“ULCER HEALING POTENTIAL OF AEGLE MARMELOS FRUIT SEED”
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ABSTRACT
The present study was aimed to investigate anti-ulcer activity of methanolic and aqueous extract of Aegle marmelos seeds using indomethacin induced ulceration, stressed induced ulceration and pylorus ligation induced ulcerations. Ranitidine (50 mg/kg) was used as standard antiulcer agent. The ulcer index and percentage protection was estimated in all three models. Volume of gastric secretion, free acidity, total acidity and pH was estimated in pylorus ligation induced ulcer model. Methanolic extract showed significant (p<0.01) ulcer protective action at the doses of 200 and 400 mg/kg b.w. in all animal model. The aqueous extract was also found to possess significant (p<0.05) ulcer healing property at the same doses as of methanolic extract. A significant reduction in volume of gastric juice, free acidity and total acidity, along with increase in pH was observed in pylorus ligated rats. The antiulcer property of both the extracts was attributed due to the presence of quercetin like (Flavonoid) contents.

Keywords: Aegle marmelos, Ulcer, Pylorus ligation, Ulcer index

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INTRODUCTION

Peptic ulcers are deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa [1]. It is generally accepted that it results from an imbalance between aggressive and defensive factors [2]. The common causes for the induction of gastric ulcers are high acid and peptic content, irritation, poor blood supply, poor secretion of mucus, H. pylori infection, long term use of NSAIDs [3] and adrenocorticosteroids [4], cigarette smoking [5, 6, 7], use of alcohol [8], abnormal diet, psychological stress [9, 10], genetic factors and life style. The gastric ulcers also have association with some disease conditions [11]. The severe physiological stressed conditions, such as burns, CNS trauma, surgery & severe medical illness may also produce gastric ulcers. [12, 13].

A number of medicaments have been used for the treatment of gastric ulcers such as antacids, proton pump inhibitors and antihistaminics, but most of these drugs produce several adverse reactions [14]. Thus, there is need for more effective and safe anti-ulcer agents. However, plants are the most important source for the new drug development because of the growing recognition that the natural products are non-toxic, less side effects and available at affordable price [15].

Bael (Aegle marmelos, Linn.), family rutacae, is also known as bale fruit tree, is a moderate sized, slender, aromatic tree, 6.0 - 7.5 m in height, and 90 to 120 cm in girth, with a some what fluted bole of 3.0-4.5 meter growing wild throughout the deciduous forests of India. It is indigenous to Indian subcontinents and mainly found in tropical and subtropical regions [16].

MATERIAL AND METHODS:

Chemical Used: All the chemicals and reagents used for the study were of analytical grade. Methanol (Merck, Germany), Indomethacin (SRL, India) were incorporated in study.

Collection and authentication of plant material

The fresh, unripened fruits of Aegle marmelos were collected from healthy trees were growing at very hygiene and polluted free area in the month of may-June, located at various regions of Jaipur, Rajasthan. The plant part was identified and authenticated from the department of Botany, University of Rajasthan, Jaipur, Rajasthan, and voucher specimen was deposited, viz no. RUBL: 20866.

Preparation of extract

Freshly collected seeds of Aegle marmelos were dried at 30 °C and at 18.9 % relative humidity condition and milled with sieve to remove excess of mucilaginous hair. The plant extract was prepared using two different laboratory grade solvents (double distilled water & methanol);

- Preparation of aqueous extract: The dried powdered plant part (1.0 kg of Aegle marmelos seeds) was extracted with 4.0 liters of double distilled water for 72 hours in a round bottom flask, by placing on water bath, attaching reflux water condenser. After filtering and concentrating under vacuum the crude extract (reddish brown) was obtained.

- Preparation of methanolic extract: The powdered plant material (1.0 kg of Aegle marmelos seeds) was extracted with 4.0 liters of analytical grade methanol for 72 hours in a round bottom flask, on water bath attaching reflux water condenser. After filtering and concentrating under vacuum the crude extract (yellow reddish) was obtained.

The % yields of both the extracts (i.e. aqueous and methanol) were 19.71% and 10.84 %, respectively.
Assessment of antiulcer activity

Animals: The male wistar rats were (180-200 g) were used for the study. They were housed in matel cages and were left for two days for acclimatization to animal room, was maintained under controlled condition of (12 hours light and dark cycle at 22 ± 3°C), and were kept on standard pellet diet and water ad libitum. Before the study the animals were fasted overnight with the free access to water. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the purpose of control and supervision of experiments on experiments on animals).

Indomethacin induced gastric ulcers: A total of 36 rats were divided into 6 subgroups. Group I; received normal saline and served as control, group II, IV received 200 mg/kg, b.w and group III, V received 400 mg/kg, b.w of aqueous and methanolic extracts of Aegle marmelos seeds, sequentially. Group VI; was treated with ranitidine (50 mg/kg b. w.) and served as reference group. All the treatments were made orally for 10 days, before gastric ulcer were induced in rats (except group-I) with indomethacin (20 mg / kg b.w.). The animals were killed under ether anaesthesia after 6 hour of administration of indomethacin and the stomach was isolated and cut opened along the greater curvature [17]. Screening of ulcer was done as; (0: normal colored stomach; 0.5: red coloration; 1.0: spot ulcers; 1.5: haemorrhagic streaks; 2.0= ulcers ≥ 3 mm but ≤ 5 mm; 3.0: ulcers ≥ 5 mm) [18]. Ulcer index was calculated using formula:

\[
UI = UN + US + UP \times 10^{-1}
\]

Where; (UI: ulcer index, UN: average no. of ulcer per animal, US: average of severity score and UP: percentage of animal with ulcer).

Stressed induced ulcers: The rats were divided into 6 groups, each containing 6 animals. Group I, received normal saline, group II, IV received 200 mg/kg, b.w and group III, V received 400 mg/kg, b.w of aqueous and methanolic extracts of Aegle marmelos seeds, sequentially. Group VI; was treated with ranitidine (50 mg/kg b. w.) and served as standard. All the treatments were made orally 30 min prior to subjection of stress. The ulcer was induced by placing animals in a restraint cage maintained at temperature of 20-4°C for 3 hours. The animals were sacrificed and scoring of ulcer was done as mentioned above [19, 20].

Pylorus ligation induced ulcers: The study was performed as method described by the Shay [21]. 36 overnight fasted rats were divided in 6 subgroups; group I, received normal saline, group II, IV received 200 mg/kg, b.w and group III, V received 400 mg/kg, b.w of aqueous and methanolic extracts of Aegle marmelos seeds, sequentially. Group VI was treated with ranitidine (50 mg/kg b. w.) and served as standard. After 30 min. under ether anaesthesia, stomach was ligated and replaced carefully. Animals were deprived of both food and water during post-operative period and were sacrificed at the end of 19-20 h, stomach was dissected out and contents were subjected to centrifugation (3000 rpm for 10 min) and then analyzed for pH, total volume of gastric secretion, free acidity, pH and total acidity [22]. The ulcer index was also calculated as described above. Acidity was expressed as;

\[
\text{Total Acidity (m Eq/L)} = \frac{\text{Vol. of NaOH} \times \text{Normality}}{0.1} \times 100
\]

Statistical Analysis: Data are expressed as the mean ± standard error of mean (S.E.M.) and statistical analysis was carried out employing one way analysis of variance (ANOVA) followed by Dunnet test.

RESULT AND DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results
from an imbalance between aggressive factors and the maintainance of mucosal integrity through the endogenous defense mechanism. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production, stabilizing the surface epithelial cells or interfering with the prostaglandine synthesis. Our present study was oriented to evaluate the antiulcer activity against NSAID’S and stress induced ulceration. In addition to this pylorus ligated ulceration were also caused.

Cyclo-oxygenase (COX), the enzyme responsible for the formation of important biological mediators called prostanoids (Including prostaglandines, prostacyclin and thromboxane). However, pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain; this is the method of action of nonsteroidal antiflammatory drugs such as well known indomethacin. Inhibition of COX at the gastric mucosa directs gastric mucosal cell damage and eventually produces gastric lesions and stress which is one of the important damaging factor induces ulcer, and ligation of pylorus which increases free acidity and total acidity and reduces the pH of gastric juice making it more vulnerable to soft lining of gastric mucosa.

In our present study methanolic extracts at both the doses had shown more than 70% protection from ulcer in indomethacin induced ulceration. The same effects were also observed in experimental animals subjected to stress induced ulceration. Those animals which were pyloric ligated in this cause only 400 mg / kg b.w.of methanolic extract had given similar results as presented by standard drug ranitidine at dose of 50 mg / kg b.w. in all the above three models. Aqueous extract of Aegle marmelos seed at 200 mg / kg b.w and 400 mg / kg b.w., had also shown significant effects, but much lesser than their comparative dose of methanolic extracts. In our observation, in above three models, we came to know, that methanolic extract may be producing protective effects due to the presence of 400% more quercetin equivalent substances than in aqueous extract (Table 1).

At this stage we cannot confirm that any compound is present in methanolic and aqueous extract act as to receptor antagonist as ranitidine do, but we can confirmly suggest that the protective measures of quercetin like substances may have presented antiulcer effects.

In our present study, we presented our observation on pylorus ligated experimental and found that volume of gastric juice was significantly reduced by methanolic extract at both the doses. The same effects have also been observed on free and total acidity of gastric juice of experimental animals, whereas the pH of gastric juice is increased in the same manner. We impart our observation that the percentage protection in pylorus ligated experimental animals were less than stress induced ulceration and indomethacin induced ulceration models, but three times rise in pH in pylorus ligated experimental animals with the dose of 400 mg / kg, b.w. of methanolic extract appear a barrier for further mucosal damage, and sudden decline in secretion of gastric juice with lower acidic also confirm that methanolic extract have major role in protective mechanism against damaged mucosa (Table 2).

Our results has been showing a fairly responsibility of presence of quercetin like substances in methanolic extract over to aqueous extract. The standard drug ranitidine at the dose of 50 mg / kg b.w., which have shown extra effects due to antagonising particular receptors. Here, we don’t claim that certain extract like methanolic extract and aqueous extract act on certain receptor level. This might be happening and in extracts the responsible constituent would be of flavonoid type.
Table 1: Effect of aqueous and methanolic extracts of Aegle marmelos seeds on Indomethacin, Stress and Pylorus ligation induced rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments</th>
<th>Dose (mg/kg, b.w.), Oral</th>
<th>Indomethacin Induced Ulceration</th>
<th>Stress induced Ulceration</th>
<th>Pylorus ligated Ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean Ulcer Index+ SEM</td>
<td>% Protection</td>
<td>Mean Ulcer Index+ SEM</td>
</tr>
<tr>
<td>I</td>
<td>Normal Saline</td>
<td>2ml /kg</td>
<td>7.116 ± 0.113</td>
<td></td>
<td>8.06 ± 0.912</td>
</tr>
<tr>
<td>II</td>
<td>Aqueous</td>
<td>200</td>
<td>4.261 ± 0.702*</td>
<td>40.12</td>
<td>5.01 ± 0.710*</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous</td>
<td>400</td>
<td>2.162 ± 0.800*</td>
<td>69.62</td>
<td>3.751 ± 0.612*</td>
</tr>
<tr>
<td>IV</td>
<td>Methanolic</td>
<td>200</td>
<td>2.001 ± 0.416**</td>
<td>71.88</td>
<td>2.251 ± 0.217**</td>
</tr>
<tr>
<td>V</td>
<td>Methanolic</td>
<td>400</td>
<td>1.812 ± 0.312**</td>
<td>74.54</td>
<td>1.512 ± 0.101**</td>
</tr>
<tr>
<td>VI</td>
<td>Ranitidine</td>
<td>50</td>
<td>1.106 ± 0.412**</td>
<td>84.45</td>
<td>0.916 ± 0.209**</td>
</tr>
</tbody>
</table>

- Values are given as Mean ± SEM for groups of six animals each.
- *P<0.05 as compared to vehicle control.
- **P<0.01 as compared to vehicle control.

Table 2: Effect of aqueous and methanolic extracts of Aegle marmelos seeds on gastric secretion following pylorus ligation

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg, b.w.), Oral</th>
<th>Vol. of gastric juice (ml)</th>
<th>Free acidity (Eq/mL)</th>
<th>Total acidity (Eq/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>2 ml/kg</td>
<td>7.83±0.191</td>
<td>86.5±2.942</td>
<td>132.17±7.098</td>
<td>2.27±0.105</td>
</tr>
<tr>
<td>II</td>
<td>Aqueous</td>
<td>200</td>
<td>5.38 ±0.358*</td>
<td>70.67±1.909*</td>
<td>79.33±3.17*</td>
<td>3.28±0.125*</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous</td>
<td>400</td>
<td>3.73±0.345*</td>
<td>63.67±1.892*</td>
<td>70.67±1.745*</td>
<td>4.27±0.143*</td>
</tr>
<tr>
<td>IV</td>
<td>Methanolic</td>
<td>200</td>
<td>3.25±0.299**</td>
<td>59.17±2.575**</td>
<td>67.67±2.186**</td>
<td>4.88±0.250**</td>
</tr>
<tr>
<td>V</td>
<td>Methanolic</td>
<td>400</td>
<td>2.17±0.176**</td>
<td>53.17±2.738**</td>
<td>59.67±2.155**</td>
<td>5.97±0.080**</td>
</tr>
<tr>
<td>VI</td>
<td>Ranitidine</td>
<td>50</td>
<td>1.67±0.193**</td>
<td>35.33±1.892**</td>
<td>46.83±1.401**</td>
<td>7.38±0.094**</td>
</tr>
</tbody>
</table>

- Values are given as Mean ± SEM for groups of six animals each.
- *P<0.05 as compared to vehicle control.
- **P<0.01 as compared to vehicle control.
Figure 1 Effect of aqueous and methanolic extract of *Aegle marmelos* seeds on Indomethacin, Stress and Pylorus ligation induced Ulcers.

**REFERENCES**


