Desmodium gangeticum: A potent anti-ulcer agent

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The present study was designed to investigate anti-ulcerogenic property of ethanolic extract of Desmodium gangeticum (DG) against cold restraint (CRU, 2 hr cold restraint stress), aspirin (ASP, 150 mg/kg orally), alcohol (AL, absolute alcohol 1ml/200gm) and pyloric ligation (PL, 4 hr pylorus ligation) induced gastric ulcer models in Sprague Dawley rats, and histamine (HST, 0.25 mg/kg) induced duodenal ulcer in guinea pigs. We found that DG at a dose of 200mg/kg, (orally), markedly decreased the incidence of ulcers in all the above models. DG showed significant protection against CRU (68.37%), AL (88.87%), ASP (38.2%), PL (40.63%) and HST (63.15%) induced ulcer models, whereas standard drug omeprazole (OMZ) showed protection index of 83.86, 56.35, 70.31 and 84.21 %, respectively in CRU, ASP, PL and HST models. Sucralfate as standard drug showed 92.64% protection in AL model. DG significantly reduced acid secretion 41.61 %, whereas OMZ produced 43.13% reduction. Treatment with DG showed increase in mucin secretion by 56.17%, whereas OMZ showed 12.45% increase. Anti-ulcer effect of DG may be due to its cytoprotective effect along with anti-secretory activity and could act as a potent therapeutic agent against peptic ulcer disease.

Keywords: Anti-ulcer Desmodium gangeticum; Gastric ulcer; Leguminosae

Desmodium gangeticum DC (Leguminosae, suborder Papilionaceae; DG) popularly known as shaparni, is a well known Indian medicinal plant and is used by Ayurvedic and Unani physician as a febrifuge and anti-catarrhal. It is a small perennial shrub growing throughout India ascending to 5000 feet in the Himalayas and also in the plains of India. Plant is of great therapeutic value in treating diseases as typhoid, piles, inflammation, asthma, bronchitis and dysentery. The root is prescribed in combination with other drugs for the treatment of snakebite in the scripts of Shranghasamhita by Shushruta, and scorpion sting in Charakasamhita by Sushruta.

Peptic ulcer is one of the major gastrointestinal disorders that occur due to an imbalance between offensive and defensive factors. Major offensive factors are acid, pepsin and H. pylori infection and defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins. Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal protection has been the major approaches for therapy of peptic ulcer disease.

Number of drugs including proton pump inhibitors, histamine receptor antagonists, prostaglandin analogs and cytoprotective agents are available for the treatment of peptic ulcer. Although these drugs have brought about remarkable changes in ulcer therapy, the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapse and adverse effects during ulcer therapy. This has been the rationale for the development of new anti-ulcer drug and has been extended to herbal drugs in search for novel molecules that could show better protection and decrease the incidence of relapse. Hitherto, there is no report regarding the anti-ulcer effect of DG. Hence, in this preliminary study an attempt has been made to evaluate the effects of extract of DG on experimentally induced gastric ulcers and its possible effect on offensive and defensive factors.

Materials and Methods

Animals—Sprague-Dawley rats of either sex, weighing 180-200 g were obtained from National Animal Laboratory Centre, of the institute. Animals were kept in raised mesh bottom cages to prevent coprophagy and kept in environmentally controlled rooms (25°C ± 2°C, 12 hr light and dark cycle) with...
free access to water. Animals were fed with standard Hind Lever diet pellets ad libitum.

All animals were deprived of food for 18 hr before subjecting to ulcerogens and were randomly allocated to different experimental groups. Six rats were used for each group.

Experimental protocols were approved by our institutional ethical committee, which follow guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) that complies with international norms of INSA.

Collection of plant—The whole plants of Desmodium gangeticum (DG) were collected from the region of Utranchal in the month of February, and were identified by Botany Division, Central Drug Research Institute, Lucknow. A voucher specimen (No. 4604) was kept in the herbarium of the Institute.

Whole plant was shade dried and powdered. About 500 g of powdered plant was placed in glass percolator with ethanol (4 I) and was allowed to stand at room temperature for about 16 hr (overnight). The percolate was collected. This process of extraction was repeated for four times. The combined extract was filtered and concentrated under vacuum using rotavapor at 40°C. Weight of extract obtained was 30.5g. The total yield of plant extract was 6.1%.

Treatment schedule—Omeprazole (OMZ; Sigma Chemicals, USA) and sucralfate (SUC; Menarini Raunaq Pharma Ltd, India) were used as reference standards. DG extract and standard drugs were administered orally, once daily as an aqueous suspension of 1% sodium carboxymethylcellulose (CMC) at a volume of 1 ml/200g of body weight. Control group of animals was treated with vehicle similar to experimental groups.

Animals were treated with DG extract, OMZ and SUC at a dose of 200, 10 and 500 mg/kg body weight, respectively.

Anti-ulcer studies

Cold restraint stress induced ulcers (CRU) model—Fasted animals (for 18 hr) were subjected to stress paradigm as described earlier. After 45 min of extract and OMZ treatment, the rats were immobilized and were placed at 4°C in an environmental chamber. The animals were sacrificed 2 hr later by cervical dislocation and stomach was taken out, cut along the greater curvature. Ulcers in the glandular portion of stomachs were scored.

Aspirin induced ulcer (ASP) model—Aspirin at a dose of 150 mg/kg body weight was used for the induction of ulcers. Aspirin was administered orally to the rats after 45 min of extract and OMZ treatment. After 5 hr, all animals were sacrificed and ulcer scoring was done.

Alcohol induced ulcers (AL) model—Gastric ulcers were induced by administration of absolute alcohol at a dose of 1ml/200g of body weight, orally, after 45 min of extract and SUC treatment to all groups of animals.

Animals were sacrificed 1 hr after and hemorrhagic length was measured to calculate ulcer index.

Pylorus ligation induced ulcers (PL) model—After 45 min of extract and OMZ treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats under pentobarbital anaesthesia at a dose of 35mg/kg, ip of body weight. Pyloric ligation was done without causing any damage to blood supply of the stomach. The stomach was replaced carefully and abdomen was closed with sutures. Animals were allowed to recover and stabilize in individual cage and were deprived of water during post-operative period. After 4 hr of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected for performing gastric secretion study.

Histamine induced duodenal ulcers (HST) model—Duodenal ulcers were induced by intraperitoneal administration of histamine acid phosphate (Sigma Chemicals, USA) at a dose of 0.25 mg/kg of body weight to guinea pigs, after 45 min of extract and OMZ treatment, at every 30 min interval for 4 hr. Promethazine hydrochloride at a dose of 2.5 mg/kg of body weight was injected (ip) to each animal 15 min prior to administration of histamine, in order to protect animals from histamine toxicity. Animals were sacrificed after 30 min of last dose of histamine. Stomach was cut along the lesser curvature down to duodenum to assess for the presence or absence of ulcers on posterior and anterior wall of duodenum.

Gastric secretion study—The gastric juice was collected 4 hr after pylorus ligation and centrifuged at 2000 rpm for 5 min. The supernatant was stored for biochemical analysis. The volume of gastric juice was expressed in terms of ml/100 g of body weight. Total acid secretion was measured by titrating with 0.01 N NaOH, using phenolphthalein as indicator and expressed in terms of μEq/ml. Peptic activity was determined by measuring the amount of liberated tyrosine by the action of enzyme on hemoglobin as a substrate and expressed in terms of units/ml. Mucin content was expressed in terms of μeq/ml.
Measurement of ulcer index—Ulcer scoring was done by viewing ulcers with magnascope under magnification (5X). Ulcers were scored with the help of arbitrary scale as described earlier. The following arbitrary scoring system was used to grade the incidence and severity of the lesions—(i) shedding of epithelium =10; (ii) petechial and frank hemorrhages = 20; (iii) one or two ulcers = 30; (iv) more than two ulcers = 40; and (v) perforated ulcers = 50.

Length of hemorrhagic band in AL model was considered as ulcer index and measured with the help of Biovis image analysis software.

Ulcer index was calculated from scorings as—
\[ UI = Us + Up \times 10^3 \]
where, \( Us \) = Mean severity of ulcer score; \( Up \) = Percentage of animals with ulcer incidence.

Percentage protection index was calculated as-\( (C-T/C) \times 100 \) (where, \( C= \) Ulcer index in control group; \( T= \) Ulcer index in treated group).

Statistical analysis—All values have been expressed as mean ± SEM. Data of ulcer index was analyzed by non-parametric ANOVA followed by Dunnett’s multiple comparison test and other data was evaluated by one-way ANOVA followed by Dunnett’s multiple comparison test using Graph Pad PRISM software. \( P \) value <0.05 was considered significant.

Results

In our pilot study, graded doses of DG extract (50, 100, 200 and 400 mg/kg body weight) were given. We found that 200 mg/kg body weight to be the most effective dose showing protection index of 68.27%, whereas standard drug OMZ has shown 83.86% protection (Fig. 1). Hence, the dose of 200 mg/kg body weight was selected for further study.

Ethanolic extract of DG showed significant anti-ulcer effect against ulcers induced in all the models. In CRU, ASP, PL and HST induced ulcer models, DG at a dose of 200 mg/kg body weight showed protection index of 68.27, 38.2, 40.63 and 63.15%, respectively, whereas standard drug OMZ at a dose of 10 mg/kg showed protection index of 83.86, 56.35, 70.31 and 84.21 % in the above mentioned models (Fig. 2).

The protection index varies between different models, but DG was found to be most protective in alcohol induced ulcer model at a dose of 200 mg/kg body weight by showing protection index of 88.87%, whereas SUC at a dose of 500 g/kg body weight showed 92.64% protection (Fig. 2).

Significant reduction in total acid secretion was also observed with no effect on peptic activity. Further, DG extract showed significant rise in mucin secretion as compared to control (Table 1).

Discussion

Peptic ulcers, are the results due to overproduction of gastric acid and/or decrease in gastric mucosal protection mechanisms. That is why, the potential anti-ulcerogenic and ulcer healing drugs are known to possess the property of decreasing offensive factors or of increasing the defensive factors. Some herbal drugs have been mentioned in Ayurveda for the treatment of peptic ulcers, hence modern science in the light of Ayurvedic knowledge can provide a much comprehensive study.

DG is known to possess various therapeutic properties and has been one of the most noteworthy
Table I—Effect of ethanolic extract of Desmodium gangeticum and omeprazole at doses of 200 and 10 mg/kg body weight respectively on total acid output, peptic activity and mucus secretion of gastric juice in pylorus ligation induced ulcer model. 

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume (ml/100g body wt)</th>
<th>Conc μeq/ml</th>
<th>Output μeq/4 hr</th>
<th>Conc μmol/ml</th>
<th>Output μmol/4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% CMC)</td>
<td>4.7±0.1</td>
<td>61.8±5.1</td>
<td>291.2</td>
<td>54.2±4.0</td>
<td>254.7</td>
</tr>
<tr>
<td>DG (200)</td>
<td>2.7±0.2</td>
<td>36.1±3.2**</td>
<td>97.5**</td>
<td>50.1±7.3</td>
<td>135.3</td>
</tr>
<tr>
<td>OMZ (10)</td>
<td>3.2±0.2</td>
<td>35.2±3.6**</td>
<td>114.0**</td>
<td>47.6±4.0</td>
<td>152.3</td>
</tr>
</tbody>
</table>

Statistical analysis was done by One-Way ANOVA followed by Dunnett's multiple comparison test. Significant at *P<0.05, **P<0.01 as compared to control.

Ethanolic extract of DG significantly reduced the formation of gastric ulcer in rats induced by various ulcerogens in the present study. We have studied anti-ulcerogenic activity of DG in five different models including CRU, ASP, AL, PL and HST induced gastric and duodenal ulcer models, where the induction of ulcers was either due to the effect of these ulcerogens on acid secretion or on cytoprotection or on both. It was found that DG in a dose dependent manner decreased the incidence of ulcer and 200 mg/kg body weight of DG was the statistically optimal dose for anti-ulcer studies.

CRU model was chosen for the initial study to ascertain the dose and duration of treatment. CRU is a well-accepted model for induction of gastric ulcer in which peripheral sympathetic activation plays an important role in induction of ulcers. In CRU, incidence of ulcers is mainly due to increased acid secretion and generation of free radicals etc. DG significantly decreased the ulcer index in this model as compared to control. DG was effective at a dose of 200 mg/kg body weight, the reason could be that lower doses may be ineffective and higher doses may be showing irritating effect on gastric mucosa by increasing gastric secretion. Efficacy of DG in this model may be because of its antioxidant activity. This was in agreement with earlier reports about antioxidant activity of DG, that suggest the free radical scavenging effect of DG. Such activity might also be responsible for anti-ulcer effect of DG.

Ethanol induced ulcers are due to direct necrotizing effect of ethanol on gastric mucosa. Ethanol causes necrosis of superficial epithelial cells on gastric mucosa and erosion. Hence, a cytoprotective agent, which increases mucus secretion, will be effective in this model. In the present study, it was observed that DG significantly reduced the ulcer index and increased mucus secretion in PL model as compared to control.

In PL, ulcers are developed due to accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa. Further, role of free radicals is also reported in induction of ulcers. Reduced acid output measured after pyloric ligation suggested that the protective mechanism of extract on gastric mucosa involved an inhibition of gastric secretion and its cytoprotective ability by virtue of increased mucus secretion.

In aspirin induced ulcer model, DG significantly reduced ulcer index that further supported cytoprotective effect of DG, which might be mediated by prostaglandins or because of blockade of back diffusion of H⁺ ion as DG significantly reduced the acid secretion in the present study.

Based on the present study, it could be concluded that the efficacy of DG in all ulcer models was mainly due to its more cytoprotective effect in comparison to its anti-secretory effect. It is well-documented that natural drug mostly augment the defensive factors and may be slow in activity, but are reliable and safe. Hence, use of DG alone or with combination with other drugs should be seriously considered.

Further, our results fortify the ethanopharmacological importance of DG as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. DG has been found effective in all models depicting its anti-ulcerogenic activity, hence DG and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence. However, more experimentation for anti-ulcer and detailed analysis is required to reach at a definitive conclusion.

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References


