GASTRIC ANTISECRETORY AND ANTIULCER ACTIVITIES OF AN ETHANOLIC EXTRACT OF MIMOSA PUDICA LEAVES.

Md. Saifuddin Khalid*1,2, Shah Jinesh Kumar1, Rajnish Kumar Singh1, S. Ramachandra Setty3, I.V. Narasimha Reddy1, Md. Hakeemuddin Khan1.

1. Dept. of Pharmacology, Luqman College of Pharmacy, Gulbarga, Karnataka, India.
2. Dept. of Biotechnology, Acharya Nagarjuna University, Nargarjuna Nagar, Guntur, AP., India.
3. Dept. of Pharmacology, Govt. College of Pharmacy, Bangalore, Karnataka. India

Corresponding author address:
Md. Saifuddin Khalid
Assistant Professor,
Department of Pharmacology,
Luqman college of Pharmacy,
Old Jewargi Road, Behind P&T Colony, P.O. Box # 86, Gulbarga-585 102, Karnataka, India.
Email Id: khalid2568@yahoo.com
Mobile: + 91-9845242820.

Summary

Objectives: The present study was undertaken to determine the anti-ulcer potential and antisecretory properties of the ethanolic extract of Mimosa pudica leaves extract. Materials: Ethanolic extract of the leaves Mimosa pudica were tested orally at the doses of 200 and 400 mg/kg, on gastric ulcerations experimentally induced by pylorus ligation, aspirin and alcohol models. Both the antisecretory and cytoprotection hypothesis were evaluated. Results: The ethanolic extract at 200 and 400 mg/kg has significantly inhibited ulcer formation. There was a significant (P < 0.001) dose-dependent decrease in the ulcerative lesion index produced by all the three models in rats as compared to the standard drug lansoprazole (8 mg/kg, b.w. orally). The reduction in gastric fluid volume, total acidity and an increase in the pH of the gastric fluid in pylorus ligation rats proved the antisecretory activity of Mimosa pudica leaves. Conclusion: The anti-ulcer property may be related to the tannin and flavonoids present in the extract. These results clearly indicated that ethanolic extract of the leaves of Mimosa pudica is effective against ulcer disease.

Key words: Mimosa pudica Linn; Anti-ulcer activity; Leaves ethanolic extract.
Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. Consequently, reduction of gastric acid production as well as reinforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitor to H2 antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffin-like cell (ECL), hyperplasia and haemopoietic changes. A medicinal plant provides an important source of new chemical substances with potential therapeutic effects. These have been used in traditional medicine for the treatment of several diseases. Several herbs and shrubs are useful as medicines as reported by many scientists.

Many such herbs, shrubs and plants are known to protect the organs from the environmental, chemical and occupational challenges. *Mimosa pudica* Linn is one such green leaf shrubs. The plant *Mimosa pudica* used in indigenous medicine for the treatment of hydrocele, scrofula, conjunctivitis, cuts, wounds, hemorrhages, bleeding disorders like menorrhagia, dysentery with blood and mucus, piles, in herbal formulations. The objective of the present study was to investigate the anti-ulcer property of the plant extract.

Methods

Plant material:

The fresh leaves of *Mimosa pudica* Linn were collected from Luqman college of Pharmacy, Gulbarga (Karnataka). The plant herbarium specimen was identified and authenticated by Mr. P. G. Diwakar, Joint Director, Botanical Survey of India, Western circle, Koregaon Road, Pune -1. Voucher No. JINSHMII1.

Preparation of the extract:

The authenticated leaves of *Mimosa pudica* Linn were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the leaves was Soxhlet extracted with the solvents with increasing order of polarity i.e. petroleum ether (60-80°C), chloroform (59.5-61.5°C), ethanol (64.5-65.5°C) and distilled water. The extracts obtained were concentrated under reduced pressure. The dried extract was weighed (the yield of the extract was 13.04 %) and stored in airtight containers in refrigerator below 10°C.
Phytochemical screening:

Preliminary phytochemical screening of the powdered leaves was performed for the presence of alkaloids, phenolics, flavonoids, saponins, carotenoids, carbohydrates and glycosides.

Animals:

Albino wistar rats of either sex weighing between 150 to 200 gm and Albino mice of either sex weighing between 20 to 25 gms were procured from registered breeders (346/ CPCSEA, Mahavir Enterprises, Hyderabad.) used for studying anti-ulcer activity and acute toxicity respectively. The animals were housed under standard conditions of temperature (25 ± 2°C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water ad libitum. Approval at the Institutional Animal Ethics Committee (IAEC) of Luqman College of Pharmacy, Gulbarga was taken for conducting antulcer and anti-secretary activity.

Drugs and chemicals:

Aspirin was obtained from Shalg pharmaceuticals, Goregaon, Mumbai, India and Lansoprazole was obtained from Lee Pharmaceuticals, Hyderabad. All other chemicals used in this study were obtained commercially and were of analytical grade.

Acute toxicity study:

Mice were kept overnight fasting prior to drug administration. A total of five animals were used which dose (2000 mg/kg, b.w.) of *Mimosa pudica* leaf extract. After the administration of *Mimosa pudica* leaf extract, food was withheld for further 3–4 hours. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 hours (with special attention during the first 4 hours) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory autonomic (salivation, lacrimation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks.

Pylorus-ligation induced gastric ulcer:

In the present study animals were divided into four groups of six rats each and fasted for 36 hours. Group-I was administered vehicle and served as control. Group-II served as standard and received lansoprazole (8mg/kg). Group-III and IV were given orally ethanolic extract (200 and 400 mg/kg) of *Mimosa pudica* leaves respectively. All groups were treated 30 minute prior to pyloric ligation. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 hours after ligation, the animals were sacrificed with excess of anesthetic ether, and the
stomach was dissected. The glandular portion was then exposed and examined for ulceration. Ulcer index was determined. Determination of total acid and free acid were estimated from gastric juice collected from the 4 hours pylorus ligated rats. Total acid output of the gastric juice was estimated by titration of 0.1 ml of gastric juice with 0.01N sodium hydroxide using phenolphthalein as indicator. Total acid output was expressed as mEq/L per 100gm of body weight

**Aspirin-induced gastric ulcer:**

The rats were randomly divided into four groups and fasted for 24 hour. Group-I was administered vehicle and served as control. Group-II served as standard and received lansoprazole (8mg/kg). Group-III and IV were given orally ethanolic extract; 200 and 400 mg/kg of *Mimosa pudica* leaves respectively. After 60 minute aspirin (200 mg/kg) was administered orally to all the animals in all the groups and 6 hours later the animal were sacrificed by anesthetic ether. The stomachs were removed, opened along the greater curvature and examined under (10X) microscope. Samples were studied for further histopathological parameters. Scoring of ulcer was done by the following method: 0 = normal stomach, 0.5 = red coloration, 1.0 = spot ulcers, 1.5 = hemorrhagic streaks, 2.0 = ulcer > 3 but < 5, 3.0 = ulcer > 5. Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula, $\text{Percentage protection} = 100 - \frac{U_t}{U_c} \times 100$; Where, $U_t$ = ulcer index of treated group, $U_c$ = ulcer index of control group.

**Alcohol induced gastric ulcers:**

In the present study animals were divided into four groups of six rats each and fasted for 24 hours. Group-I was administered vehicle and served as control. Group-II served as standard and received lansoprazole (8mg/kg). Group-III and IV were given orally ethanolic extract (200 and 400 mg/kg) of *Mimosa pudica* leaves respectively, orally. One hour after the administration of extracts and standard, absolute alcohol at the dose of 1ml/200 gms was administered to the all the animals. The animals were sacrificed 1 hour after administration of ethanol and the stomach was removed and opened along the greater curvature. Lesions were examined with the help of hand lens (10X) and sample was sending to further histopathological study. Scoring of ulcer was done by the following method: 0 = normal stomach, 0.5 = red coloration, 1.0 = spot ulcers, 1.5 = hemorrhagic streaks, 2.0 = ulcer > 3 but < 5, 3.0 = ulcer > 5. Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula, $\text{Percentage protection} = 100 - \frac{U_t}{U_c} \times 100$; Where, $U_t$ = ulcer index of treated group, $U_c$ = ulcer index of control group.

**Statistical analysis:**

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dennett’s’

test. P value < 0.05 was considered to be statistically significant, *P<0.05, **P<0.01 and ***P<0.001, when compared with control and toxicant group as applicable.

RESULTS

Phytochemical screening:
The preliminary phytochemical investigation has revealed that the ethanolic extract of leaves of Mimosa pudica Linn said to contain alkaloids, carbohydrate, steroids, tannins, flavonoids, glycoside and saponins.

Selection of dose of the extract:
LD$_{50}$ was done as per OECD guidelines for fixing the dose for biological evaluation. In LD$_{50}$ studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses (1/5$^{th}$ and 1/10$^{th}$ of LD$_{50}$ cut off values) of 200 and 400 mg/kg body weight.

Pylorus ligation-induced ulcer model:
Animals in the pylorus ligation group showed a significant (P < 0.001) increase in the ulcer index and acid secretory parameters like gastric volume, gastric pH, free and total acidity when compared with those of vehicle treated group. In the rats of this group, a number of ulcers were also observed. Administration of Mimosa pudica leaves extract produced significant (P < 0.001) decrease in ulcer index in a dose dependent manner. The extract also significantly reduced the gastric volume, total and free acidity, and increased the pH of the gastric fluid, proving its antisecretory activity. Mimosa pudica leaves extract at a dose of 200 and 400 mg/kg body weight showed protection index of 64.94% and 77.05%, respectively, than lansoprazole that showed protection index of 83.76% at a dose of 8 mg/kg body weight (Table 1 and figure 1).

Aspirin-induced gastric ulcer:
Administration of Aspirin produced severity of ulcer in the gastric mucosa of the control group. Mimosa pudica leaves ethanolic extract reduced these severities of ulcer as evidenced by a significant (P < 0.01) reduction in the ulcer index when compared with the control group. Mimosa pudica leaves extract at a dose of 200 and 400 mg/kg body weight showed protection index of 54.28% and 77.42%, respectively. Lansoprazole significantly (P < 0.001) reduced ulcer index of aspirin-induced gastric ulcers (Table 2 and figure 2).

Absolute alcohol-induced ulcer model:
Administration of ethanol produced haemorrhagic gastric lesions in the gastric mucosa of the control group. Mimosa pudica leaves ethanolic extract reduced these lesions as evidenced by a significant (P < 0.01) reduction in the ulcer index when compared with the control group. Mimosa pudica leaves extract at a dose of 200 and 400 mg/kg body weight showed protection index of
49.13% and 68.27%, respectively. Lansoprazole more significantly ($P < 0.001$) reduced ulcer index of ethanol-induced gastric ulcers (Table 2 and figure 3).

**Table 1: Effect of ethanolic extracts of *Mimosa pudica* leaves against pylorus ligated rats**

<table>
<thead>
<tr>
<th>Gr. No</th>
<th>Treatment (Dose)</th>
<th>Vol. of gastric juice (ml)</th>
<th>pH</th>
<th>Free acidity (mEq/L/100 gm)</th>
<th>Total acidity (mEq/L/100 gm)</th>
<th>Ulcer Index</th>
<th>% of protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Control -</td>
<td>4.533 ± 0.10</td>
<td>1.560 ± 0.02</td>
<td>76.83 ± 0.60</td>
<td>95.33 ± 0.71</td>
<td>8.433 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>II.</td>
<td>Lansoprazole 8 mg/kg</td>
<td>2.567 ± 0.10***</td>
<td>6.583 ± 0.14***</td>
<td>15.00 ± 0.57***</td>
<td>24.83 ± 0.83***</td>
<td>1.367 ± 0.06 ***</td>
<td>83.76%</td>
</tr>
<tr>
<td>III.</td>
<td>EEMP 200 mg/kg</td>
<td>4.083 ± 0.06**</td>
<td>3.513 ± 0.09*</td>
<td>36.33 ± 2.4**</td>
<td>42.50 ± 1.1**</td>
<td>3.003 ± 0.019**</td>
<td>64.94%</td>
</tr>
<tr>
<td>IV.</td>
<td>EEMP 400 mg/kg</td>
<td>3.083 ± 0.15***</td>
<td>4.502 ± 0.06***</td>
<td>17.00 ± 0.57***</td>
<td>31.17 ± 1.4***</td>
<td>1.96 ± 0.08***</td>
<td>77.05%</td>
</tr>
</tbody>
</table>

**EEMP:** Ethanol Extract of *Mimosa pudica*

The values are Mean ± SEM, $n = 6$ *p < 0.05, **p < 0.01 and *** p < 0.001 vs control.

**Figure 1:** Effect of ethanolic extracts of *Mimosa pudica* leaves on ulcer index and their % protection in pylorus ligation induced ulceration in rats

A: Control
B: Standard (Lansoprazole 8 mg/kg p. o.)
C: Ethanolic extract of *Mimosa pudica* leaves (200 mg/kg p. o.)
D: Ethanolic extract of *Mimosa pudica* leaves (400 mg/kg p. o.)
Table 2: Effect of ethanolic extracts of *Mimosa pudica* leaves on ulcer index and their % protection in Aspirin and Alcohol induced ulcer in rats

<table>
<thead>
<tr>
<th>Gr. No</th>
<th>Treatment (Dose)</th>
<th>Aspirin</th>
<th>Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ulcer Index</td>
<td>% Protection</td>
</tr>
<tr>
<td>I.</td>
<td>Control -</td>
<td>7.017 ± 0.1922</td>
<td>-</td>
</tr>
<tr>
<td>II.</td>
<td>Lansoprazole 8 mg/kg</td>
<td>1.287 ± 0.0080***</td>
<td>81.71%</td>
</tr>
<tr>
<td>III.</td>
<td>EEMP 200 mg/kg</td>
<td>3.238 ± 0.01138*</td>
<td>54.28%</td>
</tr>
<tr>
<td>IV.</td>
<td>EEMP 400 mg/kg</td>
<td>1.572 ± 0.0090***</td>
<td>77.42%</td>
</tr>
</tbody>
</table>

EEMP: Ethanolic Extract of *Mimosa pudica*

The values are Mean ± SEM, n = 6;

*p < 0.05, **p < 0.01 and *** p < 0.001 vs control.

Figure 2: Effect of ethanolic extracts of *Mimosa pudica* leaves on ulcer index and their % protection in Aspirin induced ulceration in rats

A: Control (Aspirin 200mg/kg, p. o.)
B: Standard (Lansoprazole 8mg/kg p. o.)
C: Ethanolic extract of *Mimosa pudica* leaves (200 mg/kg p. o.)
D: Ethanolic extract of *Mimosa pudica* leaves (400 mg/kg p. o.)
A: Control (Absolute ethanol 1ml/200gms. p. o.)
B: Standard (Lansoprazole 8 mg/kg p. o.)
C: Ethanolic extract of *Mimosa pudica* leaves (200 mg/kg p. o.)
D: Ethanolic extract of *Mimosa pudica* leaves (400 mg/kg p. o.)

**Figure 3:** Effect of ethanolic extracts of *Mimosa pudica* leaves on ulcer index and their % protection in Alcohol induced ulceration in rats

**Histopathological studies of Ethanolic extracts of *Mimosa pudica* leaves on Aspirin induced ulcer in rat.**

**Group-I:** In this study, the mucosa in control group (Aspirin 200mg/kg) showed redness, congestion, hemorrhagic sticks, sub mucosa showing intense inflammation predominant being eosinophils and mast cells, necrosis and dilation of blood vessels. (Figure: 4)

**Group-II:** - In Lansoprazole (8mg/kg p. o.) treated group, mucosa shows the mild redness, mild inflammation, submucosa and muscular layer is normal. (Figure: 5)

**Group-III:** - At 200 mg/kg p. o. of EEMP treated group, the mucosa shows the mild redness, mild inflammation, mild congestion, hemorrhage and dilation of blood vessels. (Figure: 6)

**Group-IV:** - At 400 mg/kg p. o. of EEMP treated group, the mucosa shows the mild dilation of blood vessels mild redness, no inflammation, no congestion and mild dilation of blood vessels. (Figure: 7)
Figure 4: Control Aspirin
Figure-5: Standard Lansoprazole (8mg/kg)
Figure 6: 200mg/kg EEMP
Figure 7: 400mg/kg EEMP
Histopathological studies of Ethanolic extracts of *Mimosa pudica* leaves on Absolute ethanol induced ulcer in rat.

Group-I: In this study, the mucosa in control group (Absolute ethanol 1ml/200gm) showed redness, infiltration, congestion, hemorrhagic sticks, inflammation, necrosis and dilation of blood vessels. Submucosa showing intense inflammation predominant being eosinophils and mast cells. (Figure: 8)

Group-II: In Lansoprazole (8mg/kg p. o.) treated group, the mucosa showed the mild redness, no inflammation, and dilation of blood vessels. (Figure: 9)

Group-III: At 200 mg/kg p. o. of EEMP treated group, the mucosa showed the mild redness, mild inflammation, no congestion. (Figure: 10)

Group-IV: At 400 mg/kg p. o. of EEMP treated group, mucosa shows the mild dilation of blood vessels mild redness, inflammation, congestion, hemorrhagic sticks and necrosis. (Figure: 11)
Figure 9: Standard Lansoprazole (8mg/kg)
Figure-10: 200mg/kg EEMP
Figure 11: 400mg/kg EEMP
Discussion

Peptic ulcer results due to overproduction of gastric acid (or) decrease in gastric mucosal production. Pylorus ligation induced ulcers occur because of an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion. In folk medicine, *Mimosa pudica* Linn is used for the various gastrointestinal diseases. The present study reveals that ethanolic extract of *Mimosa pudica* leaves extract treated groups showed a significant ($P < 0.01$) increase in gastric juice, pH, reduces the gastric volume, free acidity and total acidity when compared to control. *Mimosa pudica* leaves extract decreased the ulcer index more effectively in a dose dependent manner. These results show that the antiulcer activity of *Mimosa pudica* leaves extract might be due to its antisecretory activity.

Aspirin produced mucosal injury, which was confined to the glandular stomach. The ulcer produced by aspirin cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of $\text{H}^+$ ions. The non-ionizes form of salicylates is water insoluble, hence it tends to adhere to the gastric mucosa thereby producing irritation. Treatment with ethanolic extract of *Mimosa pudica* leaves extract afforded complete regeneration of mucosal glandular structure, which was evidenced through histopathological studies of the stomach. The results of our study prove that the crude extract of *Mimosa pudica* leaves extract possess antiulcer activity against experimentally induced acute gastric ulcer models. Hence, it can be suggested that the antiulcer activity of the extract may be attributed to its antisecretory activities.

Ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcers. Ethanolic extract of *Mimosa pudica* leaves extract significantly ($P < 0.01$) reduced the ulcer index and afforded significant protection against ethanol-induced ulcer. The antioxidant properties of *Mimosa pudica* leaves extract may have scavenged the free radicals produced by the metabolism of ethanol and thereby heal the ulcers. The preliminary phytochemical investigation of the Leaves of *Mimosa pudica* Linn showed the presence of tannins and flavonoids. Therefore, in our study the possible role for the significant anti-ulcer property of aqueous extract of leaves of *Mimosa pudica* may due to the presence of these phytochemicals.

Conclusion

On the basis of the present results and available reports, it can be concluded that the antisecretory and anti-ulcer activity elucidated by ethanolic extract of *Mimosa pudica* leaves could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.
Acknowledgement

The authors are thankful to Dr. P. G. Diwakar, joint director, botanical survey of India, Pune, for identification and authentication of plant, Mrs. Nirmala (Executive Q.C.), Lee pharma Ltd., Hyderabad, for gifting the Lansoprazole drug, Management Dr. M. Abdul Mujeeb, Chairman, Luqman College of Pharmacy, Gulbarga for providing me all facilities, throughout the research work.

References


No.25/1990-AWD
Government of India
Ministry of Social justice and Empowerment
(Committee for the Purpose of Control and Supervision of Experiments on Animals)

Date the 3rd January, 2001.

Shantir Bhavan, New Delhi-110001,

To

The Principal,
Lalman College of Pharmacy,
P.B. No. 86, Behind P & T Colony,
Jewargi Road,
Gulbarga 585 102,

Subject: Registration of Establishments/Breeders under Rule 5(a) of the “Breeding of and Experiments on Animals (Control and Supervision) Rules 1998”.

Sir,

With reference to your application on the above-mentioned subject, this is to inform that your Establishment is hereby registered for “Research”. Your Registration Number is 346/CPCSEA. The nominee of CPCSEA on the Institutional Animal Ethics Committee (IAEC) of your Establishment will be intimated in due course.

2. You are requested to quote the above Registration Number in all your future correspondence with the Committee.
3. You are also requested to convene IAEC meeting at the earliest.
4. For future correspondence you are requested to contact Office of CPCSEA at Chennai, at the address given below:

Office of the CPCSEA,
Ministry of Social Justice and Empowerment,
3rd Sea Ward Road, Valmiki Nagar,
Thiruvanmiyur, Chennai-600 041

Yours faithfully,

[Signature]

(A.K. JOSHI)
MEMBER SECRETARY(CPCSEA)
Tel. No.3387539
Fax No.3384918
Annexure - II

This is to certify that the plant specimen brought by Mr. Shah Jineshkumar, a M. Pharm Student of Luqman College of Pharmacy Gulbarga, Karnataka State, is identified as:

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>JINSHMI1</td>
<td>Mimosa pudica L.</td>
<td>Mimosaceae</td>
</tr>
</tbody>
</table>

(P.G. DIWAKAR)
JOINT DIRECTOR

[Signature]