Antiulcerogenic effect of *Justicia prostrata* Gamble

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Antiulcerogenic effect of the alcoholic (ALJP) and aqueous (AQJP) extracts of the whole plant of *Justicia prostrata* was studied in aspirin + pylorus ligated rat models and analysed for gastric volume, ulcer index, free and total acidity. Biochemical parameters like total proteins, total hexoses, hexosamine, fucose and sialic acid were also estimated. Both extracts (ALJP and AQJP) significantly reduced both the gastric volume and the acidity of gastric juice. It also significantly promoted gastric mucus secretion by increasing total carbohydrates and decreasing the protein concentration in aspirin+pylorus ligated rats. The results suggest that both the extracts (ALJP and AQJP) possess antiulcer activity, whereas AQJP is more effective when compared with ALJP in aspirin+pylorus ligated rat models. The results were compared with the standard drug Ranitidine, a H₂ receptor antagonist.

**Keywords:** Antiulcerogenic activity, Aspirin+pyloric ligation, *Justicia prostrata*

**IPC Code:** Int Cl. A61P

*Justicia prostrata* Gamble (Acanthaceae) is a small herb, which is widely distributed in the southern parts of India and mountains of Western Himalayas. Some species of the genus *Justicia* have been used in the traditional system of medicine for the treatment of fever, pain, inflammation, diabetes, diarrhoea and liver diseases. They also possess anti-tumoral, antiviral, analgesic and anti-inflammatory activities. In this genus about 20 species have been chemically investigated and the major secondary metabolites isolated were lignans, flavonoids, steroids and triterpenes. In the traditional system of medicine the hot water extract of the whole plant of *J. prostrata* is used as an antidepressant. The literature survey indicated that the petroleum ether extract of the aerial parts of the plant possesses four different arylnaphtalide lignans like Prostalidins A, B, C and retrochinenin. Anti-depressant activities of these four lignans have been reported. An arylnaphtalide lignan, Justicidin-E has been isolated from the petroleum ether extract of the plant. The petroleum ether extract, Justicidin-E, aqueous extract (AQJP) and alcoholic extract (ALJP) possess antistress activity, as revealed by Porsolt forced swimming test. In another study, Sanmugapriya et al. found that these two extracts (AQJP and ALJP) possess significant antinociceptive activity. Also, preliminary phytochemical studies revealed the presence of polyphenolics, steroids, lignans and triterpenes. The presence of polyphenolics prompted us to carry out the anti-ulcerogenic effect of this plant.

It is well known that most of the available antiinflammatory drugs are ulcerogenic. As the two extracts of *Justicia prostrata* were reported to have analgesic activity, the effect of these two extracts has been evaluated on aspirin+pylorus ligation induced ulcer model in rats.

**Materials and Methods**

**Plant material**—The fresh whole plant of *J. prostrata* was collected from Vellore district (12.55°N and 79.11°E), Tamilnadu during July. The taxonomic identification of the plant was established by the Botanical Survey of India, Coimbatore, Tamilnadu and voucher specimen (SP-1) has been kept in the herbarium unit of the Pharmacology Department, University of Madras, Chennai.

**Preparation of extract**—The air-dried and coarsely powdered whole plant of *J. prostrata* was extracted initially with 90% alcohol by cold maceration procedure. The marc obtained from the alcoholic extraction was further utilized for aqueous extraction.
On evaporation of the alcoholic extract, a greenish residue was obtained (3.25% w/w), whereas the aqueous extract yielded 5.43% w/w brown coloured residue. Both the extracts were kept in a desiccator and a weighed amount of the extract was suspended in 1% sodium carboxy methyl cellulose (SCMC) before the administration. The extract was subjected to qualitative chemical analysis as per Trease and Evans. 

**Animals used**—Adult male Wistar albino rats weighing 180-200 g were used for the experiment. The animals were housed in polypropylene cages at 24°±2° C and were fed with proper food and water *ad libitum* throughout the experiment. The animals were divided in 6 groups of 6 animals each. The project got clearance from the institutional animal ethical committee (IAEC).

**Drugs**—The extracts and ranitidine were suspended in 1% SCMC and given orally for 10 days during the experiment and given orally for 10 days during the experiment.

**ED₅₀ of extracts**—ED₅₀ was obtained by dose response curve and was found to be (500 mg/kg/po) for both the extracts AQJP and ALJP (Fig 1).

**Aspirin+pyloric ligation induced ulcer model**—The methods of Goel et al., Shay et al., and Parmar et al. were followed for the evaluation of anti ulcerogenic activity. Out of 6 groups, group I served as normal control and received water. Group II served as solvent control and received 1% SCMC. Group III served as aspirin control, while groups IV, V and VI served as treatment groups receiving ranitidine (50 mg/kg/po), ALJP (500 mg/kg/po) and AQJP (500 mg/kg/po) once daily, orally for 10 days, respectively. From day 8th to 10th, animals in groups III, IV, V and VI received aspirin orally as an aqueous suspension at a dose of 200 mg/kg, 2 hr after the administration of the drugs. Pylorus ligation was carried out on 18 hr fasted rats on the 11th day. After 4 hr of pylorus ligation, the animals were sacrificed by decapitation. The stomach was cut open along the greater curvature and the gastric juice was collected, centrifuged and subjected to biochemical analysis, the volume of the supernatant was expressed as ml/100g body wt. The stomach was washed with normal saline, and the lesions were observed using a binocular magnifier. The gastric ulcers were measured and the ulcer index was determined.

**Estimation of total and free acidity**—The total and free acidity were determined by titrating with 0.01 M NaOH using phenolphthalein and Topfer’s reagent. The total and free acids were expressed as mEq/l. Total acid output was also calculated.

**Biochemical investigations**—Mucin activity was estimated in the mucosubstances obtained by precipitating twice the gastric secretion with 90% ethanol in 1:9 ratio. The precipitate obtained was divided into two parts, one part was dissolved in 1 ml of 0.1N NaOH and the other part in 1 ml of 0.1N H₂SO₄. The former was used for the estimation of protein, total hexoses, hexosamine and fucose while the latter was used for the estimation of sialic acid. The results are expressed in μg/ml. The ratio of total carbohydrates (sum of total hexoses, hexosamine, fucose and sialic acid) to protein was taken as the index of mucin activity.

**Statistical analysis**—The data are expressed as mean ± SE. Results were analysed statistically by One-Way ANOVA followed by Tukey’s multiple comparison using SPSS software Student’s version. The intergroup difference was considered significant at P<0.05.

**Results**

Aspirin, which is a known ulcerogenic drug plus pyloric ligation significantly increased the gastric volume, total acidity, free acidity, ulcer index, and total acid output and protein concentration in gastric juice, but decreased the individual carbohydrate concentrations like hexose, hexosamine and...
sialic acid. However, fucose remained unaffected (Tables 1 and 2).

Administration of ALJP and AQJP at a dose of 500mg/kg normalized all these alterations observed in aspirin+pylorus ligated rats. The TC:P ratio was increased in the drug treated groups, which indicates its mucin modulatory activity. Ranitidine, the standard drug significantly \((P<0.001)\) reduced ulceration induced by aspirin+pyloric ligation.

The histopathology of stomach of aspirin treated animals showed ulceration with haemorrhage and discontinuity in the mucosal epithelial lining with exudates in the lumen. The tissue also showed submucosal edema with hyper plastic mucosal glands. The animals treated with AQJP and ALJP showed normal mucosa without ulcer and edema. The results of AQJP and ALJP are comparable to that of ranitidine treated rats (Fig. 2).

**Discussion**

In all the experimental models the precipitating factor in inducing gastric ulcer was an increase in acid-pepsin activity with or without reduction in the mucosal resistance. Ulcerogens like ACTH, cortisone, aspirin and phenylbutazone reduce the rate of secretion of mucus by the canine stomach and reduce the concentration of protein bound carbohydrates in these secretions\(^27\). These agents injure the gastric mucosa by reducing its ability to form a protective layer of mucus. In addition, the ACTH and cortisone decrease the rate of renewal of surface epithelial cells while aspirin and phenylbutazone increase the rate of exfoliation of surface epithelial cells. An increased loss of mucosal cells without a concomitant increase in cell replacement could lead to a patchy mucosal denudation, erosions and bleeding\(^27\). Aspirin induces gastric ulcers by causing back diffusion of \(H^+\) ions

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total acidity (mEq/l)</th>
<th>Free acidity (mEq/l)</th>
<th>Gastric Volume (ml/100g)</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>162.00±6.16</td>
<td>116.00±1.39</td>
<td>2.55±0.03</td>
<td>1.11±0.02</td>
</tr>
<tr>
<td>SCMC control</td>
<td>161.50±1.68*</td>
<td>119.33±1.11*</td>
<td>2.51±0.01*</td>
<td>1.10±0.04*</td>
</tr>
<tr>
<td>Aspirin + ligation control</td>
<td>251.00±3.05</td>
<td>181.50±2.39</td>
<td>4.97±0.04</td>
<td>4.27±0.05</td>
</tr>
<tr>
<td>Ranitidine (50 mg/kg)</td>
<td>180.00±1.52*</td>
<td>133.83±1.90*</td>
<td>3.09±0.03*</td>
<td>1.44±0.06*</td>
</tr>
<tr>
<td>ALJP (500 mg/kg)</td>
<td>199.50±1.17*</td>
<td>147.50±1.58*</td>
<td>3.58±0.02*</td>
<td>2.19±0.04*</td>
</tr>
<tr>
<td>AQJP (500 mg/kg)</td>
<td>192.83±2.45*</td>
<td>143.66±1.97*</td>
<td>3.29±0.03*</td>
<td>1.97±0.04*</td>
</tr>
</tbody>
</table>

*P values: <0.001 as compared to aspirin + ligation control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protein (μg/ml)</th>
<th>Hexose (μg/ml)</th>
<th>Hexosamine (μg/ml)</th>
<th>Sialic acid (μg/ml)</th>
<th>Fucose (μg/ml)</th>
<th>TC (μg/ml)</th>
<th>TC:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>258.18</td>
<td>1047.25</td>
<td>430.92</td>
<td>74.59</td>
<td>96.48</td>
<td>1634.25</td>
<td>6.31</td>
</tr>
<tr>
<td>SCMC control</td>
<td>±3.96</td>
<td>±17.56</td>
<td>±15.46</td>
<td>±1.88</td>
<td>±2.05</td>
<td>±17.20</td>
<td>±0.09</td>
</tr>
<tr>
<td>Aspirin + ligation control</td>
<td>±1.50±*</td>
<td>±5.15*</td>
<td>±8.10*</td>
<td>±3.66*</td>
<td>±0.97</td>
<td>±11.74*</td>
<td>±0.04</td>
</tr>
<tr>
<td>Ranitidine (50 mg/kg)</td>
<td>304.56</td>
<td>976.16</td>
<td>356.72</td>
<td>67.41</td>
<td>101.06</td>
<td>1501.36</td>
<td>4.92</td>
</tr>
<tr>
<td>ALJP (500 mg/kg)</td>
<td>±2.44*</td>
<td>±10.59*</td>
<td>±12.33*</td>
<td>±1.97*</td>
<td>±2.09</td>
<td>±20.34*</td>
<td>±0.07</td>
</tr>
<tr>
<td>AQJP (500 mg/kg)</td>
<td>±3.09*</td>
<td>±20.41*</td>
<td>±14.31*</td>
<td>±1.48*</td>
<td>±20.34*</td>
<td>±22.20*</td>
<td>±0.10</td>
</tr>
</tbody>
</table>

*P values: <0.001 as compared to aspirin + ligation control

TC=Total carbohydrate, P=Protein

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Table 1—Effect of extracts on total and free acidity, gastric volume and ulcer index

Table 2—Effect of extracts on mucin activity of gastric juice in aspirin + pylorus ligation induced ulcers
Fig. 2—Histopathological studies of stomach mucosa. (a) Normal control: Normal mucosa with no ulcer; (b) SCMC control: Normal mucosa, no edema; (c) Aspirin + ligation control: Ulcerated mucosa shows hemorrhage (h) and discontinuity in the lining epithelium (le) with exudates in the lumen (ex). Submucosal edema with hyperplastic mucosal glands (d) Ranitidine: Normal mucosa with no ulcer, edema in the submucosa; (e) AQIP: Normal mucosa, no edema, thick muscularis mucosa; and (f) ALJP: Normal mucosa, no ulcer, edematous submucosa.
into the mucosal cells\textsuperscript{28}. In pyloric ligation, ulcers are caused by acid and peptic activity. The stomach digestive effect of accumulated gastric juice in the induction of gastric ulcers is well documented in the pylorus ligation model\textsuperscript{29}.

In the present study, ALJP and AQIP have been shown to possess anti-ulcer activity against experimentally induced acute ulcer model (aspirin + pyloric ligation model). Both extracts significantly reduced the acid secretory parameters as well as the gastric volume, ulcer index and total acid output. They also increased the individual carbohydrate concentrations (hexoses, hexosamine, fucose and sialic acid) and total carbohydrate:protein ratio.

The increase in individual carbohydrate content in ALJP and AQIP treated groups over that of the untreated ulcer group appears to be due to stimulation of mucus secretion. The protective role of mucus on gastric mucosa was explained by Florey and Webb\textsuperscript{30}. The more the mucus production, the less was the degree of ulceration.

Aspirin causes leakage of plasma protein into gastric juice. This reflects the increased protein concentration in the gastric juice of aspirin control rats\textsuperscript{31}. Both extracts reduced the protein concentrations by reducing the leakage of proteins into gastric juice. The total carbohydrate:protein ratio serves as a direct index of gastric mucosal defense i.e. reflection of mucin activity. Its increase represents augmented mucosal protective activity\textsuperscript{32}. As the extract appears to strengthen the mucosal barrier it can be categorized as mucoprotective agent. The histopathological evaluation further suggests the mucoprotective activity of AQIP and ALJP in aspirin+pylorus ligated ulcer model in rats.

Preliminary phytochemical investigations on the plant extracts revealed the presence of polyphenolics, steroids, lignans, etc. The anti-ulcer activity of these two plant extracts ALJP and AQIP in this experimental model may be due to the presence of polyphenolic compounds like flavonoids and tannins\textsuperscript{33}. Some flavonoids have been shown to increase the mucosal content of prostaglandin and mucus in gastric mucosa, showing cytoprotective effects\textsuperscript{34}. Hence, the mucoprotective action of the plant extracts may be attributed to the presence of polyphenolic compounds like flavonoids and tannins present in the plant extracts.

References


