Anti-ulcer activity of the ethanolic extract of *Terminalia belerica* Roxb.

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ABSTRACT
The anti-ulcer activity of ethanolic extract of *Terminalia belerica* (Combretaceae) fruits ETB was investigated in pylorus ligation and ethanol induced ulcer models in wistar rats. In both models the common parameter determined was ulcer index. ETB at doses of 250, 500 mg/kg orally produced significant inhibition of the gastric lesions induced by Pylorus ligation induced ulcer & Ethanol induced gastric ulcer. The extract (250 mg/kg & 500 mg/kg) showed significant (P<0.05) reduction in free acidity and ulcer index as compared to control. This present study indicates that *Terminalia belerica* fruit extract have potential anti ulcer activity in both models.

Keywords: *Terminalia belerica*, Pylorus ligation, Ethanol induced ulcer model, Ranitidine.

I. INTRODUCTION
Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood. Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors. Gastric ulcer is among the most serious diseases in the world. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid–pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermic growth factors. Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may be responsible for the development of peptic ulcer.

*Terminalia belerica* (TB) Roxb. belonging to the family-Combretaceae, commonly known as myrobalan, is a deciduous tree found throughout the Indian forests and plains. The tree is about 30-40 m. in height and 2-3 m. in girth. The stem is straight and the leaves are broadly elliptic clustered near the end of the branches. The flowers are simple, solitary in axillary spikes. The fruit is ovoid 1-2 c.m. in diameter drupe of grey to dark brown in colour. Fruit is astringent, antiseptic, rejuvenative, brain tonic, expectorant and laxative. It is used in coughs and sore throat. Its pulp used in dysentery, diarrhoea and liver disorders. It is also useful in leprosy, fever and hair care.

Fruit contain about 20-40% of tannin, phyllemblin, β-sitosterol, anthraquinones, fixed oil, mannitol, glucose, fructose and rhamnose.
II. MATERIAL AND METHODS

II. 1. Plant material
Fruit of *Terminalia belerica* obtained from Yucca enterprises, Mumbai, were authenticated and identified by Dr. A.B. Sheerwani (Retd. Prof. and Head), Deptt. of Botany, Holkar Science College, Indore. A voucher specimen has been deposited in our laboratory for further reference.

II. 2. Extraction
Powdered fruit were soxhlet-extracted with 95% ethanol. The ethanolic extract was evaporated in vacuo and residue (yield: 31% w/w).

II. 3. Preliminary phytochemical investigation
Preliminary phytochemical analysis shows the presence of glycosides, tannins, phenolic compounds, phytosterol and flavonoids.

II. 4. Animals
Animals-Wister rats (150-250g) were obtained from the experimental animal house, School of Life Science, Devi Ahilya University, Indore. They were maintained under standard housing condition (Room temperature 25±2°C and 45-55% RH with 10:14h, L:D cycles). The animals were given standard laboratory feed and water ad libitum. The study was cleared by Animal ethics committee (School of Life Science, Devi Ahilya University, Indore). All the animals received humane care according to criteria outlined in the guide for the care and use of laboratory animals prepared by the national academy of the sciences and published by national institute of health.

II. 5. Experimental Procedure
II. 5.a. Pylorus ligation induced ulcer
Animals were divided into four groups (n=6). Group-I received 1ml of vehicle (Distilled water) that served as control, group-II received Ranitidine orally (50 mg/kg), group-III, IV received ethanolic extract (250mg/kg, 500mg/kg), respectively. Study of antiulcer activity using pylorus ligation method. Animals were fasted for 24 h and the dose was administrated 30 min prior to pylorus ligation. Animals were sacrificed 4 h later and the stomach was removed. The gastric content was collected and centrifuged. The volume, free acidity, total acidity of gastric fluid was determined. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass. Mean ulcer score for each animal was expressed as ulcer index. The ulcers were graded using the following scoring system- 0= Normal mucosa; 0.5= Red coloration; 1.0=Spot ulcer; 1.5= Hemorrhagic streaks; 2.0=Ulcer.

II. 5.b. Ethanol induced ulcer
The experiment was performed according to the method of Morimoto et al. After 12 hour of fasting, the rats were randomly divided into four groups of six animals each. First group was given 1ml of vehicle (Distilled water), and the second group was treated with Ranitidine 50 mg/kg. group-III, IV received ethanolic extract (250mg/kg, 500mg/kg), respectively. All the treatments were administered orally. One hour after treatment, all the rats received 1ml of 99.5% ethanol to induce gastric ulcer. One hour later, the animals were sacrificed by cervical dislocation, and the stomach removed and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots, for subsequent scanning. The ulcers were classified as 0 – Normal stomach, 1- spot ulceration, 1.5- Hemorrhagic streaks, 2- Ulcer.

III. Statistical analysis
The values are represented as mean ± 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<0.05 was considered statistically significant.
VI. RESULTS
VI. a. Pyloric ligation induced gastric ulcer
The effect of the ETB in Pyloric ligation induced gastric ulcer was studied and the results are tabulated in table I. The ETB significantly reduced the ulceration. The extract at the doses of 250 and 500 mg/kg afforded 68 and 74% (p<0.05) respectively, whereas ranitidine exhibited 80% protection.

VI. b. Ethanol induced gastric ulceration
In the present study ETB was evaluated for its antiulcer activity against Ethanol induced gastric ulceration in rats and the results are tabulated in table II. Oral administration of ethanol produced severe ulceration, but ETB at the doses of 250 and 500 mg/kg afforded 33 and 55% (p<0.05) respectively, whereas the reference drug ranitidine exhibited 63% protection.

V. DISCUSSION
Ulcer are caused due to imbalance aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandin. Different therapeutic agents including plant extract are used to inhibit the gastric acid secretion or to stimulate the mucosal defense mechanism by increasing the mucus production protecting the surface epithelial cells or interfering with the PG synthesis\(^1\). The treatment and prevention of these acid-related disorders are accomplished either by decreasing the level of gastric acidity or by enhancing mucosal protection\(^2\). Present study was carried out to investigate antiulcer activity of ethanolic extract of *Terminalia belerica* in pylorus ligated and ethanol induced ulceration in the rats. Pylorus ligation induced ulcer is one of the most widely used methods for studying the effect of drug on gastric secretion. Pylorus ligation induced ulcers are due to auto digestion of gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Prostaglandin E2 and I2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant-like phospholipids secretion in the gastric epithelial cells is also stimulated by the prostaglandin. Effect of pylorus ligation has caused the accumulation of gastric secretion . The total acidity, free acidity, ulcer index of gastric secretions were increases. Ranitidine and ethanolic extract of *Terminalia belerica* significantly decreased the gastric volume, total acidity, free acidity, and ulcer index. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid\(^3\). *Terminalia belerica* prevented the mucosal lesions induced by pylorus ligation. This suggests that the components present in the extract must be suppressing gastric damage. The efficacy of *Terminalia belerica* extract against gastric ulcers led us to perform yet another model i.e. ethanol induced. This model too resulted in a significant percentage protection against gastric ulcers. The percentage protection observed was very much the same as that of standard drug ranitidine (Table II). It has been postulated that histamine might be involved in the formation of pylorus ligated ulcers and plays a mediating a role in gastric secretions stimulated by gastrin vagal excitation. The H2-receptor antagonists inhibit acid production by reversibly competing with histamine for binding to H2 receptors on the basolateral membrane of parietal cells\(^4\).
Table I: Effect of *Terminalia belerica* extract on various parameters in pyloric ligation induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Ulcer index</th>
<th>% Protection</th>
<th>pH of gastric juice</th>
<th>Gastric juice (ml)</th>
<th>Free acidity meq/ltr</th>
<th>Total acidity meq/ltr</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>16.6±2.6</td>
<td>-</td>
<td>2.6±0.4</td>
<td>9.8±1.6</td>
<td>99.1±4.6</td>
<td>108.4±4.8</td>
</tr>
<tr>
<td>II</td>
<td>Ranitidine</td>
<td>3.2±1.0*</td>
<td>80</td>
<td>5.1±1.2*</td>
<td>2.9±0.4*</td>
<td>38.3±3.2*</td>
<td>55.9±3.0*</td>
</tr>
<tr>
<td>III</td>
<td>ETB (250 mg/kg)</td>
<td>5.2±1.2*</td>
<td>68</td>
<td>3.7±0.4*</td>
<td>5.1±0.98*</td>
<td>52.4±3.5*</td>
<td>64.6±4.1*</td>
</tr>
<tr>
<td>IV</td>
<td>ETB (500 mg/kg)</td>
<td>4.3±1.6*</td>
<td>74</td>
<td>4.5±1.5*</td>
<td>4.0±1.0*</td>
<td>40.1±3.8*</td>
<td>61.2±3.2*</td>
</tr>
</tbody>
</table>

Values are express as mean± SEM of 6 observations, Statistical comparisons as follows:
Significant at * p<0.05 compared to control group.

Table II: Effect of *Terminalia belerica* extract on various parameters in ethanol induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Ulcer index</th>
<th>% Protection</th>
<th>pH of gastric juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>22.8±2.1</td>
<td>-</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>II</td>
<td>Ranitidine(50mg/kg)</td>
<td>8.3±0.8*</td>
<td>63</td>
<td>5.8±0.9*</td>
</tr>
<tr>
<td>III</td>
<td>ETB (250 mg/kg)</td>
<td>15.2±1.2*</td>
<td>33</td>
<td>4.1±0.4*</td>
</tr>
<tr>
<td>IV</td>
<td>ETB (500 mg/kg)</td>
<td>10.2±1.0*</td>
<td>55</td>
<td>4.9±0.5*</td>
</tr>
</tbody>
</table>

Values are express as mean± SEM of 6 observations, Statistical comparisons as follows:
Significant at * p<0.05 compared to control group.

VI. CONCLUSION

Phytochemical investigation suggested that several species of *Terminalia* were found to exhibited anti-ulcer activity. Phytochemical investigation of this medicinal plant revealed the presence of ellagic acid and gallic acid. Ellagic acid, a widely occurring polyphenol possesses strong antioxidant activity. It has a marked inhibitory effect on acid secretion and the occurrence of stress-induced gastric lesion, and these effects may be attributed to the inhibition of H⁺ and K⁺ ATPase activity.

REFERENCES

and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. Jpn J Pharmacol 1991, 57, 495-505.


