GASTRIC ULCER PROTECTIVE EFFECT OF MADHUCA LATIFOLIA ROXB BARK IN WISTAR RATS

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ABSTRACT: The present study was carried out to investigate the protective effect of ethanolic extract of Madhuca latifolia (Roxb.) bark (MLE) and aqueous extract of Madhuca latifolia (Roxb.) bark (MLA) in aspirin induced gastric ulcer. In both extract acute toxicity performed according to guidelines for selection of doses. The oral administration (400 mg/kg) of MLE & MLA extract reduced the ulcer index and prevents the development of gastric lesions by 76.57% and 81.14% (% of protection), respectively. The present studies provide preliminary data on the antiulcer potential of bark and support the traditional uses of the plant for the treatment of gastric ulcer.

INTRODUCTION: Herbal extracts gathered lots of attention of scientist working for development of safe and effective delivery of traditional medicines. They have played a significant role in maintain human health and improving the quality of human life for thousands of years. 1 Demand of herbal medicine is increasing because of their therapeutic effectiveness wide, higher safety margin and economical. Madhuca latifolia Roxb, synonyms Madhuca indica Gmel., Madhuca longifolia Macb. commonly known as Mahua in Hindi. 2

Madhuca latifolia Roxb is a large, much branched fast growing deciduous tree that grows up to 18m high and 80cm diameter at breast height. It is considered holy tree in India by many tribal communities because of their tremendous benefits. In tribal belt of Central India it is used for cultural and economic reasons. It provides live hood security to poor households who collect it both for self consumption and for sale. 3 Its various parts are used for nutritive, medicinal and other valuable products. Corolla, fruits, flower are used for nutritional purpose. 4

Phytochemical values of plant include presence of triterpenoids in fruit pulp; beta-sitosterol glucoside, quercetin and dihydroquercetin in nut shell; sugars, vitamins, phosphorus, calcium, iron, magnesium and copper presents in corolla. The sugars
identified are sucrose, maltose, glucose, fructose, arabinose and rhamnose. The seeds yielded Saponins-2, 3-di-O-glucopyranoside of basic acid (saponin A and saponin B). Saponins mixture obtained from seeds shows spermicidal activity. Trunk bark contains lupeol, beta-amyrin acetate, alpha-spinasterol, erythrodiol monocaprylate, betulinic acid and oleanolic acid caprylates. 

The pharmacological activities reported are antioxidant activity of methanolic extract of bark, antihyperglycemic and antioxidant activity of ethanolic extract of bark, antioxidant and hepatoprotective effect of bark, antihyperglycemic activity of methanolic extract of bark, nephro and hepatoprotective effect and antioxidant activity of leaves, anti-inflammatory, analgesic and antipyretic activity of aerial parts, analgesic effect of flower, antiepileptic activity of heartwood, anticancer activity of ethanol extract of leaves, wound healing activity of leaves, anti-inflammatory, anti ulcer and hypoglycemic activities of Seed Cake, antioxidant and antimicrobial activity.

Gastric ulcer is a localized area of erosion in the stomach mucus membrane that is exposed to gastric acid and pepsin. In gastric ulcer one of the key defensive mechanisms is the secretion of a mucus layer that protects gastric epithelial cells. Gastric mucus coats the mucosal surface of the stomach, slows ion diffusion, and prevents the irritation, auto digestion mucosal damage by gastric acid and pepsin. It results probably due to an imbalance between the aggressive/injurious factors and the mucosal defensive factors.

Inhibitions of gastric acid secretion most commonly by proton pump inhibitors followed by H2-blockers, anticholinergics, and ulcer protective drugs by sucralfate and bismuth compounds are current recommendations for drug therapy of gastric ulcers.

One of the major problems in the treatment of gastroduodenal ulcer is that, despite a healing rate of 80-100% after 4–8 weeks of therapy with H2 – antagonists and in proton pump inhibitors (potent suppressors of gastric acid secretion), the rate of ulcer recurrence (40-80%) within 1 year after suspending the treatment. The currently used these type of antiulcer drugs have adverse reactions such as gynaecomastia, joint pain, menstrual disorder, sore throat etc.

There is a need for the search of newer therapeutic antiulcer agents from plant sources which are traditionally used in many tribal areas and from the alternative therapy. So Madhuca latifolia Roxb. was selected for the study.

**MATERIALS AND METHODS: Materials:**

**Plant material:** The fresh bark of plant Madhuca latifolia Roxb. was collected from Kharsia, Raigarh district of Chhattisgarh (India) in the month of July. The species was identified and authenticated by Dr. Shiddamallayya N, National Ayurveda Dietetics Research Institute, Bangalore-560011. (Reference no. is Drug Authentication/SMPU/NADRI/BNG/2010-11/341) The collected bark was thoroughly washed with water to remove the adherent impurities and shade dried at room temperature and then reduced into coarse powder with a mechanical grinder and sifted through sieve no. 22 and stored in air tight container.

**Chemicals:** Aspirin and Ranitidine hydrochloride were purchased from Healthy Life Pharma Pvt. Ltd. Mumbai, India.

**Methods:**

**Preparation of extract:** The coarsely powdered material (300gm) was subjected to extraction with ethanol (1000 ml) in a soxhlet apparatus by using hot continuous extraction method at 60°C and separately 300gm powdered drug was extracted with distilled water (1500 ml), by cold maceration method. The extracts obtained were evaporated to dryness at a temperature below 30°C to yield ethanolic extract (6.82% w/w) and aqueous extract (4.36% w/w).

**High performance thin layer chromatography (HPTLC) studies:** HPTLC studies of the MLE and MLA were carried out using HPTLC applicator Camag Linomat IV, HPTLC scanner Camag TLC scanner II and software for interpretation of data. An aluminium plate (10cm×10cm) precoated with silica gel (Merck 60 F 254) was used as adsorbent. Volume of sample loaded 10 μl. The plates were developed using hexane: ethyl acetate (8:2) and...
ethyl acetate: methanol: toluene: (6:2:2) for MLE and MLA respectively in a development mode Camag Twin trough chamber.

**Antiulcer Activity (in- vivo)**

Animals: Adult albino male Wistar rats (180-200 gm) were obtained from the animal house of School of Pharmacy, Chouksey Engineering College, Bilaspur (C.G.), India. All the animals were housed under standard laboratory condition at controlled room temperature 22±3°C and relative humidity 60±5% with 12 hrs light and 12hrs dark cycle. Animals were allowed free access to standard dry pellet diet and water ad libitum. The animals were randomly select and grouped in cages and acclimatized to laboratory conditions for seven days before commencement of the experiment. All experiments were performed in accordance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal). All animal experimental procedure were reviewed and approved by IAEC (reg. no.: 1257/AC/09/CPCSEA/2010/15).

**Acute toxicity studies:** The acute toxicity studies of MLE and MLA were carried out in Female Wistar rats (weighing180-200gm) by fixed dose method of OECD guide line no 420.

**Aspirin induced antiulcer activity:** Twenty four male Wistar rats (weighing180-200gm) were taken. They were divided into four groups of six rats each (n=6). The groups were as follows:

**Group I:** vehicle (1ml/kg) + aspirin (200mg/kg) and was kept as control.

**Group II:** Ranitidine (50mg/kg) + aspirin (200mg/kg) and was kept as standard.

**Group III:** MLE (400mg/kg) + aspirin (200mg/kg).

**Group IV:** MLA (400mg/kg) + aspirin (200mg/kg).

All the animals were fasted for 24 hours before the study but had free access to water. After fasting period ranitidine and all the sample of plant extracts were given orally. After 30 minutes of treatment with drug, aspirin was given orally. The animals were then sacrificed by cervical dislocation 5 hours after the treatment. The Stomachs were cut open along the greater curvature and rinsed with water to remove the gastric contents and blood clots & examined grossly.

The ulcer index was evaluated according to severity of lesions formed and ulceration was scored using magnifying lens and the ulcer scored according to its severity in comparison with that of standard by using the described scale. i.e. 0= Normal stomach; 0.5= Red colouration; 1= Spot ulcer; 1.5= Hemorrhagic streak, 2.0= Ulcers, 3.0= Perforation. The mean ulcer index in each group was calculated and expressed the percentage of inhibition using the following formula:

\[
\text{% Inhibition} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100
\]

Histopathological parameters were studies and compared between all four groups to confirm the ulcer score.

**Statistical analysis:** All data obtained were expressed as the Mean ± Standard error of mean (SEM). Statistical significance was analyzed by using one way Analysis of Variance (ANOVA) followed by Dunnett’s test, with the level of significance set at p<0.05 and p<0.01 was considered highly significant.

**RESULTS:**

**HPTLC studies:** The preliminary HPTLC studies of ethanolic and aqueous extract of Madhuca latifolia Roxb bark revealed that hexane: ethyl acetate (8:2) solvent system was ideal for MLE and gave six phytoconstituents having R$_f$ values 0.06, 0.35, 0.58, 0.62, 0.72 and 0.82 (Figure 1) while solvent system ethyl acetate: methanol: toluene: (6:2:2) was ideal for MLA which gave nine phytoconstituents having R$_f$ values 0.07, 0.09, 0.18, 0.31, 0.36, 0.45, 0.56, 0.69 and 0.81 (Figure 2).

**FIGURE 1: HPTLC FINGERPRINT PROFILE OF MLE**
Acute toxicity studies: The acute toxicity studies of MLE and MLA showed no animal died even at 2000 mg/kg and hence both the extracts were treated as non-toxic and 1/5th (400 mg/kg) of the 2000 mg/kg was selected for further investigations.

Aspirin induced antiulcer activity: The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug groups with that of the vehicle control and standard ranitidine. In case of aspirin induced ulcers, the both extract showed significant reduction of ulcers (Table 1). Results are also supported by histological studies (Figure 3), which showed that rats pre-treated with MLE, MLA and ranitidine significantly (P<0.01) inhibited the gastric lesions formation and erosion, induced by aspirin compared to rats pre-treated with vehicle. When compared the both extract MLA showed high % of protection (81.14%) as compared to extract MLE (76.57%).

DISCUSSION: Present study emphasized to focus protective effect of Madhuca latifolia (Roxb) bark in gastric ulcer. The antiulcer activity of MLE and MLA was assessed in aspirin induced gastric ulcer. Twenty four male Wistar rats were divided into four groups; six rats in each. Pre-treatment with (MLE) (400mg/kg), (MLA) (400mg/kg) extract of Madhuca latifolia (Roxb.) bark reduced gastric ulceration when compared with vehicle control group rats. When compared the both extract MLA showed high % of protection (81.14%) as compared to extract MLE (76.57%) (Table1). It may be due to presence of more number of phytocconstituents in extract MLA, which are identified by HPTLC fingerprint.
TABLE 1: EFFECT OF MADHUCA LATIFOLIA BARK EXTRACT ON ASPIRIN-INDUCED GASTRIC ULCER

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Oral dose</th>
<th>Ulcer index</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>1ml/kg</td>
<td>1.75±0.3</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Ranitidine</td>
<td>50mg/kg</td>
<td>0.25±0.11**</td>
<td>85.71</td>
</tr>
<tr>
<td>III</td>
<td>MLE</td>
<td>400mg/kg</td>
<td>0.41±0.15**</td>
<td>76.57</td>
</tr>
<tr>
<td>IV</td>
<td>MLA</td>
<td>400mg/kg</td>
<td>0.33±0.16**</td>
<td>81.14</td>
</tr>
</tbody>
</table>

Values in Mean±SEM with n=6, * Symbol represent Statitical significance. **p<0.01. One way ANOVA which came with significant difference in column means, supported by Dunnett test which compared control Vs std. & extract & found highly significant at p<0.01. SEM: Standard error mean, ANOVA: Analysis of variance, Vc: Versus, Std.: Standard

CONCLUSION: Madhuca latifolia Roxb. bark extracts (MLE and MLA) showed good anti-ulcer (in vivo) activities. Both the plant extracts showed significant prevention of the formation of lesions and decrease ulcer index which indicates that plant is potential for the NSAIDs induced gastric ulcer. Further HPTLC chromatogram of extracts showed good separation of phytoconstituents at different RF in optimize solvent system. This study can be extended for the isolation of pure compounds from the extracts and their biological activity.

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REFERENCES: