Anti-secretagogue, anti-ulcer and cytoprotective properties of *Acorus calamus* in rats*

S. RAFATULLAH*, M. TARIQ, J.S. MOSSA, M.A. AL-YAHYA, M.S. AL-SAID, A.M. AGEEL

Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh-11451, Saudi Arabia

Received March 19, 1992 - Accepted (revised) November 8, 1993.

**SUMMARY.** The ethanolic extract of *A. calamus* was studied in rats for its ability to inhibit gastric secretion and to protect gastroduodenal mucosa against the injuries caused by pyloric ligation, indomethacin, reserpine and cysteamine administration and cytodestructive agents including 80% ethanol, 0.6 M HCl, 0.2 M NaOH and 25% NaCl. An oral dose of 500 mg/kg of the extract showed significant anti-secretory and anti-ulcerogenic activity in rats subjected to pyloric ligation, indomethacin, reserpine and cysteamine administration. The extract had highly significant protective effect against cytodestructive agents. These findings support the use of calamus for the treatment of gastropathy in traditional medicine.

**Key words:** *Acorus calamus*; anti-secretagogue activity; anti-ulcer activity; cytoprotective activity.

The aromatic rhizome of calamus, *Acorus calamus* Linn., family Araceae, is being official in many pharmacopoeias. L locock has reported the smooth muscle relaxant activity of this plant in rats. A similar spasmylytic activity of calamus has been reported by Tyler. Keys has mentioned that the use of calamus in Chinese medicine is to aid digestion and regulating gastrointestinal fermentation and in hyperacidity. Menon and Dandiya have reported the tranquilizing action of asarone, a constituent of *A. calamus*. The chloroform extract fractions of calamus have been shown to alter behavioural response of conscious rhesus monkeys. Recently Vashi and Patel have reported the microbicidal and fungicidal action of the calamus extract. Saxena *et al.* have developed a new insect chemosterilant from the calamus.

In Greeko-Arab medicine, the calamus, known as Wadj or Bach, has been used to treat numerous disorders including gastritis, lack of appetite, epilepsy, rheumatoid arthritis; it has been used as a stomachic, carminative, antiflatulent, sedative and febrifuge; it has been also applied externally in skin diseases.

The reported constituents of the calamus rhizome are asaron, parasaron, asarylaldehyde, sesquiterpenes, acorin, eugenol. The present investigation was undertaken to study the effect of the ethanolic extract of this plant on experimentally induced gastric and duodenal ulcers using various experimental model.

**EXPERIMENTAL**

*Animals.* Wistar albino rats of either sex of approximately the same age, generally weighing between 200-230 g, were used. The rats were fed standard chow diet and water *ad libitum*. They were divided into various groups of six to eleven animals each. The distribution of the animals into groups, the sequence of the trials and the treatment allotted to each group were randomized.

*Plant material and extract preparation.* The calamus rhizomes were procured from the local market and identified in taxonomy division of Medicinal, Aromatic and Poisonous Plants

---

*An Abstract of this paper has been published in Bonn Bacans' International Joint Symposium (Abstract pp 3-79), Bonn, July, 17-22, 1990.
Research Center; a sample was deposited in herbarium for future reference. The rhizomes (500 g) were powdered and percolated with 96% ethanol, the solvent was then removed at low temperature under reduced pressure and the dry extract so prepared was stored in a refrigerator (yield 12.7%). All doses are expressed in terms of the extract itself.

**Anti-secretory study.**

**Pylorus-ligated (Shay) rats.** The animals (female) were fasted for 48 h with access to water *ad libitum* before the pylorus was ligated\(^1\) under light ether anaesthesia. The animals were killed 6 h after the pylorus ligation, and the stomachs were removed, contents collected, measured, centrifuged and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7 using a pH-meter and total acid output was calculated. Each stomach was examined for lesions in the forestomach portion and indexed according to severity.

**Experimental gastric lesions.**

**Indomethacin-induced gastric ulcers.** Ulcers were induced according to the method of Bhardwaj et al.\(^ 2\) Indomethacin (SIGMA\(^ 8\), USA) was suspended in 1% carboxymethylcellulose in water and administered orally at a dose of 30 mg/kg (5 ml/kg). Six hours after indomethacin administration, the animals were killed.

**Reserpine-induced gastric ulcers.** The method of Gupta et al.\(^ 3\) was followed. Reserpine (CIBA) was administered in a dose of 5 mg/kg i.m. (5 ml/kg) and the animals were killed 24 h later.

**Cytotoxicity studies.** The method of Robert\(^ 4\) was followed. The following necrotizing agents were administered orally to male rats in a volume of 1 ml: 80% ethanol, 0.6 M HCl, 25% NaCl and 0.2 M NaOH. One hour after the administration of necrotizing agents, the animals were sacrificed and each stomach was examined for gastric lesions.

**Experimental duodenal lesions.** The method described by Szabo\(^ 5\) was followed. Female rats, weighing 180-200 g, were used. Food and water were available *ad libitum* throughout the study. Duodenal ulcers were induced by two oral administrations of cysteamine hydrochloride (300 mg/kg, p.o.) with an interval of 4 h. All the animals were killed 48 h after the first dose of cysteamine.

**General procedures.** The ethanol extract of calamus (solution in normal saline, 100 mg/ml) was given at a dose of 500 mg/kg orally 30 min before the administration of an ulcerogenic or necrotizing agent (twice in case of cysteamine). In Shay rats it was administered i.p. immediately after pylorus ligation. Control animals received an equivalent volume (5 ml/kg) of the normal saline vehicle.

The animals were killed at the end of specified periods using anaesthetic ether and the stomach and duodenum were excised. The duodenum was opened along its anti-mesenteric side and the stomach along the greater curvature, their contents were rinsed off with saline and the linings examined with a 6.4 X binocular magnifier. Lesions were also assessed by two observers unaware of the experimental protocols.

Gastric lesions induced by all the procedures used in this study were multiple in each stomach. They were evaluated singly according to their dimensions and severity, and scored using a scale of 0 (no visible ulcers) to 10 (deep lesions with a diameter greater than 8 mm). The scores for each single lesion were then summed so that the total score per stomach could exceed the value of 10.

The duodenal ulcers were scored for intensity, using a scale of 0 to 3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated (into the pancreas or liver) ulcer.

The results refer to the average lesion score ± S.E.M. Statistical analysis of the severity of gastric ulcers was done by Student’s t-test.

**RESULTS**

Pylorus ligation of rats for 6 h showed accumulation of gastric secretory volume and increase in titratable acidity. The ligation of pylorus also produced mild ulceration, mainly located in the forestomach and only few haemorrhagic spots
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) I.P.</th>
<th>Volume of gastric content (ml)</th>
<th>Titratable acid (mEq/l)</th>
<th>Total acid output (mEq/l)</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>8.83 ± 0.78</td>
<td>71.10 ± 2.04</td>
<td>539.23 ± 25.75</td>
<td>1.00 ± 0.25</td>
</tr>
<tr>
<td><em>A. calamus</em> extract</td>
<td>500</td>
<td>1.53 ± 0.16**</td>
<td>55.44 ± 6.35</td>
<td>79.92 ± 2.16***</td>
<td>0.16 ± 0.16**</td>
</tr>
</tbody>
</table>

Six animals were used in each group.

*P<0.05 and **P<0.001, Student’s t-test.

Table 1 - Effect of an ethanolic extract of *A. calamus* on the volume of gastric secretions, free and total acid production and the degree of ulceration in 6-h pylorus ligated (Shay) rats.

were observed in the glandular stomach. The administration of extract immediately after pylorus ligation led to a significant decrease in the volume and acidity of basal gastric secretions and ulcer index (Table 1).

Administration of indomethacin and reserpine resulted in the production of gastric mucosal damage mainly in the glandular segment of the stomach in 100% of the animals. Pre-treatment of the animals with calamus extract produced a significant decrease in the intensity of gastric mucosal damage (Table 2). Administration of cysteamine hydrochloride caused 10% mortality of the rats during 24 h period and duodenal ulcers in 80% of all the rats. Rats which died had perforated duodenal ulcers. Usually two ulcers were produced close to the pylorus, the larger on the anterior and the smaller on the posterior wall of the duodenum. They were elongated, extending longitudinally down the duodenum. Treatment of the rats with the extract was found to be effective in reducing the intensity of the ulceration induced by cysteamine (Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Oral dose (mg/kg)</th>
<th>Lesion score (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indomethacin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>—</td>
<td>33.50 ± 3.00</td>
</tr>
<tr>
<td><em>A. calamus</em> extract</td>
<td>6</td>
<td>500</td>
<td>3.33 ± 2.10***</td>
</tr>
<tr>
<td><strong>Reserpine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>—</td>
<td>36.66 ± 6.77</td>
</tr>
<tr>
<td><em>A. calamus</em> extract</td>
<td>6</td>
<td>500</td>
<td>18.33 ± 4.31*</td>
</tr>
<tr>
<td><strong>Cysteamine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>—</td>
<td>1.90 ± 0.23</td>
</tr>
<tr>
<td><em>A. calamus</em> extract</td>
<td>10</td>
<td>500x2</td>
<td>1.00 ± 0.21*</td>
</tr>
</tbody>
</table>

Significance relative to respective control data: *P<0.05, **P<0.001, Student’s t-test.

Table 2 - Effect of an ethanolic extract of *A. calamus* on experimentally induced gastric and duodenal ulcers in rats.
<table>
<thead>
<tr>
<th>Procedure (necrotizing agents)</th>
<th>Ulcer index (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>80% EtOH</td>
<td>6.66 ± 0.61</td>
</tr>
<tr>
<td>0.6 M HCl</td>
<td>7.16 ± 0.30</td>
</tr>
<tr>
<td>0.2 M NaOH</td>
<td>6.33 ± 0.55</td>
</tr>
<tr>
<td>25% NaCl</td>
<td>7.00 ± 0.50</td>
</tr>
</tbody>
</table>

Six animals were used in each group.

*P < 0.001 Student’s t-test.

Table 3 - Effect of an ethanolic extract of A. calamus on the gastric lesions induced by various necrotizing agents.

Lesions induced by various necrotizing agents were grouped in varying sized patches, usually parallel to the major axis of the stomach. Treatment with calamus extract significantly reduced the severity of these lesions (Table 3). However, the cytoprotective effect of the calamus extract was reversed by pre-treatment with indomethacin.

DISCUSSION

The results of this study clearly indicate that the administration of an ethanolic extract of calamus produced significant anti-secretory, anti-ulcer and cytoprotective effects in rats. The pylorus ligation studies showed that calamus significantly decreased the volume and acidity of basal gastric secretions. On the other hand, the extract antagonized the contractile effect of acetylcholine on guinea-pig ileum suggesting its anticholinergic property. Anticholinergic drugs have been shown to inhibit gastric acid secretions and to protect gastric mucosa against chemically induced lesions.\(^\text{16, 17}\) The ability of calamus extract to protect gastric mucosa against indomethacin- and reserpine-induced mucosal damage further confirms its anti-ulcer activity. Indomethacin is a potent prostaglandin biosynthesis inhibitor.\(^\text{18}\) There is mounting evidence that an increase of certain endogenous prostaglandins can enhance gastric mucosal resistance against ulcerogenic agents.\(^\text{19}\) The calamus extract exerts a highly significant cytoprotective effect against the gastric lesions induced by various necrotizing agents, thus suggesting a protective effect on the gastric mucosa. Many of the orally effective cytoprotective agents act by increasing the generation of prostaglandins due to a mild irritant action, a phenomenon which has been described as "adaptive cytoprotection".\(^\text{20}\) Calamus appears to possess significant adaptive cytoprotective activity, as prior treatment with indomethacin in a prostaglandin biosynthesis inhibitory dose significantly diminishes the cytoprotective effect of calamus extract. The severity of duodenal ulcer was significantly reduced after an administration of calamus extract. Cysteamine-induced duodenal ulcers are considered to be due to a continuous process of hypersecretion of gastric acid.\(^\text{21, 22}\) In fact, hypersecretion of acid, disturbed gastroduodenal motility, hypergastrinaemia and
decreased mucosal resistance have all been implicated in the pathogenesis of cysteamine-induced duodenal ulceration. 23, 24 The active constituents of calamus responsible for its anti-ulcer activity are not known. However, recently some naturally occurring diterpenes lactone 25 and some terpenoidal compounds 26 have been shown to possess antipeptic ulcer activity. Calamus is known to possess sesquiterpenes 27, 26 which might be contributing to its gastroduodenal anti-ulcer activity. The present study supports the claims made by traditional medicine practitioners about the usefulness of Acorus calamus in gastrointestinal diseases. However, further studies on its acute and chronic toxicity are recommended to evaluate its safety for human consumption.

REFERENCES