Studies on anti-ulcer properties of *Cissampelos mucronata* leaf extract

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The methanolic extract of the leaves of *C. mucronata* was screened for anti-ulcer properties using animal models. On isolated guinea pig ileum the extract inhibited contractions evoked by acetylcholine, histamine and serotonin. The extract remarkably decreased the propulsive movement of gastrointestinal content. The extract exhibited significant anti-ulcer activity protecting rats from indomethacin, histamine and stress-induced ulcers. It inhibited the growth of both Gram-positive and Gram-negative microorganisms. The oral LD<sub>50</sub> value of the extract in mice was estimated to be $8.5 \pm 0.35$ g/kg. The results revealed that the plant *C. mucronata* has potential medicinal value as an anti-ulcer agent.

The fractions contained in silica gel powder were extracted with methanol through repeated washing. The extracts were filtered and the solution of the respective fraction was concentrated under reduced pressure. For pharmacological screening, only the crude (whole) methanol extract which is freely soluble in distilled water was used.

**Acute toxicity test**—The acute toxicity (LD<sub>50</sub>) of the extract was estimated in Swiss albino mice (20-25 g) by the oral route as described by Lorké<sup>4</sup>.

**Effects on isolated guinea pig ileum**—Segments of ileum (2 cm long) isolated from freshly killed guinea pigs were suspended in a 20 ml organ bath containing aerated Tyrode solution at 37°C. The composition of the Tyrode solution was (mM/L): NaCl 137, CaCl<sub>2</sub> 1.0, NaHCO<sub>3</sub> 12, NaH<sub>2</sub>PO<sub>4</sub> 0.2, KCl 0.7, MgCl<sub>2</sub> 1.0 and glucose 5.5. The tissues were set up under a tension of 0.5 g and responses were recorded on a 2-channel recorder (Gemini 7070, Ugo Basile, Italy) through an isotonic transducer. At the end of 60 min equilibration period, the responses of the tissues to acetylcholine, histamine and serotonin were established in the absence and in the presence of the extract. Each agonist was used on a separate tissue, and four separate determinations were made for each agonist.

**Effect on small intestinal transit**—Twenty albino mice of either sex were randomly divided into five groups of 4 animals per groups. The animals were starved for 24 hr prior to the experiments, but had free access to water.

One group received Tween 85 (20 ml/kg) the second group received atropine (10 mg/kg) while the
remaining 3 groups received different doses of the extract, (100-400 mg/kg). All administrations were by the oral route. Five minutes after drug administration, 0.5ml of a 5% charcoal suspension in 10% aqueous solution of tragacanth powder was administered to each animal orally. The animals were sacrificed 30 min later and the abdomen opened. The percentage distance of the small intestine (from the pylorus to the caecum) travelled by the charcoal plug in the treatment groups was determined.

Anti-ulcer activity—Three models of inducing experimental gastric ulcers were used to assess the anti-ulcer activity of the extract. These included indomethacin, histamine and stress-induced ulcers. Ten rats were used for each model. They were divided into two groups of five rats each. One group received Tween 8S.i.p. while the other group received the extract at the dose of 450 mg/kg i.p. Thirty minutes later, ulcers were induced with the respective agent. After 8 hr, (for indomethacin and histamine) the animals were killed and the stomach removed and opened along the greater curvature. The stomach was rinsed under a stream of water and pinned flat on a cork board. The stomachs were observed with a hand lens (×10). Erosions formed on the glandular portions of the stomach were counted and each given a severity rating on a 1-3 scale based on the diameter of the ulcer (viz.1<1mm; 2>1mm<2mm; 3>2mm)\(^{11}\). The overall total divided by a factor of 10 was designated as the ulcer index (UI) for that stomach\(^{11}\).

For stress-induced ulcer, 30 min after drugs administration the animals were introduced into cages containing cold water (15\(^{+}\)2\(^\circ\)C) and were made to swim for 18 hr\(^{10}\). The animals were then sacrificed, their stomachs removed and opened along the greater curvature and the ulcer index calculated as above. Cimetidine was used for comparison.

Antimicrobial activity—The following microorganisms were used for the investigation; Escherichia coli, Pseudomonas aeruginosa Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi, Candida albicans and Aspergillus niger. They were hospital strains maintained on nutrient broth agar at 4\(^\circ\)C. Prior to use, they were subcultured in nutrient broth agar plates at 37\(^\circ\)C for 24 hr. The agar disc diffusion method of Lovian\(^2\) was employed. Two doses of the extract were applied to appropriately labelled wells made in gelled agar containing 1.0x10\(^6\) organism/ml. The plates were incubated at 37\(^\circ\)C for 24 hr for bacteria and 72 hr for fungi. The effect of the extract on the growth of the microorganisms were studied by observing the zones of inhibition. The experiments were carried out in triplicate and the average clear diameter of zone of inhibition was recorded.

Statistical analysis—Results were expressed as means±standard errors. The significance of difference between means of control and treated groups was determined by Student's t-test and results were regarded as significance with P<0.05.

Acute toxicity—The oral LD\(_{50}\) of the extract in mice was calculated to be 8.5±0.35 g/kg. The extract appeared well tolerated as the animals did not exhibit any symptoms of overt toxicity.

Effect on guinea pig ileum—The extract neither contracted nor relaxed the isolated guinea pig ileum. However, the extract potently inhibited the contractions evoked by acetylcholine, histamine and serotonin. The inhibition appeared competitive with ID\(_{50}\) value (dose producing 50% inhibition) of 2.10±0.8 mg, 3.63±1.1 mg and 3.16±0.4 mg for acetylcholine, histamine and serotonin respectively.

Effect on small intestinal transit—The result of the charcoal meal test is shown in Table 1. The administration of the extract significantly (P<0.05), reduced in a dose-related manner the charcoal meal transit. The inhibition produced by 400 mg/kg of the extract was comparable to that produced by atropine (10 mg/kg).

Anti-ulcer activity—All the ulcerogenic agents induced ulcers in 100% of the control rats. The extract (450 mg/kg) had a significant (P<0.05) protective effect against ulcers. The effect of the extract was less than that of cimetidine (100 mg/kg) (Table 2).

Antimicrobial activity—The sensitivity of the microorganisms to the extract is shown in Table 3. The bacteria were sensitive to the extract while the fungi were totally resistant. This effect is dose-related. Ps. aeruginosa, S. typhi and E. coli were most sensitive.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose(mg/kg)</th>
<th>Distance travelled (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 85</td>
<td>20 ml/kg</td>
<td>22.1±4.0</td>
</tr>
<tr>
<td>Atropine</td>
<td>10</td>
<td>1.1±0.8**</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>11.6±2.1*</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>5.6±2.3**</td>
</tr>
<tr>
<td>Extract</td>
<td>400</td>
<td>0.9±0.2**</td>
</tr>
</tbody>
</table>

\(^*P<0.05, **P<0.01\)
This spasmolytic effect may partly contribute to the ulcer pain with subsequent healing of ulcer wounds. Effects through various mechanisms, which include modulation of gut motility. Anti-ulcer activity of the plant. The extract also demonstrated potent anti-ulcer activity, protecting rats from ulcerogenesis induced by various agents and mechanisms. Anti-ulcer drugs may produce beneficial effects through various mechanisms, which include among others the modulation of gut hypermotility and inhibition of microbial growth. In ulcer patients, reduction in intestinal motility helps to ameliorate ulcer pain with subsequent healing of ulcer wounds. Spasmolytic effect may partly contribute to the anti-ulcer properties of the plant. The extract inhibited the growth of Gram-positive and Gram-negative bacteria. A growing body of evidence indicates a relationship between colonization by microorganisms and a variety of gastric diseases including gastric ulcer, and "triple therapy" involving at least two antibacterial agents has been used to treat gastric ulcers. Furthermore, bacterial overgrowth due to hypochlorhydria is commonly observed with histamine (H2)-receptor antagonist during therapy of gastric ulcers. Consequently, the antibacterial property of the plant may be beneficial in ulcer management.

The protection against ulcerogenesis, as manifested in the significant reduction in the ulcer index, as well as the spasmolytic and the antibacterial activities clearly confirm the folkloric anti-ulcer activity of the extract and provide pharmacological rationale for its use in traditional medicine.

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References