Evaluation of *Pongamia pinnata* root extract on gastric ulcers and mucosal offensive and defensive factors in rats

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Effect of methanolic extract of *P. Pinnaw* roots (PPRM) was studied against various experimental gastric ulcer models and offensive and defensive gastric mucosal factors in rats. An initial dose-response study using 12.5-50mg/kg *P. Pinnata* root extract, when given orally in two divided dose for 4 days + 5th full dose on the day of experiment 60 min before the experiment, indicated 25mg/kg as an optimal regimen and was used for further study. PPRM showed significant protection against aspirin and 4 hr PE, but not against ethanol-induced gastric ulceration. It showed tendency to decrease acetic acid-induced ulcer after 10 days treatment. Ulcer protective effect of *PPRM* was due to augmentation of mucosal defensive factors like mucin secretion, life span of mucosal cells, mucosal cell glycoproteins, cell proliferation and prevention of lipid per oxidation rather than on the offensive acid-pepsin secretion.

*P. pinnata* (linn) Pierre (Syn P. glabra Vent) is a medium sized glabrous tree, found through out India and further distributed East wards mainly in the littoral regions of South Eastern Asia and Australia. Different parts of this plant have been recommended in Ayurvedic literature as a remedy for various ailments. The seeds and seed oil of this plant have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, muscular and rheumatism. Leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement. Recently the ethanolic extract of leaves of this plant is shown to have prominent anti-inflammatory activity but devoid of any ulcerogenic potential as the commonly used NSAIDs generally showed increased propensity to gastric ulceration. Recently, we have reported the ulcer protective effects of extracts of both seeds and roots of *P. pinnata* (PP) and the ethanolic extract of the roots of this plant tended to decrease acid-pepsin and increase mucin secretion. Although several factors are reported to be involved in ulcerogenesis, the causative factor is basically the imbalance between offensive and defensive mucosal factors. Several of the drugs used for the treatment of ulcers are those, which reduce or neutralize the offensive acid secretions, while some other drugs like carbadoxolone, sulphafate and prostaglandin analogs increase the defensive factors with out affecting the offensive acid secretion. They are termed as cytoprotective drugs and are proved useful in ulcer therapy. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect of mucosal defensive factors. The present work is thus, in continuation of our earlier work and includes various experimental ulcer models and parameters of mucosal offensive (acid-pepsin output and reactive oxygen species) and defensive factors (gastric mucin secretion and gastric mucosal cell shedding, cell proliferation or glycoproteins) using methanolic extract of *P. pinnata* roots.

Materials and Methods

**Collection and extractions**—Roots of *Pongamia pinnata* were collected locally in the month of November and were duly authenticated by Dr S K Tiwari, Department of Kayachikitsa, BHU, Varanasi, India. The roots were macerated (1000 g) and extracted with methanol (3 l, 5 days) by cold percolation. The % yield of dried extract was 3.2%.

**Animals**—Albino rats (CF strain) of either sex, weighing between 150-200 g were used for the present study. They were obtained from the Central Animal House of the Institute. They were kept in colony cages at room temperature (25° ± 3° C) and RH (45-56%) under 12 hr light/dark cycles for 5 days before treatment. The animals were allowed freely to...
access rodent pellet diet (Hind lever) and water ad libitum.

Drug treatment — Methanolic extract of root of P. pinnata (PPRM) was given in graded doses of 12.5, 25 and 50 mg/kg in two divided doses, daily for 4 days and the last full dose was given on 5th day of experiment, 60 min before subjecting 18 hr fasted rats to 2 hr cold restraint stress. Preliminary results indicated an optimal ulcer protective effect with 25 mg/kg. Therefore, for further study this dose and duration was chosen for detailed study, while the control animals received 1% carboxy methylcellulose. All the animals received the drugs orally with the help of an orogastric tube at a concentration of 1.0 ml/100 g. Any deviation in the duration of treatment has been mentioned in the Results/Table of the present study.

Anti-ulcer study
Cold-restraint stress (CRS)-induced ulcers — To 18 hr fasted rats, cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2 hr at 4°-6°C. The animals were then sacrificed by cervical dislocation and ulcers were scored on the dissected stomachs and ulcer index was calculated as described earlier. Statistical analysis was done using Wilcoxon Rank Sum test.

Aspirin (ASP) induced ulcers — ASP at a concentration of 200 mg/kg (20 mg/ml) was administered to the animals on 5th day of experiment and ulcers were scored after 4 hr. The stomach was taken out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach as described above.

Ethanol (EtOH) induced ulcers — Gastric ulcers were induced in rats by administering EtOH (1 ml/200g, 1 hr) on 5th day of experiment and the animals were sacrificed by cervical dislocation 1 hr after ethanol administration. Stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm²/rat). Statistical analysis of data was done using unpaired Student’s t test.

Pylorus ligated (PL) induced ulcers — Rats were kept for 18 hr fasting and care was taken to avoid coprophagy. On the 5th day of experiment, animals were anaesthetized after the drug treatment described above, using pentobarbitone (35 mg/kg, ip), the abdomen was opened and pyloric end of stomach was ligated without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period. After 4 hr, stomachs were dissected out and contents were collected into tubes for estimation of biochemical parameters. The ulcers were scored and statistically analysed as described above for cold restraint stress ulcers.

Ulcer healing study
Acetic acid induced ulcers — For induction of acetic acid ulcer, the method was followed as reported earlier. Rats were anaesthetized with pentobarbitone (35 mg/kg, ip). The abdomen was opened and the stomach was visualized. A cylindrical glass tube (6 mm in diam) was tightly placed upon the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end. 50% acetic acid (0.06 ml/animal) was instilled into the tube and allowed to remain for 60 sec on the gastric wall. After removal of acid solution, the abdomen was closed in two layers and animals were caged and fed normally. PPRM (12.5 mg/kg) was given on day one, orally, twice daily, 4 hr after the application of acetic acid and continued either up to 5 or 10 days after induction of ulcer. The animals were then sacrificed after 18 hr of the last dose of drug either on 6th or 11th day of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (mm²/rat) of ulcers. Statistical significance was calculated using unpaired Student’s t test.

Gastric secretion study — Studies on offensive factors such as acid and pepsin and defensive factors such as mucin secretion and cell shedding were carried out in gastric secretion. The gastric juice was collected 4 hr after PL and centrifuged for 5 min at 2000 rpm. The supernatant was collected and the volume of gastric juice was expressed as ml/100g body weight. Acid output was determined by titrating with 0.01 N NaOH, using phenolphtalein as indicator and expressed as μEq/4hr. Peptic output was determined using hemoglobin as substrate and expressed as μEq/4 hr. Dissolved mucous substances were estimated in 90% alcoholic precipitate of gastric juice. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 N NaOH or 1 ml of 0.1 N H₂SO₄. The former was used for estimation of protein, total hexoses, hexosamine and fucose, while the latter was used for estimation of sialic acid. The results have been expressed in μg/ml of gastric juice. Ratio of total...
carbohydrate (TC; sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity. DNA content was estimated and expressed as μg/ml gastric juice. Statistical analysis of data was done using unpaired Student’s t test.

Gastric mucosal study—Estimation of cellular mucin as glycoproteins was carried out in the gastric mucosa of 4 hr pyloric ligated rats. Mucosal scrapings of glandular portion of rat stomach were homogenized in normal saline (20 mg/ml) and treated with 90% ethanol in the same manner as described for mucin estimation in the gastric juice. The precipitate thus obtained, was subjected for estimation of carbohydrates and protein using the methods described above for gastric juice contents. The results are expressed as μg/100mg wet tissue and TC: P ratio has been taken as the index of glycoprotein activity. Statistical analysis of data was done using unpaired Student’s t test.

Cell proliferation—Estimation of DNA was carried out in gastric mucosal scrapings. Mucosal scrapings of glandular portion of rat stomach were homogenized in 2.5 ml of ice-cold 0.6 N perchloric acid. DNA and protein were then estimated. The concentration of DNA has been expressed as μg DNA/mg protein.

Estimation of free radical generation

The fundic part of the gastric mucosal scrap was homogenized (5%) in ice-cold 0.9% saline with a Potter-Elvehjem glass homogeniser for 30 sec. The homogenate was then centrifuged at 800 g for 10 min followed by centrifugation of the supernatant at 12,000 g for 15 min and the obtained mitochondrial fraction was used for following estimations.

Lipid peroxidase—LPO product malondialdehyde (MDA) was estimated using 1,1,3,3-tetraethoxypropane as the standard and has been expressed as nmole/mg of protein.

Superoxide dismutase (SOD) activity—SOD was estimated by following the procedure of Kakkar et al. Inhibition of reduction of nitro blue tetrazolium (NBT) to blue coloured formozan in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. One unit of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50 % in 1 min under the defined assay conditions and the results have been expressed as units (U) of SOD activity/mg of protein.

Catalase (CAT) activity—Decomposition of H₂O₂ in presence of catalase was followed at 240 nm. One unit of (U) CAT was defined as the amount of enzyme required to decompose 1μmole of H₂O₂ per min, at 25°C and pH 7.0. Enzyme activity has been expressed as units (U) of CAT activity/mg of protein.

Results

Methanolic extract of P. pinnata roots (PPRM; 12.5-50 mg/kg) when given in two divided doses, orally daily for 4 days and a full dose, 60 min before the experiment on day 5, showed significant ulcer protection (control ulcer index: 35.2±6.8; PPRM 12.5-50 mg/kg, % protection—48.3-67.9%; P<0.1–P<0.01). A dose of 25 mg/kg for 5 days i.e. 12.5 mg/kg, bd×4 days +25.0 mg/kg stat on 5th day 60 min before, was then selected for further study. This dose showed significant ulcer protection against aspirin and 4 hr pylorus ligation (PL), but not against ethanol induced gastric ulceration (Table 1). Sucralfate (SFT, 250 mg/kg bd, po, x 4 days+500 mg/kg, stat, 60 min before on day 5) showed significant ulcer protection in all the three above models (Table 1). Further, PPRM tended to heal acetic acid induced gastric ulcers after 10 days treatment only, while SFT decreased the ulcer index both after 5 and 10 days treatment (Table 2).

On PL induced gastric secretion, PPRM tended to increase acid–pepsin secretion, while SFT significantly decreased the pepsin output (Table 3). PPRM tended to increase mucin activity in terms of total carbohydrate and protein ratio (TC: P), but SFT increased it significantly. There was no significant effect on individual carbohydrates (total hexoses, hexosamine, fucose or sialic acid) or total carbohydrates and protein with PPRM. However, SFT either tended to increase or increased the above parameters (Table 3). DNA concentration of gastric juice is an

<table>
<thead>
<tr>
<th>Oral treatment (mg/kg×5 days)</th>
<th>Ulcer index</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Aspirin</td>
<td>PL</td>
</tr>
<tr>
<td>Control (1% CMC)</td>
<td>18.3±3.2</td>
<td>25.1±4.0</td>
<td>10.0±0.9</td>
</tr>
<tr>
<td>PPRM</td>
<td>25</td>
<td>20.5±3.6</td>
<td>15.1±1.8*</td>
</tr>
<tr>
<td>SFT</td>
<td>500</td>
<td>9.7±1.6</td>
<td>7.3±3.7b</td>
</tr>
</tbody>
</table>

P values: *<0.05, b<0.01, c<0.001 compared to respective control groups.

Table 1—Effect of methanolic extract of fresh roots of P. pinnata (PPRM) and sucralfate (SFT) on ethanol (100%, 1ml/200g, po, 1 hr), aspirin (200 mg/kg, po, 4 hr) and 4 hr pylorus ligation (PL) induced gastric ulcers in rats

[Values are mean ± SE from 8 animals in each group]
important marker of gastric mucosal damage or cell shedding. DNA content was significantly decreased by both PPRM and SFT (Table 3).

On mucosal glycoproteins, PPRM had little or no effect on individual carbohydrate or total carbohydrates but tended to decrease protein content leading to significant increase in TC: P ratio while, SFT tended to increase, or increased the individual carbohydrate. Again, SFT tended to decrease protein content leading to significant increase in TC: P ratio while, SFT tended to increase, or increased the individual carbohydrate. DNA/mg protein is a reliable index for cell proliferation. PPRM tended to increase the cell proliferation (32.4% increase), while SFT had little effect (13.1% increase; Table 4). Free radical and lipid peroxidation (LPO) play an important role in ulcerogenesis. PL tended to increase LPO, while superoxide dismutase (SOD) and catalase (CAT) levels were little affected. PPRM tended to reverse the above increase in LPO without any effect on SOD and CAT levels compared to control PL group (Table 5).

**Discussion**

The present study indicated the ability of roots of *P. pinnata* to protect the formation of gastric ulcer significantly in various experimental models except ethanol-induced ulcer model in rats. Significant protection was also observed with SFT, a known cytoprotective drug used clinically in healing of gastric ulcers and preventing the recurrence of peptic ulcers. Earlier, we reported the ulcer protective effect of ethanolic extract of *P. pinnata* roots in CRS- and 4 hr PL-induced ulcer models and the effect could be due to its effect on mucin secretion, which is one of the important mucosal defensive factors. Recently Srinivasan and associates while studying the anti-inflammatory activity of ethanolic extract of PP leaves in rats, demonstrated anti-inflammatory activity of this plant and this effect was not associated with any ulcerogenic effect which most of NSAIDS like aspirin show. In an earlier observations from our laboratory, some of the plant products like kaempferol, a flavanoid and amentoflavanone too demonstrate both anti-inflammatory and anti-ulcerogenic activity. It thus, indicates that in plant kingdom both the activities can run side by side.

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Table 2 — Effect of PPRM and SFT on 50% acetic acid-induced gastric ulcer healing

<table>
<thead>
<tr>
<th>Oral treatment (mg/kg × 5 days)</th>
<th>Acetic acid-induced ulcers (healing)</th>
<th>5 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcer index % incidence perforations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (1% CMC)</td>
<td>13.1 ± 1.1</td>
<td>40.0</td>
<td>7.0 ± 1.2</td>
</tr>
<tr>
<td>PPRM 25</td>
<td>15.7 ± 2.2</td>
<td>37.5</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>SFT 500</td>
<td>8.9 ± 1.7</td>
<td>12.5</td>
<td>2.1 ± 0.9</td>
</tr>
</tbody>
</table>

*P* values: *a* < 0.05, *b* < 0.01 compared to respective control groups

Table 3 — Effect of PPRM (25 mg/kg, po × 5 days) and SFT (500 mg/kg, po × 5 days) on gastric juice volume, acid, pepsin and DNA content in 4 hr PL rats

<table>
<thead>
<tr>
<th>Gastric juice</th>
<th>Control</th>
<th>PPRM</th>
<th>SFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid—pepsin secretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (ml/100 g)</td>
<td>1.99 ± 0.15</td>
<td>2.21 ± 0.15</td>
<td>1.87 ± 0.13</td>
</tr>
<tr>
<td>Acid output (μEq/4 hr)</td>
<td>163.4 ± 16.8</td>
<td>187.1 ± 15.8</td>
<td>193.3 ± 11.9</td>
</tr>
<tr>
<td>Pepsin output (μ mole/4 hr)</td>
<td>549.2 ± 52.8</td>
<td>603.2 ± 71.2</td>
<td>382.2 ± 47.2</td>
</tr>
<tr>
<td>Mucin activity (μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hexoses</td>
<td>350.0 ± 30.8</td>
<td>379.7 ± 24.1</td>
<td>401.2 ± 19.3</td>
</tr>
<tr>
<td>Hexosamine</td>
<td>178.7 ± 9.1</td>
<td>173.1 ± 9.9</td>
<td>204.1 ± 13.2</td>
</tr>
<tr>
<td>Fucose</td>
<td>80.3 ± 4.2</td>
<td>108.9 ± 14.6</td>
<td>99.3 ± 7.3</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>15.9 ± 1.3</td>
<td>20.0 ± 2.1</td>
<td>29.1 ± 5.4</td>
</tr>
<tr>
<td>Total carbohydrates (TC)</td>
<td>624.9 ± 36.0</td>
<td>681.7 ± 28.5</td>
<td>731.0 ± 33.4</td>
</tr>
<tr>
<td>Protein (P)</td>
<td>638.9 ± 40.5</td>
<td>573.8 ± 31.5</td>
<td>550.3 ± 23.9</td>
</tr>
<tr>
<td>TC: P</td>
<td>1.02 ± 0.09</td>
<td>1.23 ± 0.10</td>
<td>1.33 ± 0.11</td>
</tr>
<tr>
<td>Cell shedding (μg DNA/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA (μg/ml)</td>
<td>162.0 ± 8.2</td>
<td>114.2 ± 7.7</td>
<td>108.1 ± 8.9</td>
</tr>
</tbody>
</table>

*P* values: *a* < 0.005, *b* < 0.001 compared to respective control
and these drugs could be used for their antiinflammatory activities without the danger of gastric ulceration.

Damage produced by ethanol to gastric mucosa is due to number of contributing factors and includes effects on mucosal blood flow, platelet thrombi, damage to capillary endothelium and release of arachidonic metabolites. PL-induced ulcers are thought to be due to increased presence of acid and pepsin in the stomach. Stress induced ulcers are caused by a number of factors both physical and psychological in nature. Increase in gastric motility, vagal over activity, mast cell degradation, decreased gastric mucosal blood flow and increased leukotrienes synthesis were also reported for gastric ulcers caused by ethanol and stress. Hence, diverse mechanism might be involved in anti-ulcerogenic activity of PPRM. The extract had no effect on acid-pepsin secretion but tended to increase mucus secretion. It significantly increased mucosal glycoproteins and life span of mucosal cells as evidenced by decrease in DNA content of gastric juice and both could play an important role for its ulcer protective effects. Endogenous prostaglandins, mucosal blood flow or bicarbonate secretion play an important role in ulcer protection and it could be possible that this extract might have significant effect on any of these parameters.

Rapid proliferation of gastric mucosa plays an important role in mucosal protection during normal state following mucosal damage. Following extensive damage of the surface epithelial cells, repair occurs within a few hours through a process called restitution and which could be followed by cell proliferation. Increased life span of cell as evidenced by a decrease in cell shedding and increased tendency to cell proliferation as evidenced by an increase in DNA/mg protein, thus indicated gastric mucosal renewal and restitution by P. pinnata.

Increase in lipid peroxidation (LPO) levels indicate, increase of reactive oxygen species (ROS), the major radical being superoxide anion $(O_2^-)$, $H_2O_2$ and hydroxyl radical $(OH)$. These induce cell degradation by increasing peroxidation of cell membrane lipids causing loss of structural and functional integrity of cell membranes. Increase in superoxide dismutase (SOD) level is in response to increased tissue $O_2$.
speeding up their dismutation and converting it immediately into \( \text{H}_2\text{O}_2 \). (Ref. 42). Accumulation of \( \text{H}_2\text{O}_2 \) occurs in the mitochondria and cytosol and if not scavenged by catalase (CAT)\(^4\), can lead to increased generation of \( \text{OH}^- \) radical. In 4 hr PL-induced gastric damage, LPO was significantly increased with little effect on SOD and CAT levels. However, treatment of rats with PPRM tended to reverse change in LPO activity, indicating decreased lipid peroxidation and damage to cells. Thus, the present study indicated the ulcer protective effects of PPRM. The protection afforded by PPRM could be due to the augmentation of defensive mucosal factors and inhibition of lipid peroxidation.

**Acknowledgement**

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**References**

42 Fridovich I, Biological effects of superoxide radical, Arch Biochem Biophys, 247 (1986) 1.