ANTIULCER ACTIVITY OF ANNONA RETICULATE LEAVES EXTRACT IN RATS

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ABSTRACT

The present study has been undertaken to investigate the antiulcer activity of Annona reticulate leaves extract in rats. In Ethanol and indomethacine induced ulcer model, Extract or reference or vehicle was administered to 24 hours fasted rats, after 30 min, 50% alcohol or indomethacine was administered. After 1 hour, rats were sacrificed and ulcer index and related parameters were determined. Annona reticulate (100 & 200 mg/kg doses) and reference drug (3mg/kg) has shown significant decrease in ulcer index, acid volume and content and showed improvement in glutathione and pH when compared with vehicle control. Therefore, aqueous extract of leaves of Annona reticulate is a potent antiulcer agent.

Keywords: Antiulcer, Ethanol, Indomethacine, Ulcer index, Glutathione

INTRODUCTION

Peptic ulcer disease has become one of the most common gastrointestinal disorders that involves the damage to mucosal membrane or severe lesions.1 Traditionally peptic ulcers have been described as an imbalance between the luminal acid peptic attack versus the mucosal defense.2 Numerous plants have been evaluated as therapeutics for the treatment of a variety of diseases including peptic ulcer.3,4 Annona reticulate (Annonaceae) is a tree commonly known as Ramphal. The leaf decoction is given as a vermifuge. Crushed leaves or a paste of the flesh may be poulticed on boils, abscesses and ulcers. The unripe fruit is rich in tannin is dried, pulverized and employed against diarrhea and dysentery. The bark is very astringent and the decoction is taken as a tonic and also as a remedy for diarrhea and dysentery. The root decoction is taken as a febrifuge.5 The plant (mainly leaves) is rich of alkaloids, diterpenoids, acetogenins, δ-cadinene, elemol, α-copaene and δ-eudesmol in combination with monoterpenes terpinen-4-ol and α-terpineol.6 Free radical scavenger property7 and rich alkaloidal contents favours antiulcer activity of Annona reticulate.

The Annona reticulate leaves has been claimed to have antiulcer activity,3 but no detailed scientific investigations have been carried out to define the antiulcer activities of Annona reticulate. Thus the present investigation sets out to study the antiulcer activity of Annona reticulate leaves extract.

MATERIALS AND METHODS

Drugs

DTNB solution (5, 5-dithiobis-2-nitrobenzoic acid) was procured from M/S Sisco research laboratories Pvt. Ltd, Mumbai-400099, India.

Plant material

The leaves of Annona reticulate was collected from the outskirts of Tumkur. The plant was identified by a Botanist.

Animals

Wistar rats weighing 180-200 gm of either sex were used in this study. They were divided into four groups, with each group containing 6 animals. Clearance to carry out the work was obtained from the Institutional animal ethical committee bearing no. SSCPT/AEC. Clear /51/2007–08 dated 22/09/2007.

Preparation of extract

The AELAR (Aqueous extract of leaves of Annona reticulate) extract was prepared by soxhlet extraction of 100 g leaf powder in 250 ml of distilled water. The extract was concentrated dried in vacuum and residue stored in refrigerator at 2-8°C for use in subsequent experiments.

Experimental design

In this study, there are four groups (n=6). Group-1 served as vehicle control and recieved Indomethacine or ethanol. Group-2 served as reference and received famotidine (3mg/kg). Group-3 served as treated group and recieved AR (100mg/kg). Group-4 served as treated group and recieved AR (200mg/kg).

Ethanol-induced gastric lesions

Annona reticulate or vehicle was administered to 24 hours fasted rats. After 30 min, ulceration was induced by oral administration of 50 % ethanol. The animals were sacrificed after 1 h following administration of ethanol. The stomach was removed, opened along the greater curvature and ulcer index was measured. The gastric contents were collected and centrifuged. Suprenant was collected and acid volume10 and pH10 were determined. Supernatant was titrated against 0.01 N NaOH to find out the total acidity.11

Indomethacin-induced gastric mucosal damage

After fasting for 24 hours, Test or reference or vehicle has been administered. Indomethacin (10 mg/kg, p.o) was administered by gavage needle after 30 min. of administration of test or vehicle. One hour after test or reference or vehicle administration, all rats were sacrificed. The numbers of ulcer spots in the glandular portion of the stomach were counted in both control and drug treated animals and the ulcer index was calculated.

Ulcer index11

Ulcer index was calculated by using the following score and formula:

Scoring of ulcer

0 = Normal coloured stomach, 0.5 = Red colouration, 1 = Spot ulcer, 1.5 = Haemorrhagic streaks, 2 = Ulcers ≥3 but ≤ 5, 3 = Ulcers>5

Calculation of ulcer Index

U1 = UN + US + UP x 10-1

U1 = Ulcer Index

UN = Average of number of ulcer per animal, US = Average of severity score,

Calculation of ulcer Index

UP = Percentage of animal with ulcer

GSH4

Glutathione was determine by Ellman’s reaction using 5’5-dithio-bis-2-nitrobenzoic acid (DTNB).

Statistical analyses

Data of independent observations are shown as the mean±sem. Statistical analysis was performed using unpaired t test. P<0.05 was considered as statistically significant.
RESULTS

In both the models, AELAR has showed significant dose dependent decrease in ulcer index (UI) when compared with vehicle control group (P<0.001). Reference drug also shown significant decrease in UI (P<0.001). (Table-1, 2). AELAR has showed significant decrease in acid volume and gastric content when compared with vehicle group (P<0.001). However, AELAR and reference drug has showed significant increase in pH levels (P<0.001). Significant improvement in glutathione level has showed by AELAR which corroborate its free radical scavenging property (P<0.001), (Table-1, 2).

Table 1: Ethanol induced gastric ulcers

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Dose (mg/Kg)</th>
<th>UI</th>
<th>Acid volume</th>
<th>Total acidity</th>
<th>pH</th>
<th>Glutathione (mmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>---</td>
<td>14.7±1.6</td>
<td>8.4±0.20</td>
<td>116.7±3.17</td>
<td>2.00±0.09</td>
<td>4.4±0.30</td>
</tr>
<tr>
<td>2</td>
<td>Famotidine</td>
<td>3</td>
<td>3.8±1.5***</td>
<td>2.1±0.78***</td>
<td>64.23±2.48***</td>
<td>4.21±0.18***</td>
<td>7.3±0.45</td>
</tr>
<tr>
<td>3</td>
<td>AELAR</td>
<td>100</td>
<td>5.9±0.79***</td>
<td>4.1±0.24***</td>
<td>78.14±1.98***</td>
<td>3.11±0.19***</td>
<td>5.2±0.34</td>
</tr>
<tr>
<td>4</td>
<td>AELAR</td>
<td>200</td>
<td>4.2±1.02***</td>
<td>3.4±1.04***</td>
<td>65.69±3.14***</td>
<td>3.86±0.28***</td>
<td>8.7±0.22</td>
</tr>
</tbody>
</table>

n=6, Values represent the mean±sem. P<0.05*, P<0.001***.

Table 2: Indomethacine induced gastric ulcers

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Dose (mg/Kg)</th>
<th>UI</th>
<th>Acid volume</th>
<th>Total acidity</th>
<th>pH</th>
<th>Glutathione (mmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>---</td>
<td>13.4±0.79</td>
<td>6.1±0.42</td>
<td>121.4±2.4</td>
<td>2.32±0.04</td>
<td>5.17±1.04</td>
</tr>
<tr>
<td>2</td>
<td>Famotidine</td>
<td>3</td>
<td>4.1±0.95***</td>
<td>2.8±0.64***</td>
<td>57.49±2.18***</td>
<td>3.47±0.18***</td>
<td>6.87±0.74***</td>
</tr>
<tr>
<td>3</td>
<td>AELAR</td>
<td>100</td>
<td>6.4±1.12***</td>
<td>4.2±1.10***</td>
<td>74.14±3.24***</td>
<td>3.07±1.02***</td>
<td>5.50±0.96**</td>
</tr>
<tr>
<td>4</td>
<td>AELAR</td>
<td>200</td>
<td>4.0±0.74***</td>
<td>3.7±0.75***</td>
<td>60.56±2.89***</td>
<td>3.36±0.09***</td>
<td>6.94±0.47***</td>
</tr>
</tbody>
</table>

n=6, Values represent the mean±sem. P<0.05*, P<0.001***.

Fig. 1: Antiulcer effect of AELAR in Ethanol induced gastric ulcer

Fig. 2: Antiulcer effect of AELAR in Indomethacine induced gastric ulcer mucosal damage
DISCUSSION

Peptic ulcer is the most common gastrointestinal disorder which occurs as a result of an imbalance between the aggressive factors of acid and pepsin and maintenance of mucosal integrity through endogenous defensive mechanism. To regain the balance, different therapeutics including spice and plant extracts have been used.15

Ulcer index is a index of ulcer formation and ulcer formation is directly related to factors such as gastric volume, total acidity, pH and also Glutathione. In both the model of ulcers, there is a increase in acid secretion, which in turn caused increase in gastric volume, low pH, increased total acidity resulting in higher ulcer index. AELAR reduced the gastric volume, total acidity and hence ulcer index showing the anti-secretory mechanism.16 Indomethacin induced gastric mucosal injury decreased the glutathione peroxidase activity and aggravated the injury due to accelerated accumulation of H₂O₂ and lipid peroxidation.17 Furthermore, excessive peroxidation causes increased glutathione consumption.18 Peroxidation was also enhanced by ethanol. These effects were counteracted by enhancing the glutathione tissue level after treatment with AELAR which will serve as free radical scavenger.19

Ethanol induced gastric damage ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions, which is attributed mainly to the inhibition of biosynthesis of cytoprotective PG resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway.20 These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H⁺ and Na⁺ ions reducing the transmucosal potential difference and induce formation of erosions and ulcers. In this model AELAR was able to produce a significant reduction of the gastric mucosal damage, indicating a probable local increase in PG synthesis.21

Ulcer formation induced by Indomethacin, a non-steroidal anti-inflammatory agent, is known to be correlated with inhibition of cyclooxygenase, that prevents prostaglandin (PG) biosynthesis,22,23 which in turn inhibits the release of mucus,24 a defensive factor against gastrointestinal damage. In this model AELAR was produced its ulcer protective effect by counteracting the inhibition of PG synthesis and enhancing the mucus release.

In the present study, the anti ulcer effect might be due to a possible relationship between protection of mucosal damage, inhibition of acid secretion and the antioxidant property of Annona reticulate. Annona reticulate has already been reported for its antioxidant activity. Therefore, the AELAR has showed its antiulcer activity due to its antisecretory, antioxidant and cytoprotective mechanism. This study indicates that AELAR has a potential anti ulcer activity.

REFERENCES

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