Effect of *Centella asiatica* Linn on physical and chemical factors induced gastric ulceration and secretion in rats

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Centella asiatica is commonly mentioned as a Rasayana in Ayurveda, an ancient system of Indian medicine for various ailments including abdominal disorders. Rasayanas have been advocated for use in rejuvenation therapy. The present study was conducted to evaluate the possible anti-ulcerogenic activity of fresh juice of *C. asiatica* (CAJ) against ethanol-, aspirin-, cold-restraint stress- and pyloric ligation induced gastric ulcers in rats. The drug given orally in doses of 200 and 600 mg/kg twice daily for five days, showed significant protection against all the above experimental ulcer models and the results were comparable with those elicited by sucralfate (SF; 250 mg/kg, po, BD X 5 days). CAJ showed little or no effect on offensive acid-pepsin secretion. However, at 600 mg/kg CAJ significantly increased gastric juice mucin secretion and increased the mucusal cell glycoproteins signifying increase in cellular mucus. It also decreased cell shedding indicating fortification of mucusal barrier. Thus, the ulcer protective effect of CAJ may be due to strengthening of the mucusal defensive factors.

Gastric ulceration is caused by many factors like stress, drugs, alcohol etc. A rational therapy for peptic ulcer still remains elusive and search for safer potential drugs is being carried out. Use of natural drugs in gastric ulcers is well documented. Most of these drugs augment the mucosal defensive factors, which are thought to be important for protection of gastric mucosa. Peptic ulcers are reported to be due to an imbalance between offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and cell shedding. *Centella asiatica* Linn Urban (CA, Hindi-Mandukaparni), a creeping perennial is mentioned in Ayurveda for treatment of abdominal disorders and epilepsy, and as a carminative and a cardiotonic. The usefulness of CA in various diseases has been reported. Also CA had inhibited gastric ulceration induced by cold restraint stress in rats and the activity was attributed to the GABA-ergic system.

The present experimental work has been undertaken to study the effect of juice of fresh whole plants of *Centella asiatica* (CAJ) on different models of gastric ulcer in rats and its possible effects on offensive and defensive mucosal factors.

**Materials and Methods**

**Animals**—Albino rats (C-F strain) of either sex weighing between 150-180 g were procured from the central animal house of the Institute and housed in well ventilated colony cages in the departmental animal house at 25°±2°C and 45-56% RH, 10:14 hr L:D cycles for one week for acclimatization. The animals were fed with standard rodent pellet diet (Hind Lever) and water ad Libitum.

**Collection of plant**—*Centella asiatica* of cultivated variety was obtained during the month of March from the Ayurvedic gardens of the Institute and was identified with the reference herbarium maintained in the Department of Dravyaguna. Whole plants (1 kg) were size reduced, crushed and 750 ml of juice thus obtained was filtered. The dry weight in terms of solid content in the juice was 4 %. The fresh juice was stored in a refrigerator at -20°C in a glass container and was used within a week of its extraction. The juice was warmed each time at 37°C before administration to the animals.

**Experimental study**—CAJ in doses of 200 and 600 mg/kg (in terms of dry weight) and sucralfate (SF) in the dose of 250 mg/kg were administered, po twice daily at 1000 and 1600 hrs for five days. On the 6th day of experiment, the 18 hr fasted rats were subjected to the following experimental gastric ulcer studies.

**Anti-ulcer study**

(a) Ethanol (EtOH)-induced ulcers: The gastric ulcers were induced in rats by administering EtOH (1 ml/200 g, 1 hr) and the animals were sacrificed by cervical dislocation and 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 by using unpaired Student's t test.

(b) Aspirin (ASP)-induced ulcers: ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals 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 below.

Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer in pluses (+). Severity of the ulcers were scored after histological confirmation as follows: 0: no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes; ++++, ulcer size 1-2 mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; +++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscularis still remaining unaffected (Figs. 1-4). The pooled group ulcer score was then calculated. Statistical analysis was done by using Wilcoxon Sum Rank test.

(c) Cold-restraint stress (CRS)- induced ulcers: On day six, to 18 hr fasted rats cold restraint stress was given by strapping the rats on a wooden plank and keeping them at 4°-6°C for 2 hr. The animals were then sacrificed by cervical dislocation and ulcers were scored on the dissected stomachs as described above.

(d) Pylorus-ligated (PL)- induced ulcers: Drugs were administered for 5 days as described above. On day 6 after the last dose, the rats were kept for 18 hr fasting and care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbitone (35 mg/kg, ip), the abdomen was opened and pylorus ligation was done 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 as described under ASP-induced ulcers.

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 concentration and output were determined by titrating with 0.01 N NaOH, using phenolphthalein as indicator and is expressed as µEq/ml and µEq/4 hr respectively. Pepsin activity was determined using hemoglobin as substrate and has been expressed as µmol/ml and µmol/4 hr for concentration and output respectively. Dissolved mucousubstances were estimated in 90% alcoholic precipitate of the 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 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 of gastric juice. The 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/100g weight of rat.

Gastric mucosal study—Estimation of cellular mucin as glycoproteins was carried out in the gastric mucosa in 4 hr pyloric ligated rats. Mucosal scraping 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 the 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 by using unpaired Student's t test.

Results

CAJ in doses of 200 and 600 mg/kg, given twice daily for 5 days showed a significant protection against the experimental ulcers induced by EtOH, ASP, CRS, and PL and was comparable with SF a known ulcer protective drug (Table 1).

CAJ (200 and 600 mg/kg) showed little or no change in gastric juice volume, acid and pepsin
concentration or output, while SF showed significant reduction mainly in pepsin concentration and output (Table 2). A significant decrease in DNA content was observed both with CAJ and SF (Table 2).

On mucin secretion, both CAJ (600 mg/kg) and SF had pronounced effect on various fractions of mucoproteins. They tended to decrease protein (P), had little or no effect on fucose and sialic acid, while they increased the total hexoses and hexosamine significantly leading to increase in total carbohydrates (TC) and TC:P (Table 3). Similarly, higher dose of CAJ and SF tended to increase or increased total hexoses, hexosamine, sialic acid and total carbohydrates leading to significant increase in TC : P ratio of mucosal glycoproteins (Table 3).

Discussion

The juice of CA (fresh whole plants) was found to possess significant antiulcer activity against EtOH-, ASP-, CRS-, and PL-induced gastric ulcers in rats.

Figs 1-4 — Photomicrograph of rat mucosa (1) — stained with haematoxylin-eosin showing ‘+’ ulcer. Note the histological changes limited to the superficial layers with no congestion. (2) — showing ‘++’ ulcer. Note the necrotic changes in about half of the mucosal thickness. (3) — showing ‘+++’ ulcer. Note the necrotic changes in about two-thirds of the mucosal thickness. (4) — showing ‘++++’ ulcer. Note the perforations with complete destruction with necrosis and hemorrhage, and muscularis remains unaffected. [Figs 1-4 X 100; US- ulcer site; SML- superficial mucosal layer; MM- muscularis mucosae]
Ulcers caused by chemical inducers like ethanol and aspirin could be due to their direct effect or release of noxious substances including free radicals. Disruption of prostaglandin synthesis is another contributing factor for aspirin-induced ulcers. Various physical and psychological stresses cause gastric ulcers in human and experimental animals. The effect of CAJ on various models suggests its diverse role in ulcer protection, which may include its reported effect on wound healing, as an antioxidant and GABA-ergic activities.

In the healthy stomach, there is a balance between aggressive factors and the protection afforded by pre-epithelial, epithelial and sub-epithelial mechanisms of mucosa. Secretion of mucus and bicarbonate by surface epithelial constitute a mucus-bicarbonate barrier, which is regarded as first line of defense against potential ulcerogens. Although pathogenesis of ulcer is multifactorial, ulcer is considered to be due to derangement of balance between aggressive and defensive factors. Hence further study was undertaken to study the status of aggressive factors namely acid and pepsin and important defensive factors such as mucus secretion, cellular mucus and cell shedding. Cell shedding has been taken as an indicator of life span of mucosal cells.

In the gastric juice, evaluation of offensive factors showed that CAJ did not have any significant effect on acid and pepsin secretion, while SF decreased the concentration and output of pepsin. This activity may be due to its adherence to the gastric mucosa and interaction of SF polyanions with the substrate proteins thus, preventing pepsin from binding to the substrate, which is the first step in peptic hydrolysis. SF has also been reported to exert its anti-peptic effect by directly adsorbing to the enzyme.

The entire surface of the gastric mucosa is covered by a continuous layer of mucus gel. The mucin has several functions apart from being the first line of defense against noxious gastric contents. The defense is mostly due to active secretion of bicarbonates and retardation of diffusion of hydrogen ions and penetration of large molecules like pepsin, which are inactivated by the alkaline environment. The thickness of mucus gel is determined by the dynamic balance between mucus secretion and surface erosion by proteolysis and mechanical destruction.

PL-induced gastric ulcers are caused by enhanced acid-pepsin secretion leading to auto digestion of the gastric mucosa and break down of mucosal barrier. A copious amount of gastric mucus is secreted during superficial mucosal damage and provides a favorable microenvironment in repair by restitution. Hence estimation of mucin secretion is valuable for the study of mucosal defensive mechanisms against ulcerogens. TC:P ratio is taken as reliable index for mucin secretion and was significantly increased by CAJ. Increase in total carbohydrates reflects increase in dissolved carbohydrates and this was mainly due to significant increase in total hexoses and hexosamine. SF also significantly increased the TC:P indicating increased mucin secretion, which was mostly due significant increase in mucopolysachyharides such as total hexoses and hexosamine.

Surface mucous cells and mucus neck cells secrete mucus by exocytosis. The main components of gastric mucous are the acidic glycoprotein, sialic acid and neutral mucopolysaccarides like total hexoses, hexosamine and fucose. The glycoproteins are of importance for their specific properties such as gel formation and viscosity. TC:P of the mucosa, which is taken as a reliable marker for cellular mucus was significantly increased by higher dose of CAJ.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EIOH induced ulcers (mm²/rat)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.6 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>CAJ</td>
<td>200, 600</td>
<td>93.6, 100.0</td>
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<tr>
<td>SF</td>
<td>250</td>
<td>62.8</td>
</tr>
<tr>
<td>Control</td>
<td>21.5 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>CAJ</td>
<td>200, 600</td>
<td>76.7, 94.0</td>
</tr>
<tr>
<td>SF</td>
<td>250</td>
<td>83.7</td>
</tr>
<tr>
<td>Control</td>
<td>29.8 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>CAJ</td>
<td>200, 600</td>
<td>73.2, 78.5</td>
</tr>
<tr>
<td>SF</td>
<td>250</td>
<td>62.4</td>
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<tr>
<td>Control</td>
<td>15.0 ± 2.6</td>
<td></td>
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<tr>
<td>CAJ</td>
<td>200, 600</td>
<td>78.7, 89.4</td>
</tr>
<tr>
<td>SF</td>
<td>250</td>
<td>62.7</td>
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</table>

P values: * < 0.01, ** < 0.001
indicating increase in mucin content of the mucosa. This was primarily due to increase in mucopolysaccharides, specifically of sialic acid, which was significant. SF also increased sialic acid, total hexoses and total carbohydrates in the gastric mucosa, thereby increasing TC:P indicating increase in glycoprotein content and hence the augmentation of the mucosal barrier by CAJ was both due to increase in mucin content of the mucosa, thereby increasing the gastric secretion. This protective effect in turn caused less shedding of mucosal cells as evident from decrease in DNA content of the gastric juice, which is taken as a reliable indicator for cell shedding. The present investigation establishes the ulcer protective effect of juice of fresh plants of \textit{Centella asiatica} and the protective effect may be due to augmentation of defensive mucosal factors.

### Table 2 - Effect of CAJ on gastric juice volume, acid, pepsin, and DNA contents in 4 hr PL rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg, bd x 5 days)</th>
<th>Volume (ml/100g)</th>
<th>Acid Concentration (μEq/ml)</th>
<th>Acid Output (μEq/4hr)</th>
<th>Pepsin Concentration (μmol/ml)</th>
<th>Pepsin Output (μmol/4hr)</th>
<th>DNA (μg/ml/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.32±0.09</td>
<td>62.5±4.0</td>
<td>82.5±11.7</td>
<td>484.2±22.4</td>
<td>639.1±49.7</td>
<td>292.3±24.4</td>
</tr>
<tr>
<td>CAJ 200</td>
<td>1.42±0.15</td>
<td>53.3±3.3</td>
<td>75.7±10.6</td>
<td>446.3±26.6</td>
<td>633.7±83.2</td>
<td>202.2±29.6</td>
</tr>
<tr>
<td>600</td>
<td>1.76±0.23</td>
<td>52.5±3.1</td>
<td>92.4±12.6</td>
<td>426.3±14.3</td>
<td>750.3±52.8</td>
<td>159.2±18.7</td>
</tr>
<tr>
<td>SF 250</td>
<td>1.53±0.15</td>
<td>55.3±3.1</td>
<td>84.6±8.7</td>
<td>279.5±31.6</td>
<td>427.6±66.0</td>
<td>131.8±13.2</td>
</tr>
</tbody>
</table>

P values: ^a<0.05, ^b<0.01, ^c<0.001

### Table 3 - Effect of CAJ on gastric juice mucoproteins and glycoproteins in 4 hr PL rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg, bd x 5 days)</th>
<th>Protein (P)</th>
<th>Total hexoses (a)</th>
<th>Hexosamine (b)</th>
<th>Fucose (c)</th>
<th>Sialic acid (d)</th>
<th>TC (a+b+c+d)</th>
<th>TC : P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>332.7±27.9</td>
<td>203.8±18.3</td>
<td>113.0±12.9</td>
<td>68.1±5.7</td>
<td>26.6±3.2</td>
<td>411.5±49.6</td>
<td>1.25±0.17</td>
</tr>
<tr>
<td>CAJ 200</td>
<td>274.1±41.2</td>
<td>241.0±24.1</td>
<td>155.8±20.4</td>
<td>63.4±6.8</td>
<td>24.9±6.2</td>
<td>485.1±69.4</td>
<td>1.79±0.23</td>
</tr>
<tr>
<td>600</td>
<td>245.6±39.2</td>
<td>282.9±25.0^b</td>
<td>205.8±24.9^b</td>
<td>65.8±9.1</td>
<td>31.5±2.5</td>
<td>558.0±51.8</td>
<td>2.29±0.32 ^a</td>
</tr>
<tr>
<td>SF 250</td>
<td>237.5±35.9</td>
<td>297.0±22.6^b</td>
<td>184.2±20.9^a</td>
<td>71.0±11.2</td>
<td>28.6±3.1</td>
<td>580.8±63.9</td>
<td>2.48±0.26 ^b</td>
</tr>
</tbody>
</table>

Glycoprotein (μg/100mg wet tissue)

<table>
<thead>
<tr>
<th>Treatment (mg/kg, bd x 5 days)</th>
<th>Protein (P)</th>
<th>Total hexoses (a)</th>
<th>Hexosamine (b)</th>
<th>Fucose (c)</th>
<th>Sialic acid (d)</th>
<th>TC (a+b+c+d)</th>
<th>TC : P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4252±373</td>
<td>2714±262</td>
<td>1391±132</td>
<td>268±11</td>
<td>99±8</td>
<td>4472±395</td>
<td>1.06±0.09</td>
</tr>
<tr>
<td>CAJ 200</td>
<td>4349±306</td>
<td>3150±241</td>
<td>1713±145</td>
<td>240±16</td>
<td>126±11</td>
<td>5229±413</td>
<td>1.23±0.11</td>
</tr>
<tr>
<td>600</td>
<td>3876±245</td>
<td>3260±275</td>
<td>1801±151</td>
<td>280±13</td>
<td>140±13^a</td>
<td>5481±317</td>
<td>1.45±0.13 ^a</td>
</tr>
<tr>
<td>SF 250</td>
<td>4031±284</td>
<td>3554±253^a</td>
<td>1454±106</td>
<td>307±39</td>
<td>170±29^a</td>
<td>5485±284</td>
<td>1.40±0.12 ^a</td>
</tr>
</tbody>
</table>

P values: ^a<0.05, ^b<0.01
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References


