Studies on Activity of Various Extracts of *Albizia amara* against Drug induced Gastric Ulcers

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**Abstract**

*Albizia amara* is used as a medicinal herb by the tribes of forest regions of Western Ghats. It is used for headaches, backaches, stomach pain, piles and simple ulcers. The anti ulcer activity of various extracts of *Albizia amara* was investigated on ethanol, pylorus ligated and indomethacin induced pylorus ligated ulcer models in mice and rats. The common parameter determined was ulcer index. In the pyloric ligation model and indomethacin induced pyloric ligated models oral administration of both extracts such as petroleum ether and methanol, standard drug ranitidine and control group to separate groups of Wister rats of either sex was performed. Total acidity, volume of gastric juice, pH, percentage protection and ulcer index were assessed. In the case of the 90% ethanol-induced ulceration model in mice, there was a decrease in ulcer score and percentage protection in test groups of petroleum ether (46.72%), methanol (68%) and standard drug ranitidine (85.44%) when compared to the negative control. There was a decrease in gastric secretion and ulcer index among the treated groups i.e. petroleum ether (73.91%), methanol (80.72%) and in standard drug (91.59%) when compared to the negative control in pyloric ligated ulcers. In indomethacin induced pyloric ligated ulcer model in rats there was a reduction in ulcerative score in animals receiving petroleum ether (63.2%), methanolic (62.07%) and standard drug (80.02%) when compared to the negative control. The extract (250 mg/kg) showed significant (P < 0.01) reduction in gastric volume, free acidity and ulcer index as compared to control in all models.

**Key words:** Albizia amara; Pyloric ligation, Indomethacin induced ulcers, ulcer index.

**Introduction**

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors.[1] The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs.[2] These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility.[3] Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor “PAF”, leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric oxide).[4] The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

*Albizia amara* (Fabaceae) is a plant used in traditional system of medicine in India. The seeds of *Albizia amara* used as an astringent, treating piles, diarrhoea, gonorrhoea, leprosy, leucoderma, erysipelas and abscesses.[5] The flowers have been applied to boils, eruptions, swellings, ulcers, also regarded as an emetic, to tackle hair-fall and dandruff on the scalp and as a remedy for coughs and malaria. It is also known as “Kaunthia”, a native term originated from Hindi language, indicating an age-old usage of those species by Indian indigenous communities.[6] Other popular names are oil cake tree. Leaves were used to tackle hair-fall and dandruff on the scalp. It is used to make hair protective oils. A simple application involves soaking the leaves and flowers in water and using a wet grinder to make a thick paste, and used as a natural shampoo. However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native practitioners.
MATERIALS AND METHODS

Plant Material
The dried leaves of *Albizia amara* were supplied by Medicinal plants Revitalisation and Rehabilitation Centre, Sevaiyur, Tamilnadu and authenticated by Dr. S. Jha, Professor, Birla Institute of Technology, Mesra, Ranchi, India. The authenticated specimen has been deposited (PHARM/HS/14/09-10) in the department.

Preparation of extract
The crude drugs were dried under shade for 4-6 days. Then the dried materials were milled to powder. This powdered material was again dried in the oven at 40 °C for 4 h. The coarsely dried powdered leaves were extracted with Petroleum Ether (60°-80°) cold maceration for 72 h, and hot percolation by 90% methanol about 72 h. The extracts were recovered and concentrated to dryness. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of petroleum ether extract and methanolic extract was found to be 15.2% w/w and 7.2% w/w respectively and these extracts were used for further studies,[7]

Preliminary phytochemical screening
The phytochemical examinations of the extracts were performed by the standard methods.[7]

Studies of Acute Toxicity
Acute toxicity studies were carried out on Wistar rats according to standard procedures. Alcoholic extracts at doses of 50, 100, 250, 500, and 1000 mg/kg body weight were administered to separate groups of mice and rats (n = 5) after overnight fasting. Subsequent to administration of drug extract, the animals were observed closely for the first 3 h for any toxic manifestations such as increased locomotor activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 h. The animals were observed for a further week.[8]

Animals used
Wistar albino rats of either sex weighing between 150-250 gm and mice of either sex weighing between 20-50 gm were used. Institutional Animal Ethics Committee approved the experimental protocol (BIT/PH/IAEC/13/17:02:2010); animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Albino rats were used in this thesis was obtained from the Animal House of Birla institute of technology, Mesra Ranchi. The animals were housed in Polypropylene cages and maintained at 24 °C ± 2 °C under 12 h light/dark cycle and were fed ad libitum with standard pellet diet and had free access to water. The animals were given standard diet.

Indomethacin plus pylorus ligated induced ulcer in rats
Animals were fasted for 18 h but allowed access to water only prior to the experiment and divided into 4 groups (n = 6). Group I received the vehicle (1% Tween 80) Groups II and III received 250 mg kg−1 of methanolic and petroleum ether extract, while IV received ranitidine (60 mg/kg) respectively. Thirty minutes after oral administration of extract, ulcer was induced by oral administration of indomethacin (20 mg/kg). After 7 hr, the animals were scarified and the abdomen opened. The stomach was isolated and opened along the greater curvature and rinsed under a stream of water. ulceration on the gastric mucosa were observed with a hand lens (x10) and scored.[9]

Pyloric ligation in rats
Animals are divided into four groups, each consisting of six rats. First group having pyloric ligated. Second and Third Groups received methanolic extract and pet.ether extract in a dose of 200 mg/kg. Ranitidine, in the dose of 20 mg/kg was administered orally for Group Fourth as a reference drug for ulcer protective studies. After 45 min of extracts and Ranitidine treatment, pyloric ligation was be done by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. After 7 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed.[10,11]

Ethanol induced ulcer model
The ulcer was induced by administering ethanol. All the animals were fasted for 36 h before administration of ethanol. The animals were divided into four groups, each consisting of six mice. One Group represented the control group, receive ethanol. Second & third Groups received methanolic extract and Pet. ether extract 250 mg/kg respectively and, Ranitidine, in the dose of 60 mg/kg were administered orally for Fourth group as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1 ml/200 g) Orally, after 45 min of methanolic and pet.extract extract and Ranitidine treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 hr latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored. A score for the ulcer was study similar to pyloric ligation induced ulcer model.[12,13]
Effect on Indomethacin plus pyloric ligated ulcer model

The results are depicted in Table 1, which shows a decrease in ulcer score, volume of acid secretion, total acidity and pH in various extracts of *Albizia amara* i.e. methanolic extract and petroleum ether extract. In the group of animals in which ulcers were induced using indomethacin and pylorus ligature, the methanolic extract showed significant activity in all the selected parameters with % inhibition of ulcers and a significant reduction in total acidity, ulcer score and gastric secretion ($P < 0.001$). Standard drug treatment with ranitidine (60 mg/kg) also showed significant reductions in acidity, gastric secretion and ulcer score with a protective index of 66.37% when compared to positive control group. The petroleum ether extract produced protective index of 63.2%.

Pyloric ligation induced gastric ulcer

In pyloric ligation induced ulcer model, Oral administration of ME in the dose of 200 mg/kg dose showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. It was showing protection index of 80.72% at the dose of 250 mg/kg in comparison to control whereas Ranitidine as reference standard drug was reduction of ulcer 91.59%. (Results are tabulated in Table 2).

Ethanol-induced gastric ulcer

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. Methanolic extract has shown significant protection index of 68% and 46% with the dose of 250 mg/kg respectively in comparison to control, Ranitidine as reference standard drug was reduction of ulcer 85.44%. (Results are tabulated in Table 3).

Peptic ulcer disease is a chronic inflammatory disease characterized by ulceration in the upper gastro-intestinal tract. The pathophysiology of ulcers is due to an imbalance of alkaloids, flavonoids, tannins, terpenoids, phenols and steroids.

RESULTS AND DISCUSSIONS

The results of preliminary phytochemical screening of the both extracts of *Albizia amara* revealed that presence of various extract of *Albizia amara* against Drug induced Gastric Ulcers.
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The effect of *Albizia amara* extracts on the mucosal damage in the Pyloric ligation induced gastric ulcer model in rats reveals the decreases in ulcer scores. Treatment with successive extracts and standard drug shows the decreases i.e. methanol (80.72%), petroleum ether (73.91%), and ranitidine (91.59%). This indicates that the extracts have cytoprotective effects against the irritant actions caused by acids.[14]

The present investigation demonstrated the efficacy of *Albizia amara* plant extract against gastric ulceration induced by 3 experimental models viz., indomethacin plus pylorus ligated induced gastric ulceration, pylorus ligated induced ulceration and 90% ethanol induced ulceration. The plant extract *Albizia amara* and standard drugs produces a decrease in the ulcer number, total acidity, volume of gastric juice and pH in the indomethacin induced pyloric ligation ulcer model in rats. The curative ratio in this pyloric ligation model was 66.37%, 63.2% and 80.02% using methanol, petroleum ether and standard drug ranitidine, respectively. This indicates that the plant has antiulcerogenic, antisecretory and cytoprotective actions. Several investigators have reported the same results after plant extract treatment. Gastric mucus is known to protect the gastric mucosa against tissue damage by HCl produced by parietal cells. It consists of viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that covers the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant thus can reduce mucosal damage mediated by oxygen free radicals. The protective properties of the mucus barrier depend not only on gel structures but also on the thickness of the layer covering the mucosal layer. A decrease in gastric mucus renders the mucosa susceptible to injuries induced mainly by acids, NSAIDs and alcohol.[15]

### Table 2: Effect of *Albizia amara* on Pylorus Ligated Ulcers

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dosage mg/kg</th>
<th>Vol. of Gastric juice (ml/100 g)</th>
<th>PH</th>
<th>Total Acidity (mEq)</th>
<th>Ulcer index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1% Tween 80</td>
<td>4.28 ± 0.09</td>
<td>4.28 ± 0.09</td>
<td>116.8 ± 1.01</td>
<td>6.9 ± 0.78</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Methanolic Ext</td>
<td>250 mg</td>
<td>2.96 ± 0.06**</td>
<td>4.38 ± 0.06**</td>
<td>59.2 ± 1.01**</td>
<td>1.33 ± 0.11**</td>
<td>80.72</td>
</tr>
<tr>
<td>3</td>
<td>Pet. Ether Ext</td>
<td>250 mg</td>
<td>2.33 ± 0.10**</td>
<td>3.5 ± 0.03**</td>
<td>72.8 ± 0.8**</td>
<td>1.8 ± 0.30**</td>
<td>73.91</td>
</tr>
<tr>
<td>4</td>
<td>Ranitidine</td>
<td>60 mg</td>
<td>2.7 ± 0.06**</td>
<td>4.95 ± 0.02**</td>
<td>49.6 ± 1.60**</td>
<td>0.66 ± 0.11**</td>
<td>91.59</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM of 6 observations, Statistical comparison as follows, significant at **p < 0.01 compared to control group.

### Table 3: Effect of *Albizia amara* on ethanol induced ulcers

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dosage mg/kg</th>
<th>Vol. of Gastric juice (ml/100 g)</th>
<th>PH</th>
<th>Total Acidity (mEq)</th>
<th>Ulcer index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1% Tween 80</td>
<td>2.3 ± 0.04</td>
<td>3.13 ± 0.04</td>
<td>130.53 ± 1.23</td>
<td>6.25 ± 0.30</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Methanolic Ext</td>
<td>250 mg</td>
<td>1.63 ± 0.03**</td>
<td>4.6 ± 0.06**</td>
<td>102.4 ± 1.6**</td>
<td>2 ± 0.22**</td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td>Pet. Ether Ext</td>
<td>250 mg</td>
<td>2.03 ± 0.03**</td>
<td>3.5 ± 0.03**</td>
<td>76.8 ± 1.23**</td>
<td>3.33 ± 0.30**</td>
<td>73.91</td>
</tr>
<tr>
<td>4</td>
<td>Ranitidine</td>
<td>60 mg</td>
<td>1.2 ± 0.08**</td>
<td>5.26 ± 0.08**</td>
<td>49.6 ± 1.60**</td>
<td>0.91 ± 0.15**</td>
<td>91.59</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM of 6 observations, Statistical comparison as follows, significant at **p < 0.01 compared to control group.

between aggressive factors (acid, pepsin, H. pylori and NSAIDs) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). The integrity of the gastroduodenal mucosa is maintained through a hemostatic balance between these aggressive and defensive factors. The major cause of gastric ulcer is the chronic use of NSAIDs. Therapeutic and adverse effects of NSAIDs have been attributed to the ability of these drugs to inhibit the action of Cycoxygenase (COX). COX is responsible for the synthesis of prostaglandins that normally inhibit acid secretion, as well as having a protective effect on the gastric mucosa. Infection of the stomach mucosa with H. pylori- a gram-negative spiral shaped bacterium - is now generally considered to be a major cause of gastrointestinal ulcers. Treatment includes H2-receptor antagonists (Cimetidine), proton pump inhibitors (Omeprazole) and cytoprotectives (Misoprostol). Antacids, like aluminum hydroxide and magnesium hydroxide, are used often to neutralize excess gastric acidity in the stomach. Due to problems associated with recurrence after treatment, there is the need to seek an alternative drug against gastrointestinal ulcers.[14]

The present investigation demonstrated the efficacy of *Albizia amara* plant extract against gastric ulceration induced by 3 experimental models viz., indomethacin plus pylorus ligated induced gastric ulceration, pylorus ligated induced ulceration and 90% ethanol induced ulceration. The curative ratio in this pyloric ligation model was 66.37%, 63.2% and 80.02% using methanol, petroleum ether and standard drug ranitidine, respectively. This indicates that the plant has antiulcerogenic, antisecretory and cytoprotective actions. Several investigators have reported the same results after plant extract treatment. Gastric mucus is known to protect the gastric mucosa against tissue damage by HCl produced by parietal cells. It consists of viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that covers the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant thus can reduce mucosal damage mediated by oxygen free radicals. The protective properties of the mucus barrier depend not only on gel structures but also on the thickness of the layer covering the mucosal layer. A decrease in gastric mucus renders the mucosa susceptible to injuries induced mainly by acids, NSAIDs and alcohol.[15]

### CONCLUSION

Peptic ulcer is an imbalance between gastroduodenal mucosal defense mechanisms and offensive factors. Some studies have revealed that reactive oxygen species (ROS) and lipid
peroxidation are implicated in the pathogenesis of ethanol induced gastric lesions and gastrointestinal damage and that they attack and damage many biological molecules such as prostaglandins. After an initial reaction with ROS, a continuing chain reaction causes cell injury and ultimately cell death. [17,18,19] Therefore, treatment with antioxidants and free radical scavengers can decrease ethanol induced gastric mucosal damage. In the present study, a reduction in ulcer number in ethanol induced gastric ulceration in mice was found after various extract treatments, such as methanol (68%), petroleum ether (46.72%), of *Albizia amara* and the standard drug ranitidine (85.44%). This indicates cytoprotective actions in the plant extracts. Plant chemical substances such as flavonoids, tannins, terpenoids etc have been shown to scavenger free radicals and therefore are viewed as promising therapeutic drugs for free radical pathologies. Phytochemical tests revealed the presence of flavonoids and terpenoids in the extracts of *Albizia amara*. Some of the triterpenes are known as an antulcer agents and their action has been mentioned to be due to activation of cellular proteins, reduction of mucosal prostaglandin metabolism, cytoprotective actions and reduction of gastric vascular permeability. However, the mechanism by which this extract produces an antiulcer effect is not entirely clear. The result in present study seems to provide support for the use of *Albizia amara* as an antulcer drug in folk medicine. Therefore, also in view of its large use in India more detailed phytochemical and pharmacological investigations on the antiulcer effects and toxicity studies are required.

In all three ulcer experimental models the methanolic extract shows the best antiulcerogenic action, due to the presence of tannins and flavonoids, as in literature references. The present data obtained from various extracts of *Albizia amara* showed the presence of a gastro-protective effect and improved ulcer healing properties. The data also confirmed the traditional claim on the use of *A. amara* for treating gastric ulcers in the Indian subcontinent. Although at this time it is difficult to explain the exact mechanism involved with these crude extracts, the effects obtained on acute and chronic gastric lesions suggest a multifactorial mechanism, involving *A. amara* influence on free-radical scavenging properties, on endogenous prostaglandins and sulphydryl groups.[20]

**ACKNOWLEDGEMENT**

The authors are thankful to Birla Institute of Technology, Mesra, Ranchi for providing the necessary facilities.

T. Rajkumar thankfully acknowledges All India Council for Technical Education for providing Fellowship.

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