RESEARCH ARTICLE

# Evaluation of Anti-ulcer Activity of Methanolic Extract of Lagenaria Siceraria

Vivek Srivastava,* Priyanka Gupta, Deepika Sharma


## Abstract

**Introduction:** Ulcer is a common gastrointestinal disorder characterized by inflamed lesions of the mucosa and tissue that protect the gastrointestinal tract. These ulcers can develop when the imbalance occurs between the gastroprotective (mucus, bicarbonate and prostaglandins) and aggressive (acid, pepsin, bile salts and Helicobacter pylori bacteria). According to research, other species from genus Lagenaria show analgesic, anti-inflammatory, immunomodulatory, anti-hyperlipedemic, diuretic and anti-helmintic activities on the fruit of Lagenaria siceraria.

**Methods:** The anti-ulcer activity of methanolic extract of Lagenaria siceraria fruits was investigated in pylorus ligation, ethanol induced, Asprin induced and Cold-restraint stress induced ulcer models in wistar rats. In all models the common parameter determined was ulcer index.

**Results:** The extract (100 mg/kg & 200 mg/kg) showed significant ($P < 0.005$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that Lagenaria siceraria fruit extract have potential anti-ulcer activity in these models.

**Discussion:** MELS caused a significant decrease in the gastric volume, free acidity, and total acidity compared to the control group, indicating an antisecretory mechanism. Several scientific studies revealed that the phytoconstituents like flavonoids, tannins, terpenoids and saponin were responsible for gastro protective agents. Further studies are needed to find out their exact mechanism of action on gastric acid secretion and gastric cytoprotection.


**Keywords:** Asprin induced and Cold-restraint stress induced ulcer model, Ethanol induced, *Lagenaria siceraria*, Pylorus ligation, Ulcer index.

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## Introduction

Peptic ulcer disease once was a condition with great morbidity and even mortality now a days.[1,2] In the past operation was the only option to treat gastric ulcer but now this changed dramatically by the introduction of medical therapy[3,4] The most important step in the treatment of this chronic recurrent gastric disease was the discovery of H. Pylori and to inhibit its pathogenetic properties.[5-7] With anti-*Helicobacter pylori* therapy peptic ulcer disease could be treating definitely.[8,9]

Ulcer is a common gastrointestinal disorder and can be characterized by inflamed lesions of the mucosa and tissue that protect the gastrointestinal tract. Chronic alcohol intake, smoking, excessive stress, chronic usage of non-steroidal anti-inflammatory drugs and *H. pylori* bacterial infection are the crucial causes of peptic ulcer characterized by inflammation, mucosal bleeding and abdominal pain in patients. These ulcers can develop when the imbalance occurs between the gastroprotective (mucus, bicarbonate and prostaglandins) and aggressive (acid, pepsin, bile salts and *H. pylori* bacteria).[10] The goal of peptic ulcer treatment is to relieve pain, heal the ulcer and prevent recurrence of ulcer. Currently there is no cost-effective treatment or medicines that can full fill all these goals. Hence, in general, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being efficacious and safe.[11]

Department of Pharmacology, Uttarakhand Technical University, Dehradun, Uttarakhand, India

**Corresponding Author:** Vivek Srivastava, Department of Pharmacology, Uttarakhand Technical University, Dehradun, Uttarakhand, India, Email: vivek_srivastava16nov@yahoo.com.

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Lagenaria siceraria is one of the excellent fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health.[12] It is climbing herb,5 angled stem with stout, tendrils 2 fid leaves often 15cm in diameter,cordate,dentate,5 angular or 5 lobed, hairy on both surfaces, flower large, white, solitary, monocious or dioecious, fruit in large indehisant, bell shaped, seeds are smooth,1.6 to 2 cm, white with marginal groove. It is considered as laxative, cardiotonic, diuretic, bitter, emetic, lesson inflammation. These species shows presence of palmitic acid, palmitoleic acid and steraric acid and olecic acid. According to research and as other species from genus Lagenaria shows analgesic, anti-inflammatory, immunomodulatory, antihyperlipedemic,

diuretic and antihelmintic activities on the fruit of Lagenaria siceraria.[13,14]

## Materials and Methods

- *Plant material:* The fruits of Lagenaria siceraria were collected from the local market of Lucknow, and they were authenticated as Lagenaria siceraria (Bottle gourd) by Taxonomist of NBRI (Lucknow), and voucher specimen is submitted in NBRI for future reference.
- *Preparation of fruit extract:* The fresh ripe fruits were sliced using a home slicer and the slices obtained were shade dried. The dried coarsely powdered plant material was extracted with 99% methanol using rota vapour apparatus (BUCHI). The solvent was evaporated under vacuum, which gave semi-solid mass (24.49%w/w).
- *Phytochemical analysis:* The phytochemical screening of the extracts of the different seeds revealed phlobatannins, saponins, cardiac glycosides, phenols, alkaloids, flavonoids, terpenoids, deoxy-sugar, carbohydrates, and reducing sugars in varying quantities.[15]
- *Animals used:* Sprague-Dwaley rats of either sex weighing between 150–250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol for a study, and animals were maintained under standard conditions in an animal house approved by the Committee for Control and Supervision on Experiments on Animals (CPCSEA). Rats were procured from the animal house of the National Botanical Research Institute, Lucknow. The animals were housed in Polypropylene cages and maintained at 24°C ± 2°C under 12 hours light/ dark cycle and were fed *ad libitum* with a standard pellet diet and had free access to water. The animals were given a standard pellet diet supplied by Amrut India Ltd. The diet composition is protein 10%, Arachis oil 4%, Fibers 1%, Calcium 1%, Vitamin A 1000 IU/gm, and Vitamin D 500 IU/gm.

## Pyloric Ligation-Induced Ulcer Model

The experiment for inducing ulcers was conducted according to the techniques described earlier.[15] After the 21st day of drug administration, the rats were fasted for 24 hours, anesthetizing the animal to make a midline abdominal incision to ligate pylorus without causing any damage to its blood supply. The stomach was replaced carefully, and the abdominal wall was closed with sutures. The rats were deprived of water after the ligation period. Four hours after the procedure, the rats were sacrificed, and the stomachs were dissected out and cut open along the greater curvature to determine the ulcer index (UI).[16] The gastric content volume was measured after centrifugation, while the acidity was determined by titration with 0.01 N NaOH using Toppfer's reagent and phenolphthalein as indicators.[17] The percentage inhibition (PI) of the ulcer production was also calculated.[18]

Scoring of the ulcer will be made as follows Figure 1 shows the Macroscopically view of Pylorus ligation induced ulcer

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

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

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

### Aspirin-Induced Gastric Ulcer Model

The ulcer was induced by this method was according to the method described earlier.[19] Following 1 hour of administering the last dose of the test and control compounds, the rats were fasted for 24 hour and were administered aspirin in a dose of 200 mg/kg b.w. p.o. Four hours after aspirin induction, the animals were sacrificed, and their stomachs were dissected out. The UI and PI were determined as described in the previous model. Figure 2 shows the Macroscopic view of Asprin induced ulcer.

### Cold Restraint Stress-induced Ulcer Model

The experiment was conducted according to the method described earlier.[20] The test and control groups were deprived of food for 12 hours after administering the last dose of the drugs. The rats were then immobilized in a steel cage and placed at a temperature of 3–5°C for 3 hours, following which they were then euthanized by cervical dislocation. The ulcers were then examined on the dissected stomachs to determine the UI and PI described in the previous models. Figure 3 shows the macroscopic view of Cold restraint stress induced ulcer.

### Ethanol Induced Ulcer Model

The rats fasted for 24 hours before the experiment. After 1 hour of administration of methanolic extract of Lagenaria siceraria, ranitidine, and vehicle control treatment, 1ml of absolute ethanol (0.5 mL/100 g) was orally administered to each rat of every group. After 1 hour, the animal was sacrificed with an excess of anesthetic ether, and the stomach was opened along the greater curvature, cleared of residual matter with saline, and the inner surface was examined for the severity of ulceration.[21] Ulcer index and % ulcer protection were calculated. Figure 4 indicates Macroscopically view of ethanol induced ulcer.

## Results

The methanolic extract of Lagenaria siceraria in doses of 100 and 200 mg/kg b.w. caused a significant ($p < 0.05$) and dose-dependent decrease in the UI and an increase in the PI compared to the control group in all 4 models employed in the study. The ranitidine 20 mg/kg b.w. i.p. group showed

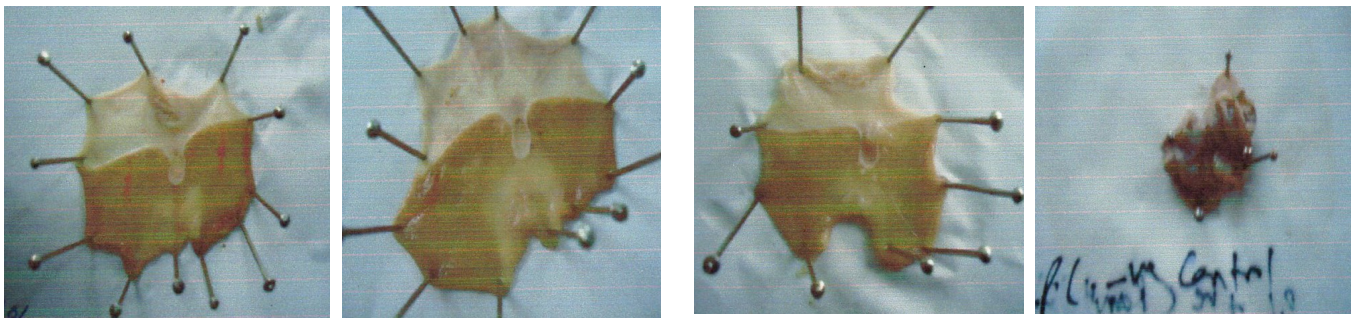
the highest PI & pH values in all 4 models employed in the study (Table 1–4). There was a significant rise in the pH with a reduction in the volume of gastric contents, free acidity, and total acidity in the AE-treated groups as compared to the control group (Table 1).

## Discussion

The cause of gastric ulcer is the increase in gastric acid (HCl) secretion, and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid.[22-24] MELS caused a significant decrease in the gastric volume, free acidity, and total acidity compared to the control group, indicating an antisecretory mechanism. The antisecretory activity could be due to the inhibition of the H + -K + -ATPase enzyme.[25,26] Pylorus ligation-induced ulcers are shown by auto-digestion of the gastric mucosa and breakdown of the gastric mucosal barrier, resulting in upper gastrointestinal lesions and ulcers. The pyloric ligation of the stomach causes the accumulation of gastric acid, which leads to the development of ulceration in stomach. The agents that decrease gastric acid secretion and increase mucus secretion effectively prevent the ulcers

**Table 1:** Effect of Lagenaria siceraria fruit extract on various parameters in pyloric ligation induced gastric ulcers

| *Group* | *Treatment* | *Ulcer index* | *Protection (%)* | *pH of gastric juice* | *Gastric juice (ml)* | *Free acidity (mEq/L)* | *Total acidity (mEq/L)* |
|---|---|---|---|---|---|---|---|
| I | Control (pyloric ligation) | 15.3 ± 1.3 | — | 2.2 ± 0.20 | 9.3 ± 0.20 | 97.8 ± 1.5 | 116.8 ± 0.23 |
| II | Ranitidine (50mg/kg) | 2.4 ± 0.04 | 84% | 4.9 ± 0.15 | 2.5 ± 0.18 | 33.8 ± 2.5 | 57.7 ± 1.4 |
| III | MELS (100mg/kg) | 3.6 ± 0.05 | 76% | 3.6 ± 0.20 | 4.2 ± 0.13 | 46.8 ± 1.3 | 67.7 ± 0.37 |
| IV | MELS (200mg/kg) | 3.1 ± 0.07 | 79% | 4.4 ± 0.18 | 3.8 ± 0.14 | 34.8 ± 1.1 | 61.2 ± 1.5 |

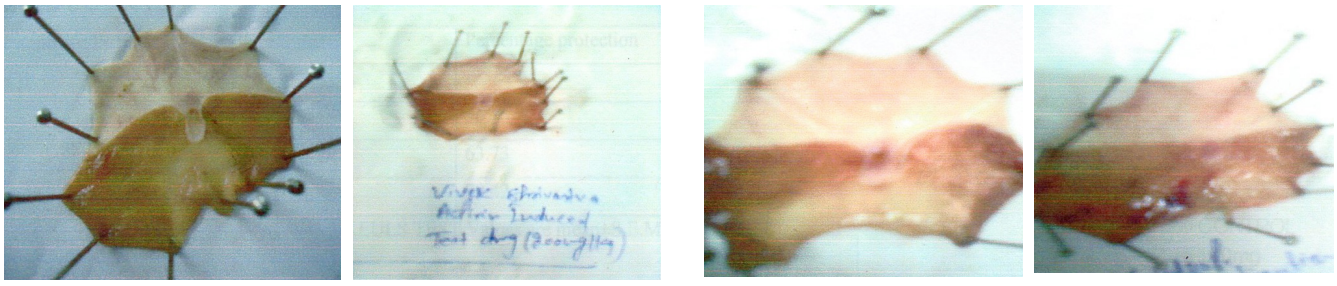


100 mg/Kg 200 mg/Kg Standard Control

**Figure 1:** Macroscopically view of Pylorus ligation induced ulcer

**Table 2:** Effect of Lagenaria siceraria fruit extract on various parameters in ethanol-induced gastric ulcers

| *Group* | *Treatment* | *Ulcer index* | *Protection (%)* | *pH of gastric juice* |
|---|---|---|---|---|
| I | Control (1 mL / 200g) | 12.475 ± 0.07 | — | 3.1 ± 0.20 |
| II | Ranitidine (50 mg/kg) | 3.5 ± 0.02* | 72% | 5.4 ± 0.09 |
| III | MELS (100 mg/kg) | 5.7 ± 0.03* | 54% | 3.8 ± 0.15 |
| IV | MELS (200 mg/kg) | 3.8 ± 0.07* | 69% | 4.9 ± 0.17 |

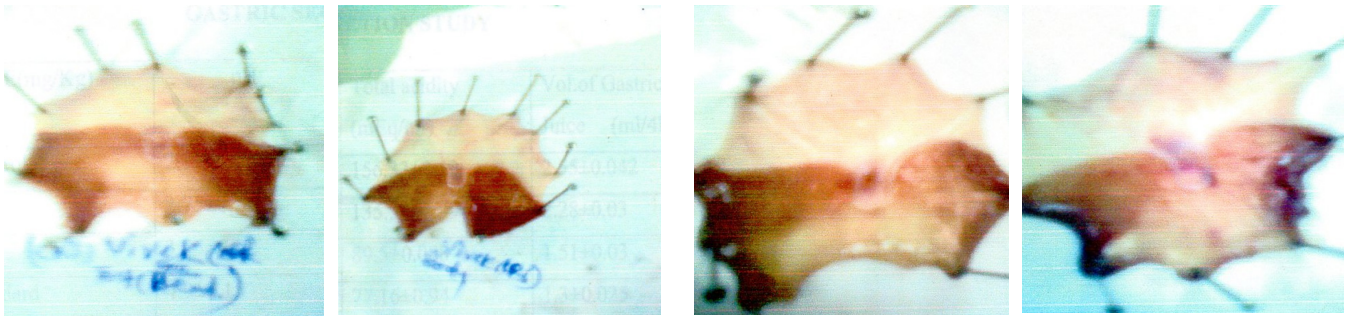


100 mg/kg 200 mg/kg Standard Control

**Figure 2:** Macroscopically view of Asprin induced ulcer

**Table 3:** Effect of Lagenaria siceraria fruit extract on various parameters in Asprin induced gastric ulcers

| *Group* | *Treatment* | *Ulcer index* | *Protection (%)* | *pH of gastric juice* |
|---|---|---|---|---|
| I | Control (20 mg/mL) | 12.33 ± .06 | – | 2.9 ± 0.20 |
| II | Ranitidine (50 mg/kg) | 2.7 ± 0.07* | 78% | 5.7 ± 0.08 |
| III | MELS (100 mg/kg) | 3.9 ± 0.04* | 68% | 3.9 ± 0.13 |
| IV | MELS (200 mg/kg) | 3.3 ± 0.06* | 73% | 4.9 ± 0.19 |

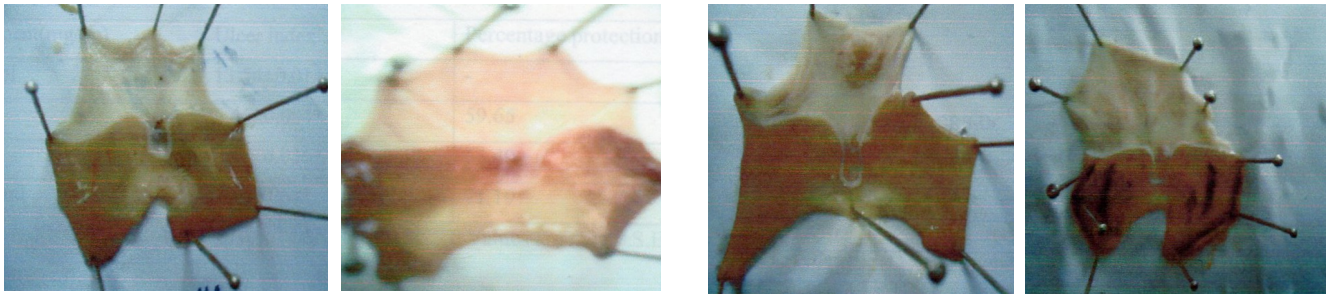


**100 mg/kg 200 mg/kg** **Standard Control**

**Figure 3:** Macroscopically view of Cold restraint stress induced ulcer

**Table 4:** Effect of Lagenaria siceraria fruit extract on various parameters in Cold restraint stress induced ulcers

| *Group* | *Treatment* | *Ulcer index* | *Protection (%)* | *pH of gastric juice* |
|---|---|---|---|---|
| I | Control | 13.54 ± 0.01 | — | 3.2 ± 0.20 |
| II | Ranitidine (50 mg/kg) | 2.46 ± 0.07* | 81% | 6.4 ± 0.08 |
| III | MELS (100 mg/kg) | 5.82 ± 0.08* | 57% | 4.2. ± 0.11 |
| IV | MELS (200 mg/kg) | 3.1 ± 0.06* | 77% | 5.9 ± 0.19 |



**100 mg/Kg 200 mg/Kg** **Standard Control**

**Figure 4:** Macroscopically view of ethanol induced ulcer

induced by this method. Like ranitidine, omeprazole acts as an anti-ulcer agent by antisecretory mechanism, inhibiting gastric secretion and pepsin activity.[27] In the present study, MELS prevents the ulcer may be by antisecretory and cytoprotective properties.

Ethanol is responsible for disturbances in gastric secretion, damage to the mucosal layer, alterations in permeability, gastric mucus depletion and free radical production. The generation of free radicals was produced by continuous release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol.[27] Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the hemorrhage and tissue injury. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric ulcer. This leads to cell death and exfoliation in the surface epithelium.[28] Alcohol can penetrate the gastric mucosa and causing cellular damage, which increases the permeability to sodium and water, leading to tissue injury. In the other hand, the accumulation of intracellular calcium causes the pathogenesis of gastric injury that leads to cell death and exfoliation of surface epithelium.[29] The present study observed that the MELS significantly reduced ethanol induced ulcer by cytoprotective action via an antioxidant effect. The MELS extract showed cytoprotection against the ethanol-induced ulceration by reducing the gastric acid

secretion or by increase of duodenal alkaline secretion or luminal prostaglandin level.[19] This study found that MELS established a cytoprotective action against ethanol-induced cellular damage in the gastric mucosa of rats. Cytoprotection of anti-ulcer drugs has been recognized due to the generation of prostaglandins.[30]

Aspirin is a non-steroidal anti-inflammatory drug that induces ulcers by inhibiting prostaglandin synthesis in the stomach by blocking the cyclooxygenase enzymes.[31] Non-steroidal antiinflammatory drugs also cause an inflammatory response increasing the reactive oxygen species in the gastric mucosa.[32] In the present study, MELS in all doses caused a significant reduction in the UI and improved PI, indicating a possible involvement of the prostaglandin pathway. Mechanistic studies measuring levels of prostaglandin E 2, myeloperoxidase, and proinflammatory cytokines (interleukin 8, tumor necrosis factor-α) could elucidate this reasoning.

Cold restraint causes both psychological and physical stress to the rats. The induced stress releases histamine in the stomach, which leads to increased acid secretion and decreased mucus production, ultimately leading to ulcers.[33] MELS caused a dose-dependent significant reduction in the UI in this model, suggesting the role of histamine in its mechanism, as suggested by a previous study.[34]

It has also been observed that MELS significantly and dose-dependently reduced the extent of gastric ulceration in pylorus ligated rats without affecting the gastric secretion or pepsin activity. The defense mechanism of mucus of gastric mucosa depends upon a balance between the synthesis and secretion of mucin constituents. MELS prevented the mucosal lesions induced by alcohol.[35] The modern approach towards a potent anti-ulcer activity of any agent involves maintaining a balance of controlling the synthesis, secretion, and metabolism of proteins, glycoproteins, and lipids to strengthen the mucosal integrity.[36]

Several scientific studies revealed that phytoconstituents like flavonoids, tannins, terpenoids, and saponin were responsible for gastroprotective agents. Previous studies have recommended that these above active compounds stimulate mucus, bicarbonate, and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in the gastrointestinal lumen.[37] Therefore, MELS possess anti-ulcer activity, may be due to the presence of flavonoids and terpenoids. Further studies are needed to determine their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

## Conclusion

The present study concluded that the anti-ulcer activity of MELS might be attributed to antisecretory, cytoprotective and antioxidant properties. MELS fruits possess anti-ulcer activity and possibly act via multiple mechanisms, including inhibition of the histamine-2 receptors/H + -K + -ATPase, prostaglandin modulation, or antioxidation. The Bioactivity-guided phytochemical screening of ESIL revealed the presence of flavonoids, and triterpenoids which may be responsible for the anti-ulcer effect.

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