Anti-ulcer and antioxidant activity of leaves of *Madhuca indica* in rats

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**SUMMARY**

The leaves of *Madhuca (M.) indica* J.f.Gmel. (Sapotaceae) have been used traditionally in folk medicine due to its astringent properties and are effective in treatment of eczema and snake bites. Methanolic extract of *M. indica* is rich in tannins and has been proven experimentally to possess antibacterial activity. The present study was undertaken to evaluate the anti-ulcer and antioxidant activity of *M. indica* in rats. The methanolic extract of leaves of *M. indica* was tested at various doses (75, 150 and 300 mg/kg, p.o.) for its effect on gastric secretion and gastric ulcers in pylorus- ligation and on ethanol- induced gastric mucosal injury in rats. The significant reduction in ulcer index in both the models along with an increase in the pH of the gastric fluid and mucin content of stomach, and the acid secretory parameters such as total acidity and volume of gastric fluid were also significantly reduced along with reduction in the pepsin activity in pylorus- ligated rats proved the anti-ulcer activity of *M. indica*. The increase in the levels of superoxide dismutase, catalase and reduced glutathione and decrease in lipid peroxidation in both the models proved the antioxidant activity of *M. indica*. Thus it can be concluded that *M. indica* possesses anti-ulcer activity, which can be attributed to its antioxidant mechanism of action.

**Key words:** Anti-ulcer; Antioxidant; Lipid peroxidation; Superoxide dismutase; Catalase; Reduced glutathione

**INTRODUCTION**

Peptic ulcer is one of the most common gastrointestinal diseases. In recent years, a widespread search has been launched to identify new anti-ulcer drugs from natural sources. Peptic ulcer disease (PUD) is caused by disruption of gastric mucosal defense and repair system. The recurrence rates of PUD are high and have been associated with several factors, including persistent *Helicobacter pylori* infection, sustained presence of mucosal damaging factors (e.g. use of non-steroidal anti-inflammatory drugs) and diminished mucosal defense ability (Hawkey *et al.*, 2000; Crespo and Suh, 2001).

Reactive oxygen species (ROS) which include superoxide anions and hydroxyl radicals have been implicated in several degenerative diseases including hypercholesterolemia, atherosclerosis, carcinogenesis, diabetes mellitus, ischemic reperfusion cardiac injury and digestive system disorders such as hypersecretion and gastric mucosal damage (Dhuley, 1999). It has been shown that there is
alteration in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus-igation induced (Rastogi et al., 1998) and ethanol induced (Pihan et al., 1987) ulceration in rats. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease (Barry, 1991).

Herbal extracts and chemical constituents have a long history of traditional use for treating ulcers, and animal studies have supported the efficacy of many herbs for the prevention and treatment of PUD (Borrelli and Izzo, 2001).

*Madhuca* (*M.*) *indica* commonly known as Mahua is a middle sized large deciduous tree, which grows to a height of 10 - 15 m. The leaves are elliptical with a pointed tip and entire margin. The plant is widely distributed in the forest of Western India. The literature review indicates that leaves of *M. indica* possess potent antioxidant principles as flavonols, myricetin and quercetin. Quercetin-3-galactoside and β-d-glucoside were identified in the ethyl acetate mother liquor (Subramanian and Nair, 1972). Sitosterol, β-carotene, stigmasterol, oleanolic acid, palmitic acid, erythrodiol, myricetin-3-O-l-arabinoside were also isolated from the leaves (Khare, 2004).

The plant of *M. indica* is known to possess various therapeutic properties and has been one of the noteworthy plants mentioned in various medicinal systems. It is a good laxative and is used in treating habitual constipation, piles and hemorrhoids. The leaves of *M. indica* have been reported to contain myricetin rhamnoside (Subramanian and Nair, 1972) and the tannin content in the leaves was found to be 4.86% (Daniel et al., 1978). Myricetin showed antioxidant property in neuroblastoma cell model of rotenone neurotoxicity (Molina-Jimenez et al., 2005), β-sitosterol modulates antioxidant enzyme response in Raw 264.7 macrophages (Moreno and Vivancos, 2005). This supports the possibility that *M. indica* may exhibit antioxidant potential against various oxidative stress conditions. Hence *M. indica* may prove to be effective in preventing ulcer formation and in ulcer healing.

*M. indica* is mentioned in ayurvedic text as a remedy for peptic ulcer (Kirtikar and Basu, 1988). There has been no scientific report available on the traditional claims of the effect of leaves of *M. indica* in gastrointestinal disorders. Thus the present study (a) evaluated the anti-ulcer effect of leaves of *M. indica* in pylorus-ligated and ethanol induced gastric lesions in rats and (b) determined the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and the levels of lipid peroxidation (LPO) and reduced glutathione (GSH) in the stomach tissues of all treated groups to check whether *M. indica* exerts anti-ulcer action by means of its antioxidant activity.

**MATERIALS AND METHODS**

**Plant material**
The leaves of *M. indica* (Sapotaceae) were collected from areas adjoining the district of Amravati (Latitude 20°56' North and Longitude 77°48' East), Maharashtra, India. Leaves were identified and authenticated by Dr. A.M. Mujumdar, taxonomist, Agharkar Research Institute (Pune, India). The voucher specimen bearing no. AHMA-R-069 was deposited in the herbarium of the institute.

**Preparation of Madhuca indica leaves extract**
The fresh shade-dried leaves were powdered in an electric grinder to coarse size. The powder was then macerated in methanol for 72 h with intermittent shaking. The solvent was allowed to evaporate at room temperature. The yield of powdered leaves of *M. indica* was found to be 10.5% w/w.

**Animals**
Female albino rats of Wistar strain weighing between 180 to 225 g were used for the experiments. The animals were purchased from the National Toxicology
Centre, Pune. The animals were housed in polypropylene cages at 22 ± 3°C and relative humidity of 45 - 55% in a clean environment under 12:12 light:dark cycle. The animals were fed ad libitum with standard pellet diet (Amrut laboratory animal feed, Sangli-India) and had free access to water. All the experiments were approved and conducted as per the guidelines of local animal ethical committee.

**Experimental design**

The animals were divided into six groups, each containing six rats. Group 1 represented the normal control group, Group 2 represented the control Pylorus-ligated control group, which received 5 ml/kg body weight of vehicle (1% gum acacia, p.o.) Groups 3 - 5 received methanolic extract of leaves of *M. indica* orally at the doses of 75, 150 and 300 mg/kg body weight, respectively. Group 6 received ranitidine (2.5 mg/kg, i.p.), which served as the standard group.

**Study of anti-ulcer and antioxidant activity using pylorus ligation method**

The method of Shay rat ulcer (Shay *et al.*, 1945) was adopted. Rats were fasted for 48 h. The methanolic extract of leaves of *M. indica* was administered to the animals. During the course of experiment, food was withdrawn but the animals had free access to water. After the pretreatment period of 1 h the animals were anaesthetized with anesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcer was measured using a vernier caliper. Ulcer index was determined by following the scoring method of Suzuki *et al.* (1976).

Score 1: an ulcer of maximal diameter of 1 mm.
Score 2: an ulcer of maximal diameter of 1 - 2 mm.
Score 3: an ulcer of maximal diameter of 2 - 3 mm.
Score 4: an ulcer of maximal diameter of 3 - 4 mm.
Score 5: an ulcer of maximal diameter of 4 - 5 mm.
Score 10: an ulcer over 5 mm in diameter.
Score 25: a perforated ulcer.

The mucin content of stomach was estimated by the method described by Rafatullah *et al.* (1994).

After macroscopic analysis, the stomach of rats of group 1 (normal control) group 2 (control) and groups 3 - 5 (*M. indica* treated groups) was then weighed and homogenized in chilled tris buffer (10 mM, pH 7.4) at a concentration of 10% w/v. The homogenates were centrifuged at 10,000 g at 0°C for 20 min using Remi C-24 high-speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation (MDA content), endogenous anti-oxidant enzymes (SOD and CAT) and reduced glutathione (GSH). The sediment was resuspended in ice cold tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of total proteins.

**Study of anti-ulcer and antioxidant activity using ethanol-induced ulcer method**

The method described by Dhuley (1999) was adopted. The rats were fed once daily for a period of 10 days with the methanolic extract of leaves of *M. indica*. On the 10th day, 1 h after the final dose of methanolic extract of leaves of *M. indica*, 96% ethanol (5 ml/kg, p.o) was administered to the overnight fasted rats of all groups. The animals were then sacrificed 1 h after the dose of ulcerogen.
The stomach was then removed, incised along the greater curvature and its mucosal erosion was determined randomly by measuring the area of the lesion. The sum of the areas was expressed as ulcer index (mm$^2$). The stomach was then weighed and processed for antioxidant estimations as mentioned in previous section.

**Biochemical parameters**
Superoxide dismutase (SOD) was determined by the method of Mishra and Fridovich (1972). Catalase was estimated by the method of Hugo Aebi as given by Colowick et al. (1984). Reduced glutathione was determined by the method of Moron et al. (1979). Lipid peroxidation or malondialdehyde (MDA) formation was estimated by the method of Slater and Sawyer (1971). Total proteins were determined by the method of Lowry et al. (1951).

**Statistical analysis**
Results of all the above estimations have been indicated in terms of mean ± SEM. Difference between the drug treated groups and the control group was statistically determined by analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparison test, with the level of significance set at $P < 0.05$.

### RESULTS

**Study of anti-ulcer and antioxidant activity using pylorus ligation method**
It was observed that in the vehicle treated control group the ulcer index was 97.16 ± 4.19 and the maximum number of ulcers were of the ulcer score 4 and 5. In the rats of this group a number of perforated ulcers (score 25) were also observed.

Methanolic extract of *M. indica* was found to produce significant decrease in the ulcer index at all the three doses; the percentage reduction being 18.35, 57.28 and 80.61% respectively. Significant reduction in ulcer index was observed at the doses of 75 mg/kg ($P < 0.01$), 150 mg/kg ($P < 0.001$) and 300 mg/kg ($P < 0.001$) as compared to the pylorus ligated control group. All the ulcers were of score 1, 2, 3 and 4 and no perforated ulcers were observed. *M. indica* also significantly reduced the volume of gastric fluid along with a significant reduction in total acidity and pepsin content of stomach as compared to the pylorus ligated control group. Whereas it significantly increased the pH of gastric fluid along with a significant elevation in mucin content of stomach as compared to the pylorus ligated control group, proving its anti-ulcer activity (Table 1). These results were comparable to the protective effect exerted by ranitidine.

Ranitidine (2.5 mg/kg, i.p.) was found to produce

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group</th>
<th>$M. indica$ (75 mg/kg)</th>
<th>$M. indica$ (150 mg/kg)</th>
<th>$M. indica$ (300 mg/kg)</th>
<th>Ranitidine (2.5 mg/kg)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index</td>
<td>97.16 ± 4.19</td>
<td>95.23 ± 4.06**</td>
<td>95.10 ± 4.68***</td>
<td>93.96 ± 2.35***</td>
<td>91.66 ± 4.61**</td>
<td>189.01</td>
</tr>
<tr>
<td>Volume of gastric fluid (ml)</td>
<td>26.10 ± 0.72</td>
<td>12.64 ± 0.80**</td>
<td>12.67 ± 1.09**</td>
<td>12.68 ± 1.12**</td>
<td>12.69 ± 1.13**</td>
<td>22.725</td>
</tr>
<tr>
<td>PH of gastric fluid</td>
<td>2.16 ± 0.27</td>
<td>2.16 ± 0.27</td>
<td>2.17 ± 0.27**</td>
<td>2.17 ± 0.27**</td>
<td>2.17 ± 0.27**</td>
<td>15.23</td>
</tr>
<tr>
<td>Total acidity (mEq/1/100g)</td>
<td>78.33 ± 4.31</td>
<td>78.06 ± 4.31</td>
<td>78.07 ± 4.31</td>
<td>78.08 ± 4.31</td>
<td>78.09 ± 4.31</td>
<td>73.271</td>
</tr>
<tr>
<td>Pepsin (µg tyrosine/ml)</td>
<td>124.25 ± 4.70</td>
<td>124.26 ± 4.70</td>
<td>124.27 ± 4.70</td>
<td>124.28 ± 4.70</td>
<td>124.29 ± 4.70</td>
<td>37.328</td>
</tr>
<tr>
<td>Mucin (µg Alcian blue/g wet tissue)</td>
<td>8.54 ± 0.40</td>
<td>12.67 ± 1.09**</td>
<td>12.68 ± 1.09**</td>
<td>12.69 ± 1.09**</td>
<td>12.70 ± 1.09**</td>
<td>32.569</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. Drug treated groups were compared with control group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = non significant. Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.
a significant ($P < 0.001$) reduction in ulcer index; the percentage reduction being 90.57%. It also significantly reduced the volume of gastric fluid, total acidity and pepsin content of stomach whereas it significantly increased the pH of gastric fluid and the mucin content of stomach as compared to pylorus ligated control group.

Pylorus-ligation was found to increase the lipid peroxidation and decrease SOD, catalase and reduced glutathione, thus leading to oxidative stress in the pylorus ligated control group. Administration of methanolic extract of *M. indica*, at the doses of 75, 150 and 300 mg/kg brought about a significant reduction in lipid peroxidation whereas it significantly increased the content of reduced glutathione as compared to the pylorus ligated control group. The activity of antioxidant enzyme SOD was found to be significantly increased at the dose of 300 mg/kg. Catalase activity was found to be significantly increased at the doses of 150 and 300 mg/kg when compared to the pylorus ligated control group (Table 2).

**Study of anti-ulcer and antioxidant activity using ethanol-induced ulcer method**

Administration of ethanol produced significant ulcers (273.33 ± 6.47) in the control group. There was a significant reduction in ulcer index at all the three doses of *M. indica* as well as in ranitidine treated group (Table 3). Ethanol administration was found to increase lipid peroxidation and decrease SOD, catalase, and reduced glutathione in the control group when compared to normal control rats. Administration of *M. indica* significantly decreased lipid peroxidation and increased the levels of SOD at all the dose levels whereas the levels of catalase and reduced glutathione were significantly increased at the dose levels of 150 and 300 mg/kg (Table 4).

**DISCUSSION**

The present study showed that pretreatment with the methanolic extract of *M. indica* exhibited both gastroprotective and ulcer healing properties in rats, probably as a result of the antioxidant action of the drug.

In most of the cases etiology of peptic ulcer is not clearly known, it is generally accepted that it results from an imbalance between aggressive factors and healing factors.

<table>
<thead>
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<th>GROUPS</th>
<th>Ulcer index</th>
</tr>
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<tbody>
<tr>
<td>Control Group</td>
<td>273.33 ± 6.47</td>
</tr>
<tr>
<td><em>M. indica</em> (75 mg/kg)</td>
<td>98.83 ± 3.34*** (63.84)</td>
</tr>
<tr>
<td><em>M. indica</em> (150 mg/kg)</td>
<td>52.28 ± 2.40*** (80.87)</td>
</tr>
<tr>
<td><em>M. indica</em> (300 mg/kg)</td>
<td>21.23 ± 1.36*** (92.23)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. Drug treated groups were compared with Control group, ***$P < 0.001$ Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.

### Table 3. Effect of *M. indica* on the ulcer index in stomach of ethanol-treated rats

<table>
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Values are expressed as mean ± S.E.M. Control group was compared with Normal Control group. Drug treated groups were compared with Control group. $*P < 0.05; ** P < 0.01; *** P < 0.001; ^NS = non significant.$
the maintenance of the mucosal integrity through the endogenous defense mechanisms (Piper and Stiel, 1986). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production.

The anti-ulcer effect of M. indica was tested against gastric lesions induced by pylorus-ligation and ethanol, the experimental models related to lesion pathogenesis with production of reactive species.

Pylorus-ligation induced ulcers are caused due to the accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa (Bhattacharya and Goe, 1991). In addition to gastric acid secretion, reflux or neurogenic effect has also been suggested to play an important role in the formation of gastric ulcer in this model (Goswami et al., 1997). Methanolic extract of leaves of M. indica prevented the mucosal lesion induced by pylorus-ligation and ethanol. The M. indica reduced the ulcer index and pepsin content of stomach along with significant reduction in acid secretory parameters like total acidity and volume of gastric fluid indicating the inhibition of aggressive factors. There was an increase in the pH of the gastric fluid and mucin content of stomach indicating cytoprotective effect of M. indica. These effects of M. indica treatment on the parameters that influence the initiation and induction of ulceration may be considered as highly desirable property of anti-ulcerogenic agent.

ROS are involved in the pathogenesis of pylorus-ligation induced (Rastogi et al., 1998) and ethanol-induced (Pihan et al., 1987) gastric mucosal injury in vivo. Results in the present study also indicate similar alterations in the anti-oxidant status after pylorus-ligation induced ulcers. Much attention has been recently focused on ROS contents, such as superoxide, hydroxyl radicals (OH) and singlet oxygen (Ames et al., 1993). ROS cause lipid peroxidation in membranes by attacking unsaturated fatty acids (Takeuchi et al., 1991; Ames et al., 1993). Therefore antioxidant defense system, including anti-oxidant enzymes, foods and drugs are important in the prevention of the toxic ROS effects (Takeuchi et al., 1991; Kviets and Smith, 1998; Bafna and Balaraman, 2004).

Preventive anti-oxidants, such as SOD and CAT enzymes are the first line of defense against reactive oxygen species. GSH is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation (Halliwell, 1995). Administration of methanolic extract of leaves of M. indica resulted in a significant increase in the SOD, CAT and GSH levels as compared to the control animals, which suggests its efficacy in preventing free radical-induced damage.

Table 4. Effect of M. indica on the antioxidant parameters in stomach of ethanol-treated rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SOD (Unit/mg protein)</th>
<th>Catalase (µmoles of H₂O₂ consumed/min/mg protein)</th>
<th>Reduced glutathione (µg of GSH/mg protein)</th>
<th>Lipid peroxidation (nmoles of MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>5.78 ± 0.42</td>
<td>8.82 ± 0.19</td>
<td>2.42 ± 0.16</td>
<td>2.78 ± 0.27</td>
</tr>
<tr>
<td>Control Group</td>
<td>2.17 ± 0.12***</td>
<td>5.52 ± 0.34***</td>
<td>0.72 ± 0.26***</td>
<td>7.69 ± 0.64***</td>
</tr>
<tr>
<td>M. indica (75 mg/kg)</td>
<td>3.58 ± 0.18*</td>
<td>6.23 ± 0.23NS</td>
<td>1.01 ± 0.16NS</td>
<td>4.48 ± 0.82**</td>
</tr>
<tr>
<td>M. indica (150 mg/kg)</td>
<td>5.03 ± 0.38***</td>
<td>7.31 ± 0.17***</td>
<td>1.68 ± 0.14**</td>
<td>2.67 ± 0.36***</td>
</tr>
<tr>
<td>M. indica (300 mg/kg)</td>
<td>6.28 ± 0.34***</td>
<td>7.89 ± 0.29***</td>
<td>2.57 ± 0.15***</td>
<td>2.19 ± 0.14***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. Control group was compared with Normal Control group. Drug treated groups were compared with Control group. *P < 0.05; **P < 0.01; ***P < 0.001; NS = non significant.
Superoxides produced by peroxidases in the tissues might damage the membranes and stomach tissues by increasing the lipid peroxidation (Takeuchi et al., 1991). Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids. Biological membranes are often rich in unsaturated fatty acids. Therefore it is not surprising that membrane lipids are susceptible to peroxidative attack (Cheesman, 1993).

Similarly, present study showed that there was a significant increase in lipid-peroxidation in rat stomach tissues of control animals. However, significant decrease in lipid-peroxidation was observed by the administration of all the doses of M. indica in both the experimental models, which suggests its protective effect.

Thus the study proved that leaves of M. indica possess anti-ulcer activity, which may be due to its antioxidant mechanism of action. However, further work is warranted to identify the active constituent(s) responsible for such an effect.

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