Evaluation of antiulcer activity of various extracts of *Semecarpus anacardium* seeds

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**ABSTRACT**

Methanolic and aqueous extracts of *Semecarpus anacardium* seeds were investigated for gastric protective activity on ethanol plus pylorus ligation induced ulcer models. A number of biochemical parameters such as gastric volume, pH of gastric content, free acidity and total acidity, dissolved mucous substances such as total protein, hexoses, hexosamine, fucose and sialic acid were estimated in 90% alcoholic precipitate of gastric juice and macroscopically sections were examined on the test and control group animals. The extract at a concentration of 200mg/kg produced a protective effect on ulcer-induced models and was comparable with the standard drug cimetidine. Some of the antioxidant enzyme levels (lipid peroxidation, superoxide dismutase and catalase) were also determined. The present study revealed that the methanolic extract of *Semecarpus anacardium* had ulcer protective activity comparable with standard drug cimetidine.

**Key words:** *S. anacardium*, ulcer, ethanol plus pylorus ligation, Biochemical parameters, Methanolic extract

**INTRODUCTION**

Peptic ulcer disease is a serious gastrointestinal disorder. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defences. There are two major factors that can disrupt the mucosal resistance to injury: non-steroidal anti-inflammatory drugs (NSAIDs) e.g aspirin and *Helicobacter pylori* infection.[1] As a matter of fact, many drugs were used to treat this disease but many of them cause adverse effects and recurrent infections frequently occur within a few weeks because of difficulty in eradication of *H. pylori*.[2] This has been rationale for the development of new antiulcer drugs and search for novel molecule. Drugs of plants origin are gaining popularity and investigating for the various disorders including peptic ulcer. The objective of present study was to evaluate the effectiveness of seeds extract in preventing the formation of gastric ulcer experimentally by ethanol-induced gastric damage in rats. Cimetidine was used as reference drug for comparison.

Seeds of *Semecarpus anacardium* (Marking nut) are used in Indian traditional medicines (Ayurveda and Sidha) either alone or as an ingredient of many polyherbal formulation for treating various ailments. *S. ancardium* of Anacardiaceae family is a medium sized tree grown in arid parts of tropical and subtropical regions. Ayurveda describes it as a potent drug for neuritis, arthritis, leprosy, helmintic infection and venereal disorders[3,4] But supporting data are lacking. Recently antioxidant,[5,6] anti-inflammatory,[7] anti-cancer,[8,9] antibacterial,[10] anti-rheumatic[11] and anthelmintic[12] activities of its seeds have been reported. A variety of flavonoids such as tetrahydroamentoflavone(THA),[13] jeediflavanone,[14] semicarpouflagonone,[15] galluflagonone,[16] nallaflagonone,[17] semecarpeti[18] and anacardioflavonone[19] along with other phenolic compounds such as bhilawanols and anacardic acids[20] have been reported. The present study was undertaken to assess the antiulcer activity of the extracts of *S.anacardium* using rats.

**MATERIALS AND METHODS**

*Semecarpus anacardium* seeds were purchased from Haryana Agricultural University, Hisar in Feb, 2009. The seeds were identified and authenticated by Dr. S. Sharma, Deptt. of Botany, HAU, Hisar.
Preparation of extracts
The air-dried Semecarpus anacardium seeds were powdered using a mechanical grinder. The dried powdered plant material (500 g) was refluxed with methanol (2L) and the semisolid brown mass was concentrated with rotary evaporator, yielding 80.50 g methanol extract (ME). The drug was treated with water (1.5 L), yielding 46.40 g aqueous fraction (AE).

Experimental Animals
The albino Wistar rats of either sex weighing 150-200 g were used for the study. Animals were divided into five groups, in each group six animals. They were maintained in the departmental animal house at 25 ± 2°C and relative humidity 45-55%, respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Hindustan Lever) and water ad libitum. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

Ethanol plus pylorus ligation method (EPPL)
The animals were placed in cages with grating floor to avoid coprophagy and divided into five groups each group six animal viz, Group I receiving 0.5% acacia gum served as vehicle control, group II ulcer induced group (EPPL), Groups III received aqueous extract at dose of 200 mg/kg and IV received the methanolic extract of Semecarpus anacardium seeds (MESA) at dose of 200 mg/kg respectively by oral route. Group V received Cimetidine 40 mg/kg orally serving as standard drug control for EPPL model. All the extracts and reference drug were suspended in 0.5% acacia gum for animal administration. Ethanol was administered once daily for 5 days. Cimetidine and the extracts were administered 30 min before each ethanol administration. On day 6 after last dose, the rats were kept for 18 h fasting. Pylorus ligation was done by following the method as described. The animals were deprived of water during the postoperative period. After 4 h, stomachs were dissected out and contents were collected in tubes for estimation of biochemical parameters. Ulcers were scored as described.

Biochemical estimation
In EPPL induced ulcer models the following were estimated by procedures described, gastric volume, pH of gastric content, free acidity and total acidity. Dissolved muco substances such as total protein, hexoses, hexosamine, fucose, sialic acid were estimated in 90% alcoholic, precipitate of gastric juice and expressed as μg/ml.[21]

Statistical analysis
The data are expressed as mean ± SEM. Statistical comparisons were performed by one-way ANOVA followed by Dunnett’s t test. The results were considered statistically significant if the p-values were less than 0.05.

RESULTS
Estimation of acid secretry parameters such as pH, gastric volume, free acidity and total acidity was increased significantly in the ethanol administered group. Administration of MESA a significant (p < 0.01) reduction in all the parameters and the results were comparable with the standard drug Cimetidine 40 mg/kg (Table 1). Determination of the concentrations of several muco proteins such as total protein, total hexoses, hexosamine, fucose and sialic acid revealed a decrease in ulcer induced group. The extract at 200 mg/kg increased the level of the muco proteins significantly and comparably with the standard drug (p < 0.01) (Table 2). The ulcer scores obtained in ulcer induced group of EPPL increased score. Administration of the extract showed a significant decrease in EPPL models (p < 0.05 and p < 0.01). Table 3 shows Effect of MESA on level of SOD, LPO and CAT. The histopathological sections of the drug treated group EPPL had shown a reduction in ulcer focus and a hyperplastic gastric mucosa with regenerating mucosal epithelium (Figure 1, 2, 3 and 4).

DISCUSSION
MESA showed significant dose-dependent ulcer protective effect against ethanol plus pylorus ligation induced gastric ulcers. Ulcers are caused due to imbalances between offensive and defensive mucosal factors and hence the
Table 2: Effect of MESA on gastric juice mucoprotein (μg/mL) in ELP rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Protein</th>
<th>Total hexoses</th>
<th>Hexosamine</th>
<th>Fucose</th>
<th>Sialic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>264.92 ± 3.330</td>
<td>421.36 ± 5.678</td>
<td>207.27 ± 5.870</td>
<td>96.324 ± 1.656</td>
<td>72.983 ± 1.179</td>
</tr>
<tr>
<td>EPPL</td>
<td>473.36 ± 3.265 a***</td>
<td>195.51 ± 5.750 a***</td>
<td>110.21 ± 4.026 a***</td>
<td>6.347 ± 0.961 a***</td>
<td>21.161 ± 1.520 a***</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>399.38 ± 4.867 b**</td>
<td>399.38 ± 4.867 b**</td>
<td>162.19 ± 6.036 b**</td>
<td>81.423 ± 0.702 b***</td>
<td>48.322 ± 1.400 b**</td>
</tr>
<tr>
<td>MeOH extract</td>
<td>373.14 ± 11.91 b**</td>
<td>7.656 ± 6.085 b**</td>
<td>171.30 ± 4.973 b**</td>
<td>85.716 ± 1.073 b***</td>
<td>56.276 ± 0.869 b**</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>329.83 ± 6.006 b**</td>
<td>403.14 ± 6.256 b**</td>
<td>175.53 ± 4.699 b**</td>
<td>116.14 ± 1.564 b***</td>
<td>64.096 ± 0.775 b**</td>
</tr>
</tbody>
</table>

Data are mean ± SEM., n = 6, Statistical significance *p < 0.05, **p < 0.01, ***p < 0.001, a - Group I vs. Group II; b indicates Group II vs. Groups III, IV and V

Table 3: Effect of S.anacardium seeds extracts on induction of gross lesions in the ethanol plus pylorus ligation method (EPPL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose orally (mg/ kg)</th>
<th>Ulcer index (Mean ± S.E.M)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR1</td>
<td>Control (0.5% Acacia gum)</td>
<td>200</td>
<td>0 49.157 ± 1.275</td>
<td>0</td>
</tr>
<tr>
<td>RR2</td>
<td>Absolute ethanol-HCl (ulcer control)</td>
<td>200</td>
<td>59.742 ± 1.655</td>
<td>0</td>
</tr>
<tr>
<td>RR3</td>
<td>Aqueous extract (AE)</td>
<td>200</td>
<td>49.157 ± 1.275*</td>
<td>30.42</td>
</tr>
<tr>
<td>RR4</td>
<td>Methanolic extract (ME)</td>
<td>200</td>
<td>28.959 ± 1.391**</td>
<td>71.06</td>
</tr>
<tr>
<td>RR5</td>
<td>Cimetidine</td>
<td>40</td>
<td>25.907 ± 1.485**</td>
<td>76.19</td>
</tr>
</tbody>
</table>

*p < 0.05 significant from (Absolute ethanol-HCl) ulcer control; **p < 0.05 significant from aqueous extract

Figure 1: Control group
Figure 2: Aq. extract treated group
Figure 3: Methanol extract treated
Figure 4: Cimetidine treated group
effects of MESA can be explained based on these factors. Mucin is a viscous glycoprotein with physochemical properties producing relatively resistant acid barrier. [21] It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defence against ulcerogens. Increase in mucin can be due to increased levels of individual mucopolysaccharide like sialic acid and total hexoses. The increase in mucosal defence may also be due to decrease in cell exfoliation. [23] Hence, the protection afforded by MESA in EPPL induced ulcers may be predominantly due to strengthening mucosal defense. The ability of MESA to protect stomach against ulcerogens by neutralizing intra gastric acidity can as well lead it to classify as a cytoprotective agent. Prostaglandins have often been quoted as a model cytoprotective agent, although this has been disputed. To ascertain this effect, the activity of MESA was studied on Ethanol plus pylorus ligation model, where ethanol is known to further aggravate mucosal damage caused by pylorus ligation. [24] It was found that MESA was effective in this model, suggesting that the activity of MESA may also involve other defensive factors apart from PG synthesis. The role of the free radicals in gastric ulcerations is well-documented. MESA significantly reduced lipid peroxidation in rat gastric mucosa. S. anacardium has been reported to possess antioxidant activity. SOD scavenges the super oxide radical \( \text{O}_2^- \), one of the reactive oxygen species (ROS) responsible for lipid peroxidation. CAT and other peroxidases further reduce \( \text{H}_2\text{O}_2^- \). The antioxidant activity of plant is already reported in literature. Thus the activity of MESA can be explained based on these factors. Hence, it can be suggested that MESA have antiulcer potential in rats and further in future, isolate the phytoconstituents which responsible for antiulcer activity may be studied. [21]

**CONCLUSION**

The present study reveals that the methanolic extract of *Semecarpus anacardium* seeds shows ulcer protective effect in ethanol plus pylorus ligation method. Hence, it can be suggested that methanolic extract (MESA) of *S. anacardium* have anti-ulcer potential in rats.

**REFERENCES**