ANTIULCER ACTIVITY OF SAPONINS OF ALBIZZIA LEBBECK BARK AND LEAVE

T.P. PATEL*1, B.K. PATEL2, P.M.PATEL3

Abstract

This study was designed to determine the antiulcer activity of n-butanol soluble fractions of Albizia lebbeck bark and leaf in pylorus ligation, ethanol and Indomethacin induced models in rats. The various extracts were prepared and extracts containing flavanoids, tannins and saponins (Methanolic extract) was selected for isolation of chemical constituents. n- butanol soluble fractions of Albizia Lebbeck bark extract (BFALB) and leaf extract (BFALL) were selected for further study. Both fractions were subjected to acute toxicity study. The 60 mg/Kg BFALB and 20 mg/Kg BFALL were selected. In pylorus ligation induced ulcer model, the parameters studied were gastric volume, pH, free acidity, total acidity and ulcer index. Ulcer index was also determined in ethanol and Indomethacin induced ulcer models. Pretreatment with BFALB and BFALL have shown decrease in ulcer index in all the experimental models of ulcer. The prior administration of both the fractions also reduced the total acidity, free acidity and increased the pH. However, the gastric volume was not reduced with 60 mg/kg of BFALB and significantly reduced with 20 mg/kg of BFALL (p<0.001). These results suggest that the antiulcerogenic compound(s) present in methanolic extract of Albizia Lebbeck bark and leave may be clustered in the n-butanol fraction, which will be investigated for the probable mechanisms of action.
INTRODUCTION:

Gastric ulcers, one of the most widespread disease states, are believed to be due to an imbalance between acid and pepsin along with weakness of the mucosal barrier \[1\]. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products, and drugs \[2\]. These agents have been implicated in the pathogeneses of gastric ulcer, including increased gastric acid and pepsin secretion, decreased gastric blood flow, the suppression of endogenous generation of prostaglandins, inhibition of mucosal growth and cell proliferation, and alteration of gastric mobility \[3\]. Although there are many products used for the treatment of gastric ulcers, most of these drugs produce several adverse reactions \[4\]. Plant extracts, however, are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers \[5\]. In traditional medicine for example, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers \[6, 7\].

Albizia lebbeck benth. (Mimosaceae) is a large, erect, unarmed and deciduous tree. Upon literature review it was found that, the leaves are used in ophthalmia \[8\]. The bark is used in bronchial asthma & other allergic disorders \[9\]. The flowers are useful in chronic cough & bronchitis \[10\]. The seeds are aphrodisiac \[8\], useful in inflammation, scrofula, skin disease, leprosy, leucoderma, chronic catarrh, seminal weakness, ophthalmopathy & poisoning \[9\]. The leaves of the plant Albizia lebbeck are rich in flavon, echinocystic acid, β-sitosterol and vicenin II etc \[9\]. The modern literature revealed that the plant is reported to possess anti-inflammatory \[11\], nootropic \[12,13\], anxiolytic \[13\], anticonvulsant \[14, 15\], antifertility \[16\], antidiarrhoeal \[17\] and antiasthamatic activity \[18\]. There is no data reference available in the literature regarding the antiulcer activity of isolated constituents of Albizia lebbeck bark and leaf extract either in humans or in any animal model. The present study has, therefore, been conducted to evaluate the antiulcer activity of isolated constituents of Albizia lebbeck bark and leaf extract using different in vivo ulcer models in rat.

MATERIALS AND METHODS:
Plant Material:

*Albizia lebbeck* bark and leaf were collected from Sarsa, near Sarsa Cross road, Gujarat in the month of December. The plant was authenticated by Dr. G. C. Jadeja, Prof. and Head of Botany, Department of Agricultural Botany, B.A. College of Agriculture, Anand Agriculture University, Anand. A herbarium specimen (Skcop-2010-1) is deposited in the college herbal museum for future reference.

Isolation of saponins:

The bark and leaf were shade dried separately at room temperature and pulverized. The powdered plant material (1 kg) was sequentially extracted three times with 3 L of petroleum ether, chloroform, methanol and water at room temperature for 48h in a soxhlet apparatus. All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven at 40°C. Each time before extracting with the next solvent, the marc was dried in an air oven below at 40°C. The marc was finally macerated with water for 24 hours to obtain the aqueous extract. The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on watch glass to observe that no residue remained after evaporation of the solvent. The extracts obtained with different solvents were collected and stored in a refrigerator. As described by Pal et al. [19], the residue was suspended in water, extracted with ethyl acetate and n-butanol (3 x 300 ml each) and the solution was evaporated to dryness in vacuum. The n-butanol soluble fraction of *Albizia Lebbeck* bark extract (BFALB) and n-butanol soluble fraction of *Albizia Lebbeck* leave extract (BFALL) were tested for the presence of saponins using haemolysis test and foam test as described earlier by Evans (1996) [20].

Animal:

Albino rats (Wistar) weighing 150-200g and albino mice weighing 20-25g of either sex were used in this study. They were procured from Flair Labs, Palsana, Surat. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C under 12 hrs dark/light cycle. They were fed with standard rat feed (VRK Nutritional Solutions) and water *ad libitum* was provided. The husk in the cages was renewed thrice a week to ensure hygiene and maximum comfort for animals. Ethical clearance for handling the
animals was obtained from the Institutional animal ethical committee prior to the beginning of the project work (Protocol No: IAEC/SKCOP/11-12/02).

Acute Toxicity studies:
The acute toxicity for \( n \)-butanol soluble fraction of *Albizia Lebbeck* bark extract (BFALB) and \( n \)-butanol soluble fraction of *Albizia Lebbeck* leave extract (BFALL) were determined on albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose method of OCED Guideline No.425 was adopted for toxicity studies \(^{[21]}\).

Anti-Ulcer Activity:
Indomethacin induced ulcer
The albino rats of either sex weighing between 180 – 200 gm were divided into four groups of six animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals were pretreated with Vehicle, standard drug (lansoprazole 8 mg/kg), 60 mg/kg of BFALB and 20 mg/kg of BFALL one hr before the administration of Indomethacin 30 mg/kg orally. The animals were then sacrificed by cervical dislocation after 4 hrs. The stomach was taken out and cut open along the greater curvature of stomach \(^{[22]}\). The number of ulcers per stomach were noted and severity of the ulcers were observed microscopically and scoring was done as per S. K. Kulkarni \(^{[23]}\): 0 for normal colored stomach, 0.5 for red coloration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcer between > 3 but < 5mm and 3 for ulcer > 5mm. Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated.

Ethanol (EtOH) induced ulcer
The albino rats of either sex weighing between 180 – 200 gm were divided into four groups of six animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals were pretreated with Vehicle, standard drug (lansoprazole 8 mg/kg), 60 mg/kg of BFALB and 20 mg/kg of BFALL. Ethanol (100%, 1ml/200 g, p.o) was administered to all the animals of group 1–4, 60 minutes after the respective treatments. The animals were sacrificed by cervical dislocation after one hour of EtOH administration and stomach was incised along the greater curvature and examined for ulcers \(^{[24]}\). The ulcer index was scored as mentioned above by the method of
Pylorus-ligated (PL) induced ulcer

Albino rats of either sex weighing between 180 – 220 g were divided into four groups of six animals each and fasted for 18 hrs and care was taken to avoid caprophagy. The animals were pretreated with Vehicle, standard drug (lansoprazole 8 mg/kg), 60 mg/kg of BFALB and 20 mg/kg of BFALL one hr prior to pylorus ligation. Animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period. After 6 hrs, stomach was dissected out; contents were collected into tubes for estimation of biochemical parameters. The stomach was taken out and cut open along the greater curvature and ulcers were scored and percentage protection was reported as mentioned in the above explained models.

Gastric acid Secretion:

The gastric juice was collected 6 hrs after pylorus ligation and centrifuged for 5 minutes at 2000 rpm and the volume of supernatant was noted. The pH of the gastric juice was recorded by the pH meter. Then the contents were subjected to analysis for free and total acidity. Free acidity and total acidity were determined using 0.01N NaoH and Topfer’s reagent containing phenolphthalein as indicator.

Statistical analysis:

Results were expressed as mean ± SEM, (n=6). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test by using Graph Pad Instat Software. p value less than 0.05 was considered to be statistically significant.

RESULTS:

Acute toxicity:

The mice treated with the 40 and 60 mg/kg of BFALB and 10 and 20 mg/kg of BFALL orally, exhibited normal behavior, i.e. they were alert, with normal grooming, touch response, and pain response. There was no sign of passivity, stereotypy, and vocalization. Their motor activity and secretory signs were also normal. In the
Animals treated with 300 mg/kg of BFALB and 100 mg/kg of BFALL, 50% of animal could not survive. Therefore 60 mg/kg of BFALB and 20 mg/kg of BFALL was selected for further study i.e. for screening of antiulcer activity.

**Antiulcer activity:**

The observations of control group indicated that Indomethacin (30 mg/kg) induced gastric ulcerations to the extent of 6.63 ± 0.51 (ulcer index). Pretreatment with BFALB and BFALL reduced the ulceration in a significant manner. The extent of gastro-protective effect of the BFALB and BFALL are 57.17% and 66.97% at 60 mg/kg and 20 mg/kg doses respectively. Similar results were obtained with ethanol induced ulcer model also. The BFALB and BFALL have shown gastro-protection 67.01% and 71.60% protection at 60 mg/kg and 20 mg/kg respectively. The results are compiled in Table No 1. The pyloric ligation has caused the accumulation of gastric secretions (9.49 ml) with pH 2.32. The total acidity and free acidity of the gastric secretions were 106.66 ± 6.82 and 99.25 ± 7.61 respectively. Pretreatment with BFALL reduced the volume of gastric secretion (1.95 ml at 20 mg/kg dose) and the pH was elevated up to 5.15. In addition, total acidity and free acidity were also reduced significantly. The results are compiled in Table No 2. Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with BFALB and BFALL have reduced them significantly (Table No 1).

**DISCUSSION:**

Nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin and indomethacin remain among the most commonly used pharmacological agents. These classes of substances do, however, have the ability to cause gastrointestinal ulceration, and this effect is linked to the ability of these agents to suppress prostaglandin synthesis. The co-administration of cholinomimetic agents, such as bethanechol, promotes a synergism with NSAIDs in the gastric lesion induced by the increased secretion of acid and pepsin in the stomach. Table 1 shows the antiulcer activity of the BFALB and BFALL in the Indomethacin induced ulcer model, a phenomenon which demonstrated significant reductions of 57.17% and 66.97%, respectively, in the damage to these gastric mucosa (p<0.001) as compared to the control value.
model, rat treated with lansoprazole showed the best results (85.53%). The increased synthesis of mucus and/or prostaglandins can be explained as the probable cytoprotective mechanism in this activity.

In EtOH-induced gastric ulcers, the lesions were characterized by multiple-hemorrhage red bands of different sizes along the long axis of the glandular stomach. As shown in Table 1, the treatment with lanzoprazole, BFALB and BFALL demonstrated significant inhibition of ulcerative lesion by 81.78%, 67.01% and 71.60%, respectively, as compared to the control value. The ability of the gastric mucosa to resist injury by endogenous secretions (acid, pepsin and bile) and by ingested irritants (e.g., alcohol) can be attributed to a number of factors that have been referred to collectively as mucosal defense [27]. The formation of gastric mucosal lesions by necrotizing agents such as HCl and EtOH has been reported to involve the depression of these gastric defensive mechanisms [29]. EtOH-induced gastric ulcers also promote stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissue injury [30]. EtOH-induced ulcers are not inhibited by antisecretory agents such as cimetidine, but are inhibited by agents that enhance mucosal defensive factors such as prostaglandins [31]. These results show that BFALB and BFALL probably have an antiulcerogenic effect related to cytoprotective activity, as the extract presented significant results in the ethanol model.

In the next step, we examined the biochemical alterations promoted by BFALB and BFALL in gastric-juice parameters in all of the treatments (Table 2). Ligation of the pylorus for 6 h produced accumulation of gastric juice, whereas BFALB showed lesser significant activity as compared to the BFALL. BFALB and BFALL, as well as Lansoprazole, did however decrease the gastric-acid secretion significantly; increase pH values and promote reduced acid output. The activities exhibited by these fractions are probably responsible for the synthesis of mucus, phospholipid, bicarbonate and prostaglandins, as well as reduced acid and pepsin outputs, consequently promoting the inhibition of gastric-acid secretion [31]. However the role
of antioxidant activity of BFALB and BFALL cannot be ruled out.

CONCLUSION:

In conclusion, the present study demonstrates that BFALB and BFALL possess antiulcer activity. The mechanism underlying this antiulcerogenic effect remains unknown, but the n-butanolic fractions contain substances, which increase endogenous prostaglandins and mucus synthesis. The antisecretory mechanism cannot, however, be dismissed. In addition, the property may be attributed to the antioxidant principles of plant, namely saponins, tannins and flavanoids. The n-butanolic fraction derived from the methanol extract is the effective fraction of crude plant material (bark and leave) and may lead to a novel gastroprotective drug. Further work is obviously required to fractionate, purify and identify the structure of the active principle(s) present in these fractions, as well as to isolate enough pure substance(s).

ACKNOWLEDGEMENTS:

The authors are thankful to H.H Shri Avichaldasji Maharajshri, President, Gnan Sampradaya Kelavani Khatu (Trust) for their constant support and providing all the facilities to carry out this research work.
Table No. 1: Effects of BFALB, BFALL and lansoprazole on Indomethacin, ethanol, and pylorus ligation induced gastric ulcers

<table>
<thead>
<tr>
<th>Gastric Lesion models</th>
<th>Treatment</th>
<th>Mean ulcer index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>Vehicle</td>
<td>6.63 ± 0.51</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lansoprazole 8 mg/kg i.p.</td>
<td>0.96 ± 0.48*</td>
<td>85.53%</td>
</tr>
<tr>
<td></td>
<td>BFALB 60 mg/kg p.o.</td>
<td>2.84 ± 0.58*</td>
<td>57.17%</td>
</tr>
<tr>
<td></td>
<td>BFALL 20 mg/kg p.o.</td>
<td>2.19 ± 0.41*</td>
<td>66.97%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Vehicle</td>
<td>6.97 ± 0.59</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lansoprazole 8 mg/kg i.p.</td>
<td>1.27 ± 0.24*</td>
<td>81.78%</td>
</tr>
<tr>
<td></td>
<td>BFALB 60 mg/kg p.o.</td>
<td>2.30 ± 0.47*</td>
<td>67.01%</td>
</tr>
<tr>
<td></td>
<td>BFALL 20 mg/kg p.o.</td>
<td>1.98 ± 0.45*</td>
<td>71.60%</td>
</tr>
<tr>
<td>Pylorus ligation</td>
<td>Vehicle</td>
<td>6.20 ± 0.27</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lansoprazole 8 mg/kg i.p.</td>
<td>0.50 ± 0.34*</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>BFALB 60 mg/kg p.o.</td>
<td>3.15 ± 0.38*</td>
<td>49.20%</td>
</tr>
<tr>
<td></td>
<td>BFALL 20 mg/kg p.o.</td>
<td>2.05 ± 0.27*</td>
<td>66.94%</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of six rats /treatment.

*P <0.001 Vs. Control
Table No. 2: Effects of BFALB, BFALL and lansoprazole on the biochemical parameters of gastric juice obtained from pylorus-ligature rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gastric Juice (ml)</th>
<th>Free Acidity (mEq/L/100g)</th>
<th>Total Acidity (mEq/L/100g)</th>
<th>Gastric pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>9.49 ± 0.62</td>
<td>99.25 ± 7.61</td>
<td>106.66 ± 6.82</td>
<td>2.32 ± 0.14</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>1.53 ± 0.26***</td>
<td>31.63 ± 3.06***</td>
<td>38.33 ± 3.76***</td>
<td>7.01 ± 0.21***</td>
</tr>
<tr>
<td>8 mg/kg i.p.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BFALB 60 mg/kg p.o.</td>
<td>4.15 ± 0.35**</td>
<td>51.15 ± 4.31***</td>
<td>58.15 ± 3.15***</td>
<td>3.17 ± 0.26**</td>
</tr>
<tr>
<td>BFALL 20 mg/kg p.o.</td>
<td>1.95 ± 0.67***</td>
<td>42.55 ± 3.27***</td>
<td>49.25 ± 2.61***</td>
<td>5.15 ± 0.58***</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of six rats / treatment

**P<0.01, *** P<0.001 Vs. Control

REFERENCES:


models in rats and mice. Phytomedicine 2001; 8, 94–100.


29. Kinoshita, M., Tsunehisa, N., Tamaki, H: Effect of a combination of ecabet sodium

