Prevention of HCl-ethanol induced gastric mucosal injury in rats by *Garcinia cambogia* extract and its possible mechanism of action

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Oral pretreatment of rats with *G. cambogia* fruit extract (1 g/kg body weight/day at interval of 7 and 15 days) protected gastric mucosa against HCl - ethanol induced damage by decreasing the volume and acidity of gastric juice. Increased lipid peroxidation, decreased activity of antioxidant enzymes, altered levels of protein and glycoproteins in the ulcerated mucosa, and gastric juice were maintained at near normal levels in *G. cambogia* pretreated rats. The results suggest the anti-ulcer activity of *G. cambogia* by virtue of its ability to decrease acidity and increase mucosal defense.

Peptic ulcer is a benign lesion of gastric or duodenal mucosa occurring as a result of disturbance of the natural balance between aggressive acid/pepsin and mucosal defense/mucosal turnover. Administration of HCl and ethanol produces ulcerative lesions and increases lipid peroxidation in the gastric mucosa, which plays a significant part in the pathogenesis of the mucosal lesions. Depletion of glutathione (GSH) in gastric mucosa results in enhanced lipid peroxidation and this can cause increased GSH consumption and increased susceptibility of the gastric mucosal cells to oxygen metabolites and acid mediated cell damage. Free radicals created by HCl/ethanol injury, attack the protein in gastric mucosa which leads to a reduction in the protein levels. Although a number of antiulcer drugs like H2 receptor antagonists, proton pump inhibitors, cytoprotectants are available for ulceration, all these have side effects and limitations.

Rind of the fruits of *Garcinia cambogia* (Gaertn.) Desr. (Clusiaceae), commonly known as Brindall berry, is astringent and contains the principle organic acid (--)-erythro-L-s-hydroxycitric acid, an effective appetite suppressor which brings early satiety via vagus nerve and decreases the acidic condition in stomach. The rind of the fruit is used in treating diarrhoea, haemorrhoids, dysentery, tumors and rheumatism. Initially, *G. cambogia* extract was screened for its antiulcer activity against indomethacin-induced gastric-ulcer. High concentration of acid in the gastric lumen is the prime factor for augmenting gastric mucosal damage by indomethacin. Since *G. cambogia* extract has acid neutralizing property, in the present study, direct ingestion of HCl-ethanol with respect to acid consuming capacity and gastric mucosal stability was analysed in rats.

**Materials and Methods**

*Drugs and chemicals*—*Garcinia cambogia* fruit extract was obtained from Siris Herbex, Vijayawada, India. Ethanol was obtained from Anilax Chemicals, USA. All other chemicals were of analytical grade.

*Animals*—Male Wistar rats (24), weighing 150-180 g were fed with standard pelleted diet (M/s. Hindustan Lever Foods, Bangalore, India) and water *ad libitum* and housed under standard environmental conditions. Animals were deprived of food for 24 hr prior to ulcer induction.

The animals were divided into following 4 groups of six animals each.

**Group I** — Normal control, received only standard diet.
**Group II** — Drug control: *G. cambogia* extract—(1g/kg body wt./day, po for 15 days).
**Group III** — Ulcer: Oral administration of 1.5 ml of HCl-ethanol mixture containing 0.15 N HCl in 70% v/v ethanol.
**Group IV** — Pretreated ulcer: Pretreatment with *G. cambogia* (1g/kg body wt/day po for 15 days) before induction of ulcer.

**Chemical analysis**—Acid neutralising capacity described for the standard antacid magnesium trisilicate in pharmacopoeia (USP 24/NF 19, 2000) was adopted to screen *G. cambogia* extract for its acid consuming capacity.
Dosage fixation — G. cambogia fruit extract was administered at different dosages (0.5, 1, 1.5 g/kg body wt/day) orally at two different time intervals of 7 and 15 days, and 1 hr prior to ulcer induction. After the treatment period, rats received oral administration of 1.5 ml of HCl-ethanol mixture containing 0.15 N HCl in 70% v/v ethanol. The animals were sacrificed after 4 hr and functional indices of ulcer activity was studied in all rats and compared with that of non-ulcer induced ones. The dosage and duration of treatment period which exhibited maximum antiulcer activity (number of lesions, peptic activity, volume of gastric juice, acid output; Table 1) was fixed up as the optimum dosage schedule for the fruit extract. The optimum dosage was found to be 1 g/kg body wt/day orally for 15 days.

Experimental procedure — At the end of the experimental period, rats in all the four groups underwent surgery as per Takeuchi et al.13 for collection of gastric juice. Briefly, the abdomen of the animals was opened under light anesthesia by a small midline incision below the xiphoid process, pyloric portion of the stomach was slightly lifted out and ligated avoiding damage to its blood supply. The stomach was replaced and the abdominal wall closed by interrupted sutures. The animals were sacrificed 4 hr after pylorus ligation. The stomach was dissected out after tying the oesophageal end; it was cut open and the gastric juice drained into a small beaker, centrifuged and the volume was noted. The stomach was inflated with formal saline and then incised through the greater curvature and examined under a dissecting microscope for the number of lesions.

The total acidity was determined by titrating the gastric juice with 0.1 N NaOH with phenolphthalein as indicator. Protein14, pepsin15, hexose and hexosamine16, sialic acid17 and fucose18 contents were estimated both in the gastric juice collected and gastric mucosa scraped from the stomach. Lipid peroxides (LPO)19, Glutathione20, superoxide dismutase (SOD)21, catalase (CAT)22, glutathione peroxidase (GSH-Px)23, and glutathione-S-transferase (GST)24 were estimated in gastric mucosa.

Statistical analysis — Student's t-test was used to assess statistical significance and the results are expressed as mean ± SE.

Results

The acid consuming capacity of 1 g of G. cambogia extract to neutralise 1 N HCl is 7.013 meq, while the acid consuming capacity of 1 g of standard reference substance, antacid, magnesium trisilicate11 is 5.0 meq (each 1 ml of 1 N HCl is equivalent to 1 meq of acid consumed) which reveals the antacid property of G. cambogia extract.

The results of initial trials carried out at different dosages of extract (0.5, 1, 1.5 g/kg body wt po/day) for two different time intervals are presented in Table 1. Dosage of 1 g/kg, po/day of G. cambogia given for 15 days markedly inhibited the acid output, number of lesions along with a reduction in the volume of gastric juice and pepsin, reflecting a positive effect of extract on HCl-ethanol induced gastric ulcer.

Oral administration of HCl-ethanol caused a significant (P < 0.001) increase in the number of lesions, volume of gastric juice and acidity and increased activity of pepsin in gastric mucosa and gastric juice (Tables 2 and 3).

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Table 1 — Effect of G. cambogia fruit extract on the number of lesions, activity of pepsin, volume of gastric juice and acid output on HCl-ethanol induced gastric ulcer in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HCl-ethanol induced ulcer (A)</th>
<th>7 days</th>
<th>15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Number of lesions</td>
<td>13.35 ± 0.78</td>
<td>12.18 ± 0.97NS</td>
<td>10.47 ± 1.09*</td>
</tr>
<tr>
<td>Pepsin (µmol tyrosine/4 hr)</td>
<td>667 ± 28.2</td>
<td>655.3 ± 21.7NS</td>
<td>642 ± 24.3NS</td>
</tr>
<tr>
<td>Volume of gastric juice (ml/4 hr)</td>
<td>3.82 ± 0.18</td>
<td>3.66 ± 0.09NS</td>
<td>3.25 ± 0.13**</td>
</tr>
<tr>
<td>Acid output (µeq/4 hr)</td>
<td>284.6 ± 19.8</td>
<td>261.3 ± 16.6NS</td>
<td>246.2 ± 15.7NS</td>
</tr>
</tbody>
</table>

P values: * < 0.05; ** < 0.01; *** < 0.001 vs Ulcer (HCl-ethanol)
A : 1.5 ml of HCl/ethanol mixture (0.15 N HCl in 70% v/v ethanol)
NS: Non significant.
Discussion

Ulcers are thought to be due to imbalances in gastric offensive and defensive mucosal factors. While acid and pepsin make up the offensive factors, the defensive factors include mucin secretion, mucosal glycoprotein, cell proliferation etc.25. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production, stabilising the surface epithelial cells26.

The number of lesions present on the gastric mucosa are indicative of the severity of ulcer disease27. Ethanol contributes significantly to the hemorrhagic and necrotic aspects of the tissue injury28. A significant reduction in the number of lesions in G. cambogia pretreated rats (group IV) may be due to the acid neutralizing capacity of the drug, thereby reducing the gastric tissue damage and ulcer formation, revealing its antacid property. Antacids have been reported to enhance mucosal prostaglandin levels, to stimulate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (control)</th>
<th>Group II (extract pretreated)</th>
<th>Group III (HCl-ethanol induced ulcer)</th>
<th>Group IV (extract + HCl-ethanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lesions</td>
<td>0</td>
<td>0</td>
<td>13.35 ± 1.07***</td>
<td>4.18 ± 0.54***</td>
</tr>
<tr>
<td>Volume of gastric juice (ml/4 hr)</td>
<td>2.80 ± 0.07</td>
<td>2.16 ± 0.10***</td>
<td>3.82 ± 0.18***</td>
<td>2.64 ± 0.09***</td>
</tr>
<tr>
<td>Acid output (tgc/4 hr)</td>
<td>186.8 ± 9.6</td>
<td>171.2 ± 5.2***</td>
<td>284.1 ± 15.3***</td>
<td>195.7 ± 9.8***</td>
</tr>
<tr>
<td>Pepsin (µmol tyrosine/4 hr)</td>
<td>548.3 ± 19.4</td>
<td>536.2 ± 16.1NS</td>
<td>667.4 ± 26.2**</td>
<td>571.2 ± 28.4*</td>
</tr>
<tr>
<td>Protein (µg/ml)</td>
<td>271.8 ± 8.3</td>
<td>274.3 ± 7.6NS</td>
<td>352.5 ± 10.1***</td>
<td>297.6 ± 8.6***</td>
</tr>
<tr>
<td>Hexose (µg/ml)</td>
<td>372.3 ± 11.7</td>
<td>386.2 ± 9.5NS</td>
<td>283.4 ± 13.9***</td>
<td>356.2 ± 10.5***</td>
</tr>
<tr>
<td>Hexosamine (µg/ml)</td>
<td>194.9 ± 7.1</td>
<td>195.1 ± 6.4NS</td>
<td>116.5 ± 9.9***</td>
<td>178.9 ± 4.7***</td>
</tr>
<tr>
<td>Sialic acid (µg/ml)</td>
<td>48.5 ± 1.52</td>
<td>45.2 ± 1.98NS</td>
<td>34.7 ± 2.05***</td>
<td>40.4 ± 1.36*</td>
</tr>
<tr>
<td>Fucose (µg/ml)</td>
<td>42.4 ± 1.03</td>
<td>44.6 ± 1.15NS</td>
<td>35.3 ± 1.56**</td>
<td>37.1 ± 1.42NS</td>
</tr>
<tr>
<td>Total carbohydrate (µg/ml)</td>
<td>658.20 ± 18.7</td>
<td>670.10 ± 19.6NS</td>
<td>469.9 ± 11.2***</td>
<td>612.6 ± 14.3***</td>
</tr>
<tr>
<td>TC : P</td>
<td>2.42 ± 0.12</td>
<td>2.45 ± 0.14NS</td>
<td>1.33 ± 0.09***</td>
<td>2.05 ± 0.10***</td>
</tr>
</tbody>
</table>

P values: * < 0.05; ** < 0.01; *** < 0.001; Group I vs. Group II & Group III; Group IV vs. Group III.
NS = Non significant; TC : P = Total Carbohydrate : Protein.

<table>
<thead>
<tr>
<th>Parametersa</th>
<th>Group I (control)</th>
<th>Group II (extract pretreated)</th>
<th>Group III (HCl-ethanol induced ulcer)</th>
<th>Group IV (extract + HCl-ethanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin</td>
<td>169.3 ± 6.9</td>
<td>156.1 ± 7.2NS</td>
<td>291.0 ± 9.3***</td>
<td>184.2 ± 8.1***</td>
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<tr>
<td>LPO</td>
<td>3.61 ± 0.08</td>
<td>3.56 ± 0.10NS</td>
<td>8.76 ± 0.23***</td>
<td>3.98 ± 0.12***</td>
</tr>
<tr>
<td>SOD</td>
<td>4.73 ± 0.05</td>
<td>4.89 ± 0.03NS</td>
<td>2.31 ± 0.09***</td>
<td>4.82 ± 0.04***</td>
</tr>
<tr>
<td>CAT</td>
<td>3.84 ± 0.11</td>
<td>4.03 ± 0.14NS</td>
<td>1.86 ± 0.07***</td>
<td>3.51 ± 0.16***</td>
</tr>
<tr>
<td>GSH</td>
<td>3.76 ± 0.09</td>
<td>3.61 ± 0.07NS</td>
<td>1.27 ± 0.05***</td>
<td>3.44 ± 0.11***</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>218.3 ± 7.5</td>
<td>227.6 ± 8.1NS</td>
<td>146.2 ± 5.9***</td>
<td>206.8 ± 8.7***</td>
</tr>
<tr>
<td>GST</td>
<td>4.26 ± 0.21</td>
<td>4.21 ± 0.08NS</td>
<td>3.37 ± 0.10***</td>
<td>3.96 ± 0.18*</td>
</tr>
<tr>
<td>Proteins</td>
<td>19.3 ± 0.74</td>
<td>18.7 ± 0.82NS</td>
<td>9.96 ± 0.35**</td>
<td>18.1 ± 0.36**</td>
</tr>
<tr>
<td>Hexose</td>
<td>14.8 ± 0.57</td>
<td>15.6 ± 0.61NS</td>
<td>8.23 ± 0.52**</td>
<td>12.9 ± 0.41**</td>
</tr>
<tr>
<td>Hexosamine</td>
<td>8.72 ± 0.23</td>
<td>8.59 ± 0.25NS</td>
<td>4.86 ± 0.17**</td>
<td>7.73 ± 0.20**</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>1.66 ± 0.08</td>
<td>1.70 ± 0.05NS</td>
<td>0.74 ± 0.01**</td>
<td>1.62 ± 0.06**</td>
</tr>
</tbody>
</table>

P values: * < 0.05; *** < 0.001; Group I vs. Group II & Group III; Group IV vs. Group III.
NS = Non Significant

*aPepsin = µmol tyrosine/4 hr; LPO = nmol/mg protein; SOD = one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation; CAT = µmol of H2O2 consumed/min/mg protein; GSH = nmol/g tissue; GSH-Px = nmol GSH oxidised/min/mg protein; GST = µmol of 1-chloro-2,4 dinitrobenzene conjugate formed/min/mg protein; proteins, hexose, hexosamine and sialic acid = mg/g.
mucus and bicarbonate secretion and inhibit pepsin activity. The volume and total acidity of gastric juice significantly increased in the untreated ulcer group (group III) relative to the normal group (group I). The acid consuming capacity of \textit{G. cambogia} extract is 7.013 meq which effectively neutralized the acidic pH of HCl-ethanol thereby decreasing the acidity of gastric juice and the mucosal injury as evident in group IV rats.

\textit{G. cambogia} extract containing the principle organic acid \textit{(−)-erythro hydroxyx citric acid (HCA)} significantly inhibited lipid biosynthesis and diverted the metabolism of carbohydrate towards glycogen production in the liver thereby controlling the appetite and HCl output. The increased production of glycogen and concomitant stimulation of glucoreceptors in the liver, resulted in early satiety through signals sent to the brain via the vagus nerve. The decrease in acid output reduced the intensity of injury to the gastric mucosa as observed on indomethacin induced mucosal injury.

HCl-ethanol induced peptic ulcer (group III) caused corrosion of gastric mucosal cells resulting in their disruption and disintegration. Ulcerative lesions of gastrointestinal tract may be associated with increased loss of protein. The prior administration of extract (group IV) resulted in almost normal protein levels, suggesting its cytoprotective activity by preventing the erosions of the mucosa and loss of protein.

Depletion of glutathione in ulcerated rats is known to result in enhanced lipid peroxidation, and excessive lipid peroxidation can cause increased glutathione consumption, as observed in the present study. The prior administration of extract (group IV) protects the gastric cells against ethanol injury by decreasing their susceptibility to free radicals and acid. The extract maintained the activity of glutathione peroxidase almost at near normalcy, and its ability to increase the level of reduced glutathione and to decrease lipid peroxidation.

Carbohydrate : protein (C:P) ratio serves as a good indicator of gastric mucosal defense and its increase represents augmented mucosal protective activity. A near normal C:P ratio was observed in pretreated ulcer (group IV) which indicates its mucoprotective property.

Relative to the normal levels, hexose, hexosamine and sialic acid contents of the gastric juice decreased considerably in the ulcer group, while the protein level increased. The increase in protein content of the gastric juice indicates the damage to the gastric mucosa as a result of which plasma protein leak into the gastric juice. The decrease in the glycoprotein moieties in the gastric juice may be attributed to the decreased activity of defense mechanisms as a result of damage to the gastric mucosa. In other words, disintegration and degradation of glycoprotein moieties into simpler components in the process of HCl-ethanol induced injury may have resulted in minimal quantities of glycoprotein in the gastric juice. The levels of protein, hexose and hexosamine were maintained at near normalcy levels in the group pretreated with extract (group IV).

Gastric mucosa preexposed to an aqueous suspension of extract (group IV) showed a decrease in lipid peroxidation, an important cause of destruction and damage to cell membranes and an increase in the activity of superoxide dismutase and catalase. \textit{Garcinia} extract with appetite suppressing activity and acid neutralizing capacity, protects the defensive mucosal barrier against offensive assault of acid.

The antiulcerogenic effect of \textit{G. cambogia} extract could be due to the modulation of defensive factors through an improvement of gastric cytoprotection, acid inhibition, free radical scavenging properties and its ability to maintain the near normal status of GSH which protects mucosa against oxidative damage, by decreasing lipid peroxidation and strengthening the mucosal barrier which is the first line of defense against exogenous and endogenous ulcerogenic agents.

\textbf{Acknowledgement}

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\textbf{References}


