Ulcer protective effect of *Leucas aspera* in various experimental ulcer models

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**Objective:** To investigate the ulcer protective effect of *Leucas aspera* (*L. aspera*) aerial parts in various experimental ulcer models.

**Methods:** Aerial parts of *L. aspera* were collected and extracted with methanol (70% v/v). Preliminary phytochemical screening and toxicity evaluation of methanolic extract of *L. aspera* were performed. Ulcer protective effect of methanolic extract of *L. aspera* was checked using various *in vivo* experimental ulcer models (indomethacin plus restraint model, swimming induced stress ulcer, serotonin induced gastric ulcer, cysteamine induced duodenal ulcer, ethanol induced gastric ulcer). Antioxidant and histopathological studies of gastric mucosa were conducted in ethanol induced ulcer model.

**Results:** Methanolic extract of *L. aspera* did not show any toxic reactions in both acute and short term toxicity studies. Moreover, methanolic extract of *L. aspera* showed powerful antisecretory and ulcer protective effect in all the tested ulcer models. Results of antioxidant and histopathological studies further confirmed the ulcer protective effect of methanolic extract of *L. aspera*.

**Conclusions:** The present study justifies the folkloric use of *L. aspera* in various gastric disorders. Further studies should be conducted to find the mechanism of action.

**Keywords**

*Leucas aspera*, Lansoprazole, Anti-ulcer, Anti-secretory, Antioxidant

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**I. Introduction**

*Leucas aspera* Willd. (*L. aspera*) belonging to the family Lamiaceae is widely distributed throughout India from the Himalayas down to Ceylon. It is an annual, branched, herb erecting to a height of 15–60 cm with stout and hispid acutely quadrangular stem and branched[11]. This herb is traditionally used as antipyretic, stimulant, expectorant, aperient, diaphoretic, insecticide and for various gastric problems. Phytochemical studies reported the presence of triterpenoids, oleanolic acid, ursolic acid and 3–sitosterol in the entire plant[2,3]. Aerial parts are reported to contain nicotine, stero,
cysteamine, super oxide dismutase (SOD) assay kit and catalase models.

Gastric and duodenal ulcers are common health problems that may be induced by a variety of factors such as stress, smoking, nutritional deficiencies and non-steroidal anti-inflammatory drugs. Although many synthetic drugs are available to treat peptic ulcers, most of these drugs have adverse reactions when used for long term[8]. So indigenous drugs possessing fewer side effects should be regarded as a better alternative for the treatment of peptic ulcer. The present study aimed to investigate the ulcer protective effect of L. aspera in various experimental models.

2. Materials and methods

2.1. Drugs and chemicals

All reagents used in the study were of analytical grade. Lansoprazole, indomethacin, serotonin creatinine sulphate, cysteamine, super oxide dismutase (SOD) assay kit and catalase assay kit were purchased from Sigma Aldrich. Carboxy methyl cellulose (CMC), xylene and acetone were obtained from Himedia.

2.2. Plant material

Aerial parts of L. aspera were collected from local areas of Guwahati, Assam, India in the month of January 2012. The plant material was authenticated by Dr. GC Sharma, Curator, Department of Botany, Gauhati University, Guwahati (voucher specimen No. 17701).

2.3. Preparation of the extract

The aerial parts of L. aspera were washed with water, shade dried in open air and pulverized using electric grinder. About 500 g of the powder was extracted with 4 L of methyl alcohol (70% v/v) by cold maceration for 7 d. The extract was filtered through Whatman filter paper No. 40, evaporated using vacuum rotary evaporator (Buchi) and heated on water bath at (45±5) °C and stored in vacuum desiccator. The yield of methanolic extract of L. aspera was obtained was 9.3% w/w.

2.4. Animals

Swiss albino mice (25–30 g) and Wistar albino rats (150–200) g of either sex were used for present investigation and were obtained from Central Animal Facility, the National Institute of Pharmaceutical Education and Research, Guwahati. Animals were housed under standard environmental conditions of temperature [(25±2) °C] and light and dark cycle (12:12 h). Animals were fed with standard pellet diet and water ad libitum. All experimental studies were done after getting permission from the Institutional Animal Ethics Committee, Gauhati Medical College, Guwahati.

2.5. Preliminary phytochemical investigation

Preliminary phytochemical screening of extract was performed using standard procedures and tests with little modifications[9,10]. Test for flavonoids: 1 mL of the extract was mixed with dilute NaOH, and golden yellow precipitate confirmed the presence of flavonoids. Test for saponins: 1 mL of the extract was mixed with 10 mL of warm distilled water, and formation of persistent foam indicated the presence of saponins. Test for glycosides: 1 mL of extract was mixed with 1 mL of water and 5–6 drops of 10% sodium hydroxide solution, and a yellow colour confirms the presence of glycosides.

2.6. Acute dose toxicity study

The acute toxicity study was carried out as per the OECD guidelines 425. Initially methanolic extract of L. aspera was administered orally at a limit dose of 2000 mg/kg to a single female rat. The rat was observed closely for the first 4 h and then periodically up to 24 h for any toxic symptoms and mortality. After 24 h same dose was administered to four more female rats[11].

2.7. Subacute dose toxicity study

Animals were divided in to 3 groups each consisting of six female rats (130–150) g[12]. Group I was kept as control, groups II and III received 400 and 800 mg/kg of the extract respectively for 14 d. The behaviours of the animals were closely observed for 1 h after daily dosing. Initial and final body weights, water and food intake, state of faecal matter and body temperatures were monitored. The animals were sacrificed on the 15th day. Hematological and serum biochemical parameters were estimated using automated counter cell (Invitrogen) and automated analyser (Dexa) respectively.

2.8. Anti ulcer screening

2.8.1. Experimental design

The animals were divided into 4 groups each consisting of six animals for all the ulcer models. Apart from the ulcerogen, different groups of animals received vehicle (0.5% CMC), methanolic extract of L. aspera 100 mg/kg, methanolic extract of L. aspera 200 mg/kg and standard (lansoprazole 30 mg/kg) respectively. CMC (0.5%) was used as solvent to prepare different doses of extract and given orally.

2.8.2. Indomethacin plus restraint induced gastric ulcer

Groups of rats, previously fasted for 24 h were given different treatments as described above. After 30 min, indomethacin (50 mg/kg) dissolved in 0.5% CMC was administered orally and then rats were immobilized by using rat restrainer. After 6 h of restraint, the animals were sacrificed and their stomach
was incised along the greater curvature to expose the mucosal surface. The gastric contents were collected and centrifuged. The gastric volume and pH of supernatant was measured. After a gentle wash with normal saline, the mucosal surface of the stomach was carefully examined under illumination using a magnifying hand lens. After identification of ulcerative areas and the length of the ulcer were measured along the greater diameter. Number of hemorrhagic spots was also calculated and every five hemorrhagic spots were considered equivalent to 1 mm of ulcer. The mean ulcer size was calculated by dividing the total length (in mm) of ulcers for all the animals divided by the total number of animals[13].

2.8.3. Swimming stress induced gastric ulcer

Swiss albino mice were fasted for 24 h and then treated with test drug, standard drug or vehicle. Thirty minutes later, they were placed inside a vertical glass cylinder filled with water up to a height of 10 cm. The temperature of the water was maintained at 20–25 °C. After 3 h animals were removed from the cylinder and sacrificed. The mean ulcer size and pH of gastric juice were estimated as described previously[14].

2.8.4. Serotonin induced gastric ulcer

Albino rats were fasted for 24 h and received different doses of the test drug, standard drug or vehicle orally. After 30 min serotonin creatinine sulphate (20 mg/kg) dissolved in saline was injected subcutaneously. The animals were sacrificed 4 h later then mean ulcer size and pH were calculated as described previously[13].

2.8.5. Cysteamine induced duodenal ulcer

Albino rats received different doses of the test drug, standard drug or vehicle orally. After 1 h, duodenal ulcers were induced by two oral administrations of cysteamine hydrochloride (400 mg/kg) in saline solution at an interval of 4 h. All animals were sacrificed 48 h after the first dose of cysteamine. The duodenum was cut open along the anti-mesenteric side and rinsed with saline[15]. Mean ulcer size was calculated as described previously.

2.8.6. Ethanol induced gastric ulcer

The gastric ulcers were induced in rats of either sex by the administration of absolute ethanol (8 mL/kg). Groups of rats, previously fasted for 24 h, were given different doses of the test drug, standard drug or vehicle prior to the administration of alcohol. After 6 h, animals were sacrificed and stomach was incised along the greater curvature and mean ulcer size was scored. The stomach was then weighed and homogenized in chilled tris buffer (10 mmol/L, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 5000 t/min at 4 °C for 20 min using high speed cooling centrifuge (Thermo scientific). The clear supernatant obtained was used for the estimation antioxidant parameters[12].

2.8.6.1. Antioxidant assays

SOD and catalase levels were measured using assay kits supplied by Sigma Aldrich, according to manufacturer’s instruction. Lipid peroxidation (LPO) in terms of thio barbituric acid reactive substances was estimated by Ohkawa method using malondialdehyde as standard and expressed as nmol/mg of protein[16]. Reduced glutathione (GSH) in the gastric mucosa was determined by Elman’s method using 5–5-dithio-bis–2–nitro benzoic acid[17]. Briefly, the homogenate was precipitated with 25% tri chloro acetic acid and centrifuged. The supernatant obtained was treated with 5–5-dithio-bis–2–nitro benzoic acid solution. The intensity of the yellow colour formed was read at 410 nm using a micro plate reader (Thermo scientific). Tissue protein was estimated by micro Lowry method[18].

2.8.6.2. Histopathological studies

Two animals from each group were sacrificed and the stomach was isolated, washed with saline and preserved in 10% buffered formalin for histopathological studies. The sections of the stomach stained with haematoxylin and eosin were assessed for histopathological changes such as congestion, edema, hemorrhage and necrosis[19].

2.9. Statistical analysis

Values were expressed as mean±SEM. Statistical analysis was performed using One-way ANOVA (Graph pad prism version 6) followed by Dunnet’s post hoc test and values of P<0.05 were considered to be statistically significant.

3. Results

3.1. Preliminary phytochemical investigation

Preliminary phytochemical investigation of methanolic extract of L. aspera showed the presence of glycosides, tannins, flavonoids and saponins.

3.2. Acute dose toxicity study

The methanolic extract of L. aspera did not show any toxic reactions and mortality up to a dose of 2000 mg/kg. No changes in food consumption, water intake or behaviour (tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma) were observed in the female rats after dose administration. Hence, methanolic extract of L. aspera at 100 mg/kg and 200 mg/kg were taken as treatment dose for the current study.

3.3 Sub acute dose toxicity study

Treatment with high doses of methanolic extract of L. aspera (400 mg/kg and 800 mg/kg for 14 d did not cause any mortality. No sign of toxicity were observed in treatment groups during the experimental period. The hematological and serum biochemical parameters of the treated groups showed no significant (P≤0.05) changes compared to the control group (Table 1).
The lesions produced in ulcer control rats were deep with massive hemorrhage. Table 2 shows that the rats pre-treated with methanolic extract of *L. aspera* 100 mg/kg and 200 mg/kg had lower mean ulcer size and gastric volume in comparison with the ulcer control group. It was also observed that treatment with methanolic extract of *L. aspera* at 200 mg/kg and lansoprazole 30 mg/kg raised the pH of gastric juice significantly as compared to ulcer control group (*P*<0.001).

### 3.5. Swimming stress induced gastric ulcer

In swimming stress induced gastric ulcer model, ulcer control group showed superficial ulcers especially in the glandular part. Methanolic extract of *L. aspera* at 100 mg/kg and 200 mg/kg reduced the mean ulcer size significantly as compared to ulcer control group (*P*<0.01 and *P*<0.001 respectively). The pH of gastric juice was also elevated by methanolic extract of *L. aspera* 200 mg/kg treatment (Table 3).

### 3.6. Serotonin induced gastric ulcer

The ulcer protective effect of methanolic extract of *L. aspera* was checked in serotonin method also. Both doses of methanolic extract of *L. aspera* significantly reduced the mean ulcer size (*P*<0.001) as compared to ulcer control. Volume of gastric juice was also reduced by the treatment with methanolic extract of *L. aspera* (both doses), but statistically not significant as compared to ulcer control group. pH of gastric juice was elevated by methanolic extract of *L. aspera* pre-treatment (Table 4).

### 3.7. Cysteamine induced duodenal ulcer

Effect of methanolic extract of *L. aspera* in duodenal ulcer was checked using cysteamine method in rats. Methanolic extract of *L. aspera* treatment reduced the mean ulcer size in a dose dependant fashion. Treatment with standard drug lansoprazole 30 mg/kg significantly reduced the mean ulcer size (*P*<0.001) as compared to ulcer control (Table 5).

### 3.8. Ethanol induced gastric ulcer

Both doses of methanolic extract of *L. aspera* exhibited marked gastro protective activity in ethanol induced gastric ulcer model. Table 6 shows effect of methanolic extract of *L. aspera* in mean ulcer size, gastric juice volume and pH of gastric juice. Methanolic extract of *L. aspera* 200 mg/kg treatment reduced the mean ulcer size and volume of gastric juice significantly as compared to ulcer control (*P*<0.001 and *P*<0.05 respectively).
Table 6
The effect of different doses of Leucas aspera methanol extract on ethanol induced gastric ulcer in rats

<table>
<thead>
<tr>
<th>Test treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean ulcer size (mm)</th>
<th>Volume of gastric juice (mL)</th>
<th>pH of gastric juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer Control</td>
<td>---</td>
<td>10.47±1.39</td>
<td>5.09±0.74</td>
<td>1.72±0.17</td>
</tr>
<tr>
<td>Methanolic extract of L. aspera 100</td>
<td>4.78±0.96 **</td>
<td>2.83±0.41 *</td>
<td>2.74±0.24 **</td>
<td></td>
</tr>
<tr>
<td>Methanolic extract of L. aspera 200</td>
<td>4.02±0.67 ***</td>
<td>2.45±0.37 **</td>
<td>3.04±0.33 **</td>
<td></td>
</tr>
<tr>
<td>Lansoprazole 30</td>
<td>3.21±0.54 ns</td>
<td>3.21±0.74 ns</td>
<td>2.81±0.08 ns</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001.
*: Not significant as compared with ulcer control.

3.8.1. Antioxidant assays

Antioxidant status of ethanol treated rats was checked. Ethanol administration was found to increase the LPO level in ulcer control group but levels of SOD, catalase and GSH were decreased. Treatment with methanolic extract of L. aspera (both doses) prevented the alteration of antioxidant molecules in the gastric mucosa (Figure 1–4).

Figure 1. Effect of different doses of L. aspera on GSH level in ethanol induced gastric ulcer model. Values are expressed as mean±SEM (n=6). ***P<0.001 as compared with ulcer control.

MeLA: methanolic extract of L. aspera.

Figure 2. Effect of different doses of L. aspera on lipid peroxidation in ethanol induced gastric ulcer model. Values are expressed as mean±SEM (n=6). **P<0.01 as compared with ulcer control.

MeLA: methanolic extract of L. aspera.

Figure 3. Effect of different doses of L. aspera on super oxide dismutase in ethanol induced gastric ulcer model. Values are expressed as mean±SEM (n=6). **P<0.01, ***P<0.001 as compared with ulcer control.

MeLA: methanolic extract of L. aspera.

Figure 4. Effect of different doses of L. aspera on catalase level in ethanol induced gastric ulcer model. Values are expressed as mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared with ulcer control.

MeLA: methanolic extract of L. aspera.

3.8.2. Histopathological studies

Photographs of gastric mucosa shows that methanolic extract of L. aspera treatment prevented the ulcer development in ethanol induced ulcer model (Figure 5).

Figure 5. Photographs of gastric mucosa in control and treated groups (ethanol induced ulcer method in rats).
A) Normal stomach, B) Ulcer control group (ethanol 8 mL/kg), C) Methanolic extract of L. aspera 100 mg/kg-ethanol 8 mL/kg, D) Methanolic extract of L. aspera 200 mg/kg-ethanol 8 mL/kg, E) Lansoprazole 30 mg/kg-ethanol 8 mL/kg.
Histopathological studies further confirmed that treatment with the methanolic extract of *L. aspera* inhibited the ethanol induced ulcer, congestion, oedema, hemorrhage and necrosis in gastric mucosa (Figure 6).

Sub acute dose toxicity studies were performed in Wistar rats with high doses of methanolic extract of *L. aspera* (400 mg/kg and 800 mg/kg). There was no mortality in the above mentioned doses at the end of the 15 d of observation. The biochemical and haematological parameters like serum glucose, protein, urea, serum glutamic pyruvic transaminase, serum glutamic–oxaloacetic transaminase, hemoglobin and white blood cell count of the methanolic extract of *L. aspera* treated (both doses) rats showed no significant changes compared to the normal control rats. From these results it is concluded that methanolic extract of *L. aspera* extract has very high margin of safety in rats.

Mechanisms of acute gastric mucosal injury are not yet fully understood, it may be caused by the imbalance between protective factors and damage factors. The main protective factors including adequate mucosal blood flow, mucosal bicarbonate barrier, endothelial cell regeneration and prostaglandins while destructive factors including gastric acid, pepsin, bile, reperfusion injury and oxygen free radicals[21]. Results obtained from the indomethacin plus restraint induced gastric ulcer model shows the ulcer protective and antisecretory effect of methanolic extract of *L. aspera*. It is reported that, indomethacin induce gastric ulcer by depleting cytoprotective prostaglandins (PGE, and PGI), which are produced in the cyclooxygenase pathway of arachidonic acid metabolism[22]. PGE and PGI, of gastric mucosa are responsible for mucus production and maintaining cellular integrity of the gastric mucosa.

4. Discussion

Research on natural products often is guided by ethno pharmacological knowledge, and has brought substantial contributions to drug innovation by providing novel chemical structures and/or mechanisms of action[12,20]. Many tribal peoples of North east India have been using the juice of *L. aspera* for various stomach ailments, but no detailed scientific information is available regarding its ulcer preventive activity. The present study has, therefore been conducted to evaluate the anti ulcer activity of methanolic extract of *L. aspera* using different *in vivo* ulcer models and further confirmed by antioxidant studies and histopathology.

Preliminary phytochemical screening of the crude extract gave positive results for the presence of flavonoids, saponins and glycosides. The presence of these phytochemicals may be responsible for the gastro protective effect of the *L. aspera*. Acute dose toxicity study, in which the animals treated with the methanolic extract of *L. aspera* at a higher dose of 2000 mg/kg, did not produce any significant toxicity signs, behavioural changes, body weight changes or macroscopic findings during observational period. So the LD₅₀ of methanolic extract of *L. aspera* should be more than 2000 mg/kg.
Ethanol ingestion damages the superficial epithelial layers and inhibits the release of mucosal prostaglandins and depresses the gastric defensive mechanisms[26]. Moreover, the antioxidant studies revealed that methanolic extract of *L. aspera* treatment counteract the oxidative damage caused by ethanol. Preventive antioxidants, such as SOD and catalase enzymes are the first line of defence against reactive oxygen species. Reduced glutathione is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation[27]. Histopathological studies further confirmed that pre-treatment with methanolic extract of *L. aspera* prevented the ethanol induced ulcer formation, congestion, oedema, hemorrhage and necrosis in gastric mucosa.

We conclude that, the aerial parts of *L. aspera* has promising gastric protective effect and is comparable to the standard drug lansoprazole. The study results justified the folkloric use of *L. aspera* and this plant’s anti ulcer properties merit further investigations like mechanism of action.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgements**

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**Comments**

**Background**

Gastric ulcer, which affects thousands of people, is becoming one of the most important diseases of the digestive system and a medical–social problem of global economic importance due to its higher morbidity and mortality rate. The drugs currently used in the treatment of gastric ulcer are antacids, anti-cholinergics, proton pump inhibitors and H2 receptor antagonists. However, there are innumerable adverse effects caused by these allopathic medicines (e.g. hypersensitivity, gynecomastia, impotence, arrhythmia and hematopoietic changes). So drugs from plant origin are good alternative to conventional drugs because of fewer side effects.

**Research frontiers**

Ulcer protective effect of methanolic extract of *L. aspera* was studied (100 and 200 mg/kg) in two species (Swiss albino mice and Wistar rat). The results showed that methanolic extract of *L. aspera* administration significantly protects the ulcer formation in both species. Histopathological pictures further confirm the ulcer protective effect of methanolic extract of *L. aspera*. Acute and sub–acute toxicity studies revealed that methanolic extract of *L. aspera* use is safe.

**Related reports**

Literature survey showed that, a preliminary study of anti ulcer activity of *L. aspera* was already conducted by Reddy *et al* in 1992. But they investigated the anti ulcer effect in only two models (pyloric ligation and aspirin induced ulcer model). But in the current study authors investigated the plant using six experimental ulcer models along with antioxidant assays and histopathological studies. The results of the current study agree with reports of Reddy *et al* that *L. aspera* has the potential to prevent experimental ulcer.

**Innovations & breakthroughs**

The results of the current study provided enough scientific bases to the traditional use of *L. aspera* in gastric ulcer. Data also showed that *L. aspera* is useful in stress induced ulcer (nowadays it is common). It was also found that *L. aspera* increases the antioxidant molecules in the gastric mucosa (free radical accumulation is one of the main causes of cell damage).

**Applications**

The ulcer protective constituent present in *L. aspera* can be developed into a pharmaceutical formulation for the treatment of stress induced and other types of gastric ulcers. Isolation of active constituent and a series of pre–clinical and clinical studies are required to develop the *L. aspera* into a pharmaceutical dosage form.

**Peer review**

In this study authors checked the ulcer protective effect of *L. aspera* in almost all the experimental models with special preference in ethanol induced model. In ethanol model, they have studied the modulation of antioxidant molecules by *L. aspera* and then confirmed the antiulcer
effect through histopathological studies. The results of the current study are promising and interesting. In conclusion further studies should be carried out to isolate the active constituent and elucidate the mode of action.

References


