N NaOH and diluted to 10 mL with distilled water. An aliquot of 1 mL was taken and 1 mL of acetyl acetone solution was added to it, mixed well and heated on a boiling water bath for 15 min, avoiding evaporation. After cooling, 5 mL of 95% ethanol and 1 mL Ehrlich's reagent were added. The mixture was diluted to 10 mL with 95% ethanol, allowed to stand for 30 min, and its absorbance was measured at 530 nm.[16]

### *Catalase Assay*

The gastric tissue was weighed and the homogenate was prepared (10%w/v) with 0.15 M Tris buffer (pH 7 by HCl). The gastric tissue homogenate was centrifuged at 1500 rpm at 4 °C for 15 min. The supernatant (100 µL) was added to cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 mL of freshly prepared 30 mM $H_2O_2$. Decrease in absorbance was read at 240 nm for 3 min at intervals of 30 sec. The rate of decomposition of $H_2O_2$ was measured spectrophotometrically from changes in absorbance at 240 nm.[17]

### *GSH (Glutathione) Assay*

The gastric tissue was weighed and the homogenate was prepared (10%w/v) with 0.15 M Tris buffer (pH 7 by HCl). The gastric tissue homogenate was centrifuged at 1500 rpm at 4 °C for 15 min. The supernatant (40 µL) was mixed with 400 µL Tris and 3360 µL water. Then, 0.2 mL (200 µL) DTNB solution was added and the absorbance was measured at 412 nm.[17]

## RESULTS AND DISCUSSION

The developed and developing countries are facing an explosive increase in the incidence of peptic ulcer day to day. One cause of peptic ulcer is bacterial infection, but some ulcers are caused by long-term use of Nonsteroidal anti-inflammatory agents (NSAIDs), like aspirin and ibuprofen. There may be change in life style like drinking, smoking stress, spicy, food also causes ulcers.[18,19] The extract was found to be semisolid, dark brown in colour with bitter taste. This extract was not soluble in water and soluble in DMSO. Thus, DMSO was selected as the solvent for all further pharmacological studies.

Phytochemical investigation of extract revealed that the extract was rich in alkaloids, glycosides, phytosterols, tannins, phenolic compounds and flavonoids. There has been considerable pharmacological research into the antiulcer activity of alkaloids, glycosides, flavonoids, tannins, phytosterols and phenolic compounds.

In the acute toxicity study, the extract was found to be safe up to the dose of 3000 mg/kg. Hence, the doses selected for present study were 300 and 150 mg/kg.

The extract was investigated for its antiulcer potential against indomethacin 30 mg/kg induced ulcer. 150 mg/kg and 300 mg/kg dose of extract and 50 mg/kg dose of ranitidine were used in the present investigation.

Indomethacin significantly caused the induction of ulcer ($P<0.05$). Histopathological changes on indomethacin induced ulcer model showed the degeneration, hemorrhage, edematous appearance of the gastric tissue, whereas, the *Laurus nobilis* leaf extract (150 and 300 mg/kg) and ranitidine (50 mg/kg) treated groups showed regeneration and prevented the formation of hemorrhage and edema Fig 1 and 2.

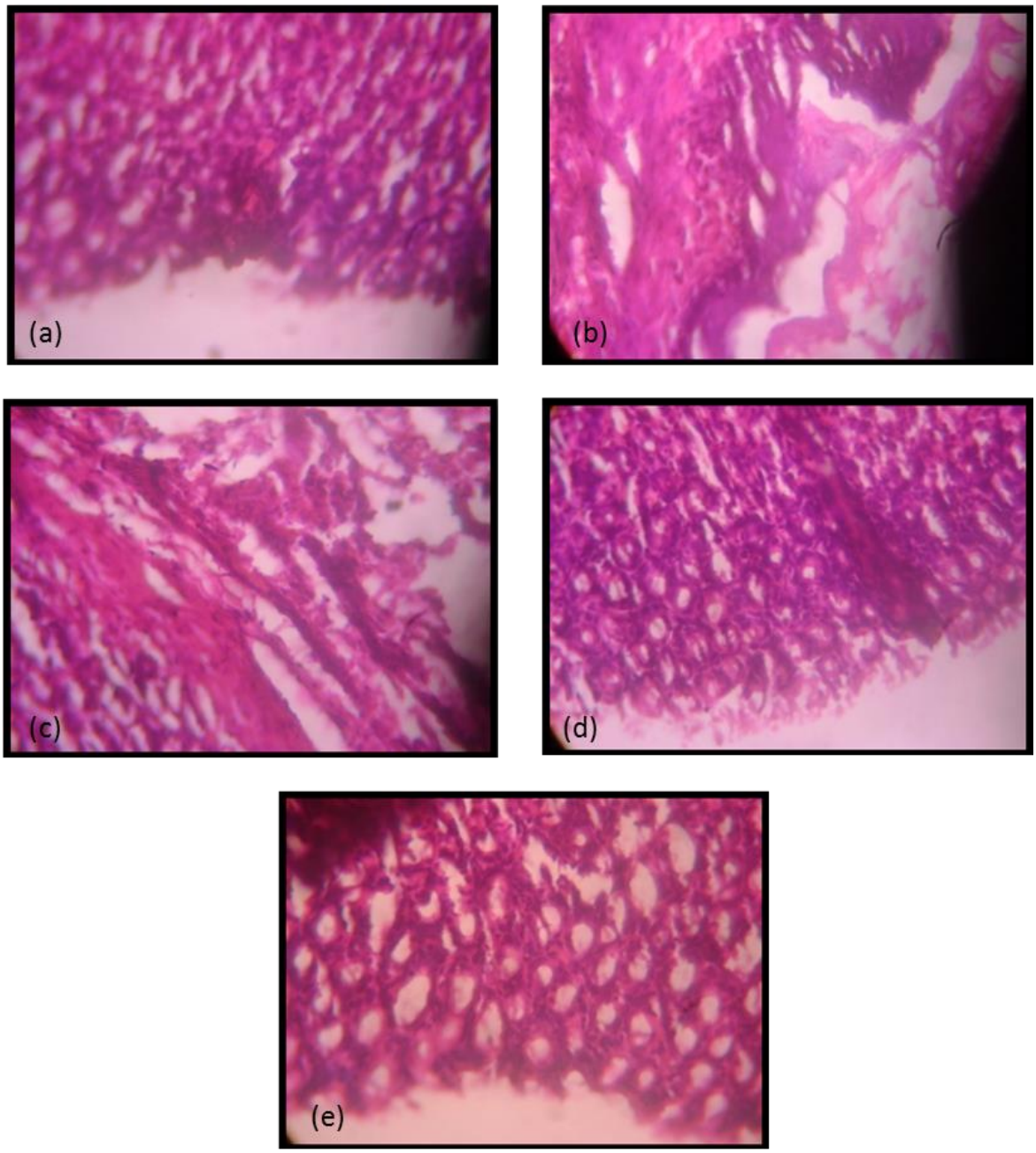


Fig 1 Microscopy and histopathological changes in rat stomach in indomethacin induced ulcer model; vehicle treated group (a), control group (b), extract treated (150 mg/kg) group (c), extract treated (300 mg/kg) group (d) and standard (ranitidine) treated group (e)

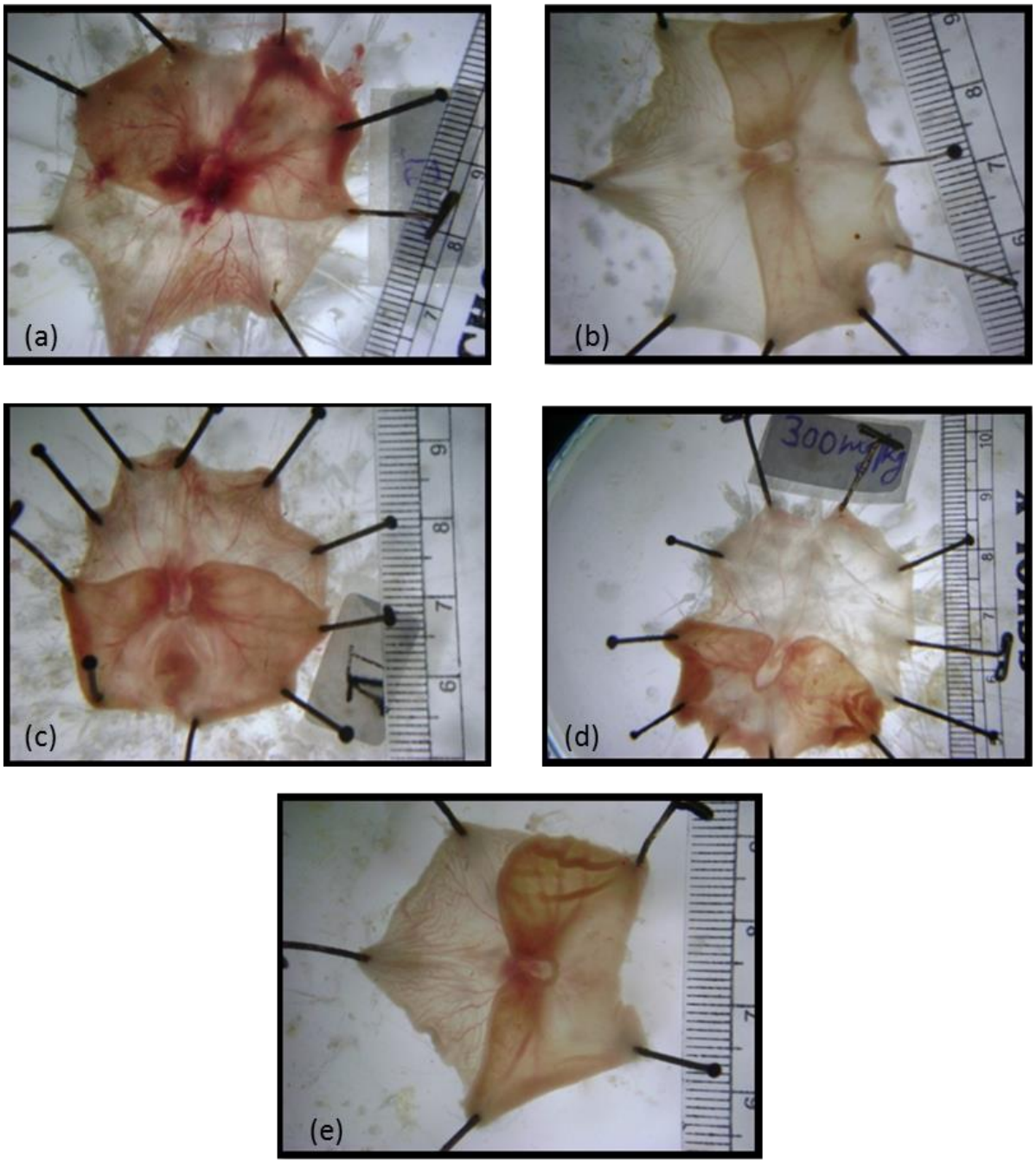


Fig 2 Macroscopy of rat stomach and ulcer score on Indomethacin induced ulcer model; control group (a), vehicle treated group (b), extract treated (150 mg/kg) group (c), extract treated (300 mg/kg) group (d) and standard (ranitidine) treated group (e)

The ulcer scores for different groups of treated animals are given in Table 3. The extracts significantly decreased ulcer score at both the dose of 150 mg/kg and 300 mg/kg ($P<0.05$). Treatment with extract at 150 mg/kg and 300 mg/kg significantly increased gastric wall mucosal content ($P<0.05$) (Table 4). Indomethacin significantly decreased the level of hexosamine. Extract at both the doses, i.e., 150 mg/kg and 300 mg/kg, increased this decreased level of hexosamine significantly as compared to indomethacin treated group ($P<0.05$) (Table 5).

Table 3 Effect of *Laurus nobilis* leaf extract on ulcer score in indomethacin induced ulcer model

| S.No. | Treatment | Dose(mg/kg) | Ulcer Score (mean±SEM) |
|---|---|---|---|
| **1.** | Vehicle | - | 1.67 ± 0.333 |
| **2.** | Control | 30 mg/kg b.w. | 4.00 ± 0.365 [a] |
| **3.** | LN 150mg/kg b.w. | 150 mg/kg b.w. | 3.50 ± 0.223 [b*] |
| **4.** | LN 300mg/kg b.w. | 300 mg/kg b.w. | 2.17 ± 0.401 [b] |
| **5.** | Standard(Ranitidine) | 50 mg/ kg b.w. | 1.16 ± 0.166 [b] |

a – significant induction as compared to vehicle treated group ($p<0.05$)
b – significant protection as compared to control group ($p<0.05$)
b* - significant protection as compared to control group ($p<0.05$)

Table 4 Effect of *Laurus nobilis* leaf extract on gastric wall mucous in indomethacin induced ulcer model

| S.No. | Treatment | Dose(mg/kg) | Gastric wall mucous (mean±SEM) |
|---|---|---|---|
| **1.** | Vehicle | - | 4.39 ± 0.074 |
| **2.** | Control | 30 mg/kg b.w. | 1.96 ± 0.051 [a] |
| **3.** | LN 150mg/kg b.w. | 150 mg/kg b.w. | 2.98 ± 0.340 [b] |
| **4.** | LN 300mg/kg b.w. | 300 mg/kg b.w. | 4.20 ± 0.067 [b] |
| **5.** | Standard(Ranitidine) | 50 mg/ kg b.w. | 4.24 ± 0.061 [b] |

a – significant induction as compared to vehicle treated group ($p<0.05$)
b – significant protection as compared to control group ($p<0.05$)

Table 5 Effect of *Laurus nobilis* leaf extract on hexosamine in indomethacin induced ulcer model

| S.No. | Treatment | Dose(mg/kg) | Hexosamine (mean±SEM) |
|---|---|---|---|
| **1.** | Vehicle | - | 279.60 ± 9.409 |
| **2.** | Control | 30 mg/kg b.w. | 80.27 ± 8.993 [a] |
| **3.** | LN 150mg/kg b.w. | 150 mg/kg b.w. | 140.00 ± 16.15 [b] |
| **4.** | LN 300mg/kg b.w. | 300 mg/kg b.w. | 209.10 ± 16.95 [b] |
| **5.** | Standard(Ranitidine) | 50 mg/ kg b.w. | 271.30 ± 9.211 [b] |

a – significant induction as compared to vehicle treated group ($p<0.05$)
b – significant protection as compared to control group ($p<0.05$)

When the extract were investigated for its protection in this antioxidant aspect, it was found that the significantly declined level of catalase and GSH by indomethacin was significantly protected by the extract at both the dose levels ($P<0.05$) (Table 6).

## CONCLUSIONS

The leaf part of *Laurus nobilis Linn* belonging to the family Lauraceae was taken for the pharmacognostic and pharmacological studies. Exhaustive extraction of the leaf part was done with acetone-methanol. The

extract shown the presence of alkaloids, flavonoids, glycosides, phytosterols, tannins and phenolic compounds. The present study demonstrated that the leaf extract possess antiulcer activity, which might be due to any of these phytoconstituents or may be a synergistic effect.

Table 6 Effect of *Laurus nobilis* leaf extract on GSH in indomethacin induced ulcer model

| S.No. | Treatment | Dose(mg/kg) | GSH (mean±SEM) |
|---|---|---|---|
| **1.** | Vehicle | - | 1.576 ± 0.014 |
| **2.** | Control | 30 mg/kg b.w. | 0.857 ± 0.011 [a] |
| **3.** | LN 150mg/kg b.w. | 150 mg/kg b.w. | 1.057 ± 0.065 [b] |
| **4.** | LN 300mg/kg b.w. | 300 mg/kg b.w. | 1.440 ± 0.033 [b] |
| **5.** | Standard(Ranitidine) | 50 mg/ kg b.w. | 1.553 ± 0.011 [b] |

a – significant induction as compared to vehicle treated group ($p<0.05$)
b – significant protection as compared to control group ($p<0.05$)

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## DECLARATION OF INTEREST
It is hereby declared that this paper does not have any conflict of interest.

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