Gastroprotective and antioxidant activities of *Ceropegia juncea* leaf ethanol extract

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ABSTRACT

The present study was aimed to evaluate gastroprotective and antioxidant activities of ethanolic extract of *Ceropegia juncea* (L.) Taub in rats. Effect of various doses (100, 200, 300 and 400 mg/kg p.o.) of *Ceropegia juncea* leaf ethanolic extract (CJEE) were studied in pylorus-ligation and ethanol-induced gastric mucosal injury in rat. The effect of CJEE on free radical induced lipid peroxidation determined by malondialdehyde estimation method. Amount of antioxidant enzymes (viz. superoxide dismutase (SOD), Catalase (CAT), reduced glutathion (GSH)) along with various membrane bound enzymes in tissue homogenate was also determined using previously described methods. Treatment with CJEE showed significant reduction in ulcer index ($P<0.01$) in both the models along with the reduction in volume and total acidity, and an increase in gastric juice pH. The animals treated with different doses of CJEE showed an increase in the levels of SOD, CAT, GSH and membrane bound enzymes like Ca$^{2+}$ ATPase, Mg$^{2+}$ ATPase, Na$^{+}$ ATPase and decrease in lipid peroxidation in both the models suggest its antioxidant activity of CJEE. These effects of CJEE suggest its gastroprotective activity, which can be attributed to its antioxidant properties. Further, the polypenolics of the plant may be held responsible for these effects, which has been found active against various ulcerogenic agents in previous reports.

Key Words: Antiulcer, Lipid Peroxidation, Catalase, Reduced Glutathion, Superoxide Dismutase, free radicals.

INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmias, impotence, gynaecomastia, and haematopoietic changes) of modern medicine [1], indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. There are evidences for the participation of...
reactive oxygen species in the etiology and pathophysiology of human disease, such as neurodegenerative disorders, inflammation, viral infections, autoimmune gastrointestinal inflammation and gastric ulcer [2]. Drugs with multiple mechanism of protective action, including antioxidant activity, may be highly effective in minimizing tissue injury in human diseases. It has been demonstrated that many drugs and formulations possess potent antioxidant action are effective in healing experimentally induced gastric ulcers [3],[5],[6].

*Ceropegia juncea* (Asclepiadaceae) is a fleshy twining herb, distributed throughout pannisular India. *C.juncea* is reported to be the source of ayurvedic drug ‘Soma’ used in variety of ailments. The plant reported to possess antipyretic, analgesic, local anesthetic, hepatoprotective activities [7]. The present work has been carried out to evaluated the claims regarding its antiulcer potential [8], further an attempt has been made to find out the mechanism involved in its gastroprotective effect.

**MATERIALS AND METHODS**

*Ceropegia Juncea* whole plant was collected from the surrounding fields of the Tirupati district of Andhra Pradesh, India, during the month of June 2008. The plant was identified and authenticated at SOS in Botany, Jiwaji University, Gwalior, India (Voucher No. 2010-11/123).

**Extraction and preliminary phytochemical analysis**

About 200 g of powdered leaves were extracted with 95% ethanol by using Soxhlet apparatus. The extract was concentrated in a rotary flash evaporator (Buchi type, model RE-2000A) under reduced pressure (174.7 mmbar). Phytochemical analysis was performed as per conventional protocol [9].

**Animals**

Female albino rats of Wistar strain weighing between 150 and 200 g were used for the study. Animals were purchased from commercial suppliers and housed under standard laboratory condition (25 ± 2°C temperature, 55 ± 5% relative humidity, and 12 h light and dark cycles). The animals had free access to food (commercial dry pellets, Rattan Brothers, India) and water. The institutional animal ethical committee has approved the protocol of the study (Clearance no: NIPS/2010-11/clear/05).

**Acute-toxicity studies**

The experiment was performed as per OECD guideline no. 423. Swiss Albino mice were fasted overnight and divided into four groups of six animals each. The ethanolic extract was suspended in vehicle (1% Tween 80) and administered orally at the dose of 1000, 2000 and 3000 mg/kg body weight to animals of group I to III. Group VI was served as control and received vehicle only. Then the mice were observed continuously at an interval of 1hr, for 6hrs and at the end of 24 hr for gross behavioral change and deaths [10].
**Experimental procedure**

The animals were divided into six groups each consisting of six rats. Group 1 represented the normal animals, which received 5 ml/kg body weight of vehicle (1% gum acacia, p.o.). Group 2 served as positive control received vehicle along with ulcerogenic treatment. Group 3 to 5 was received *C. juncea* ethanolic extract (CJEE) orally once a day at the doses of 100, 200, 300, and 400 mg/kg body weight, respectively.

**Study of anti-ulcer activity**

**Pylorus ligation method**

The method described by Ghosh (1984) was adopted [11]. Rats were fasted for 48 h. The test drug and extract were administered one hour before the experiment. After the pretreatment period animals were anaesthetized with anesthetic ether. The abdomen was opened and pylorus portion of stomach was slightly lifted out and ligated. The stomach was placed carefully in the abdomen and the wound was sutured. After four hours of pylorus ligation the rats were sacrificed and the stomach was removed. The gastric content was collected and centrifuged. The volume, pH, and total acidity of gastric fluid were determined. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers was measured using a vernier caliper. Ulcer index was determined as described by Suzuki et al. (1976) [12].

Score 1: Maximal diameter of 1 mm.
Score 2: Maximal diameter of 1 to 2 mm.
Score 3: Maximal diameter of 2 to 3 mm.
Score 4: Maximal diameter of 3 to 4 mm.
Score 5: Maximal diameter of 4 to 5 mm.
Score 10: An ulcer over 5 mm in diameter.
Score 25: A perforated ulcer.

The stomach was then homogenized in Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 rpm at 0 °C for 20 min. The supernatant was collected and used for the assays of lipid peroxidation, endogenous antioxidant enzymes superoxide dismutase, catalase, peroxidase and reduced glutathione (GSH). Histopathological examination of the stomach has been carried out as described by Rezq et al. 2010 [13].

**Ethanol-induced ulcer method**

The method described by Dhuley (1999) was adopted [3]. Test drug and extract was administered orally for a period of 10 days. On the 10th day, 1 h after the final dose, 96% ethanol (5 ml/kg, p.o.) was administered to the overnight fasted rats of all groups. The animals were then sacrificed 1 h after the dose of ulcerogen. The stomach was then removed, incised along the greater curvature and its mucosal erosion was determined randomly by measuring the area of the lesions. The sum of the areas was expressed as ulcer index (mm$^2$) [4]. The stomach was then
weighed and processed for antioxidant estimations and histopathological evaluation as mentioned in previous section.

**Biochemical estimations**
Superoxide dismutase was determined by the method of Mishra and Fridovich (1972) [14]. Catalase was estimated by the method given by Colowick et al. (1984) [15]. Reduced glutathione was determined by the method of Moron et al. (1979) [16]. Lipid peroxidation or malondialdehyde formation was estimated by the method of Slater and Sawyer (1971) [17]. Membrane bound enzymes namely Na+K+ATPase, Ca\(^{2+}\)ATPase, and Mg\(^{2+}\)ATPase were assayed according to the methods of Bonting (1970) [18], Hjerten and Pan (1983) [19], and Ohnishi et al. (1982) [20], respectively.

**Statistical analysis**
Results of all the above experiments have been expressed as mean±S.E.M and Student’s *t*-test. Results were considered statistically significant when *P* ≤ 0.05.

**RESULTS**

**Phytochemical Screening**
Phytochemical screening of the powdered leaves showed the presence of alkaloids, triterpenes, phenolics, flavonoids, tannins, saponins, and carbohydrates.

**Acute toxicity studies**
In toxicity studies, it was founded that the animals were safe up to a maximum dose of 3000 mg/kg body weight. There were no changes in normal behavior pattern and no sign of toxicity and mortality were observed. The biological evaluation was carried out at a dose of 100, 200, 300, 400 mg/kg body weight.

**Study of anti-ulcer and antioxidant activity using pylorus ligation method**
It was observed that in the control group, the ulcer index was 89.36±9.87 and the maximum number of ulcers was of the ulcer score 3 and 4. In the rats of this group, a number of perforated ulcers (score 25) were also observed. CJEE was found to produce significant decrease in ulcer index. All the ulcers were of scores 1 and 2 and no perforated ulcers were observed. CJEE also significantly reduced the volume and total acidity, and increased the pH of the gastric fluid, proving its anti-ulcer activity (Table 1). As compared to normal rats, pylorus-ligation was found to increase lipid peroxidation and decrease SOD, catalase and reduced glutathione in the control group, thus leading to oxidative stress. Administration of CJEE at the doses of 100, 200, 300 and 400 mg/kg brought about a significant reduction in lipid peroxidation and an increase in the activities of antioxidant enzymes namely, SOD and catalase in a dose dependent manner. An
increase in the level of reduced glutathione along with the enhancement in the membrane bound ATPases was also observed at all the dose levels of CJEE (Table 2).

Study of anti-ulcer and antioxidant activity using ethanol-induced ulcer method
Administration of ethanol produced significant ulcers (16.20±7.56) and found to increase lipid per-oxidation and decrease SOD, catalase, and reduced glutathione in the control group when compared to normal rats. There was a significant reduction (p<0.05) in ulcer index at all the four doses of CJEE. Administration of CJEE significantly decreased lipid peroxidation and increased the levels of SOD, catalase, reduced glutathione and all membrane bound ATPases at all the dose levels (Table 3).

DISCUSSION AND CONCLUSION

Peptic ulcer disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder [21] Peptic ulcer results due to overproduction of gastric acid or decrease in gastric mucosal production. Further, the role of free radicals is also reported in the induction of ulcers. Tissue damaging free radicals are produced due to conversion of hydroperoxyl to hydroxy fatty acids, which leads to cell destruction. The hydroperoxyl fatty acids are generated from the degeneration of mast cells and generalized lipid peroxidation accompanying cell damage [22], [23].

Pylorus ligation induced ulcers occurs because of an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion [24]. Present study demonstrated that CJEE treated groups showed a significant (P<0.01) increase in gastric juice pH, reduced the volume of secretion and total acidity in a dose dependent manner. These effects of CJEE may be considered highly desirable properties of an anti-ulcerogenic agent.

Ethanol induced gastric ulcers have been widely used for the experimental evaluation of plant extracts for their antiulcer potency and cytoprotective effect. Disturbance in gastric secretion, damage to gastric mucosa, alteration in permeability, gastric mucus depletion and free radical production are reported to be the pathogenic effect of ethanol [25]. CJEE significantly reduced the ulcer index (P<0.01) and afforded significant protection against ethanol-induced ulcers.

It is of interest to note that administration of anti-oxidant inhibits gastric injury causes by various ulcerogenic agents [26], [27]. Preventive anti-oxidant like superoxide dismutase and catalase enzymes are the first line of defense against reactive oxygen species. Reduced glutathion is also a major, low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation [28].
Ceropegia Juncea has been reported to possess secondary metabolites like flavonoids, tannins and other polyphenolics. Flavonoids are reported to decrease malondialdehyde concentration, an indicator of lipid peroxidation in stomach homogenate [29]. Apart from the flavonoids tannins are also reported to possess antiulcer and anti-oxidant properties [30]. These activities of plant polyphenols could be due to their ability to absorb, neutralize and to quench free radical [31].

Their ability as free radical scavenger could also be attributed to their redox properties, presence of conjugated ring structure and carboxlic groups, which have reported to inhibit lipid peroxidation [32].

SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals [33]. Administration of CJEE causes significant increse in SOD, Catalase reduced glutathion levels with all doses in comparision to control animals, which suggest its efficacy in preventing free radical induced damage.

Results showed significant decrease in lipid peroxidation after administration of CJEE in both models indicates its protective effect as membrane lipids are susceptible for per-oxidative attack. CJEE has also increased the activities of Na\(^+\)K\(^+\) Atpase, Ca\(^{2+}\) Atpase and Mg\(^{2+}\) Atpase (membrane bound enzymes) in both the models.

Phytochemical screening showed the presence of flavonoidal and other polyphenolic which are known to responsible for their antioxidant effect. Thus it can be concluded that the gastro-protective and antioxidant activities of CJEE may be attributed to the presence of these constituents in the extract and there is a scope of further research for identification of lead constituent(s) responsible for these activities.

### Table 1: Effect of CJEE on the various gastric parameters of pylorus-ligated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer Index</th>
<th>Volume of Gastric fluid (ml)</th>
<th>pH of gastric fluid</th>
<th>Total acidity (m Eq/l per 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.36±9.87</td>
<td>14.02±1.96</td>
<td>1.3±0.09</td>
<td>129±4.26</td>
</tr>
<tr>
<td>CJEE (100mg/kg)</td>
<td>63.46±5.27*</td>
<td>8.25±1.43*</td>
<td>2.6±1.02*</td>
<td>96.92±3.66*</td>
</tr>
<tr>
<td>CJEE (200 mg/kg)</td>
<td>47.61±3.51*</td>
<td>6.46±1.52**</td>
<td>3.1±0.85*</td>
<td>45.21±2.87**</td>
</tr>
<tr>
<td>CJEE (300 mg/kg)</td>
<td>34.97±2.19**</td>
<td>3.62±1.09**</td>
<td>3.56±0.67**</td>
<td>30.19±1.62**</td>
</tr>
<tr>
<td>CJEE (400 mg/kg)</td>
<td>19.26±1.99**</td>
<td>1.9±0.56**</td>
<td>4.80±0.42**</td>
<td>22.82±1.07**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. DHC-1 treated groups were compared with control group.

*P < 0.05; **P < 0.01.
### Table 2 Effect of CJEE on the antioxidant parameters in stomach of pylorus ligated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Control</th>
<th>CJEE 100 mg/kg</th>
<th>CJEE 200 mg/kg</th>
<th>CJEE 300 mg/kg</th>
<th>CJEE 400 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (unit/mg protein)</td>
<td>4.83 ± 0.43</td>
<td>1.98 ± 0.21*</td>
<td>2.24 ± 0.18</td>
<td>2.76 ± 1.09*</td>
<td>2.89 ± 0.98*</td>
<td>5.11 ± 1.40**</td>
</tr>
<tr>
<td>Ctalase (µ mols of H₂O₂ Consumed/(min mg protein))</td>
<td>7.66 ± 0.21</td>
<td>4.62 ± 0.82*</td>
<td>5.89 ± 1.09*</td>
<td>6.18 ± 0.80</td>
<td>6.78 ± 1.11**</td>
<td>7.92 ± 1.02**</td>
</tr>
<tr>
<td>Reduced glutathione (µg of GSH/mg protein)</td>
<td>3.34 ± 0.18</td>
<td>0.62 ± 0.14**</td>
<td>1.26 ± 0.28*</td>
<td>2.01 ± 1.14**</td>
<td>2.51 ± 0.43**</td>
<td>3.32 ± 0.34**</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol of MDA/mg protein)</td>
<td>4.77 ± 0.33</td>
<td>9.08 ± 0.38**</td>
<td>8.86 ± 0.72</td>
<td>5.09 ± 0.91**</td>
<td>4.01 ± 0.14**</td>
<td>3.74 ± 0.12**</td>
</tr>
<tr>
<td>Na+ K+ ATPase (µmol of inorganic phosphorus liberated/(min mg protein))</td>
<td>5.39 ± 0.23</td>
<td>1.54 ± 0.14**</td>
<td>1.95 ± 0.10*</td>
<td>3.42 ± 0.06**</td>
<td>4.23 ± 0.07**</td>
<td>7.02 ± 0.11**</td>
</tr>
<tr>
<td>Ca²⁺ ATPase (µmol of inorganic phosphorus liberated/(min mg protein))</td>
<td>3.56 ± 0.18</td>
<td>1.89 ± 0.81*</td>
<td>2.10 ± 0.18*</td>
<td>3.10 ± 0.14*</td>
<td>3.68 ± 0.16**</td>
<td>4.02 ± 0.36**</td>
</tr>
<tr>
<td>Mg²⁺ ATPase (µmol of inorganic phosphorus liberated/(min mg protein))</td>
<td>2.99 ± 0.41</td>
<td>1.39 ± 0.42*</td>
<td>1.75 ± 0.31**</td>
<td>2.98 ± 0.21**</td>
<td>3.26 ± 0.16**</td>
<td>3.60 ± 0.14**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. Control group was compared with normal group. CJEE treated groups were compared with control group. * P < 0.05; ** P < 0.01

### Table 3 Effect of CJEE on the antioxidant parameters and ulcer index in Ethanol treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Control</th>
<th>CJEE 100 mg/kg</th>
<th>CJEE 200 mg/kg</th>
<th>CJEE 300 mg/kg</th>
<th>CJEE 400 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (unit/mg protein)</td>
<td>7.23 ± 0.80</td>
<td>2.34 ± 0.60*</td>
<td>3.56 ± 0.30</td>
<td>4.21 ± 0.95*</td>
<td>4.96 ± 1.12**</td>
<td>6.78 ± 1.74**</td>
</tr>
<tr>
<td>Ctalase (µ mols of H₂O₂ Consumed/(min mg protein))</td>
<td>0.80 ± 0.32 *</td>
<td>0.42 ± 0.28*</td>
<td>0.54 ± 0.20</td>
<td>0.78 ± 0.22</td>
<td>0.92* ± 0.13*</td>
<td>1.31* ± 0.06**</td>
</tr>
<tr>
<td>Reduced glutathione (µg of GSH/mg protein)</td>
<td>4.20 ± 0.42</td>
<td>1.21 ± 0.08*</td>
<td>1.87 ± 1.02*</td>
<td>2.35 ± 0.04*</td>
<td>2.89 ± 0.05**</td>
<td>3.42 ± 0.14**</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol of MDA/mg protein)</td>
<td>4.56 ± 0.21</td>
<td>8.02 ± 0.09*</td>
<td>7.12 ± 0.89</td>
<td>6.09 ± 0.52</td>
<td>5.67 ± 0.14**</td>
<td>5.38 ± 0.08**</td>
</tr>
<tr>
<td>Na+ K+ ATPase (µmol of inorganic phosphorus liberated/(min mg protein))</td>
<td>6.12 ± 0.17**</td>
<td>1.69 ± 0.38*</td>
<td>2.07 ± 0.38*</td>
<td>2.89 ± 0.08*</td>
<td>3.98 ± 0.07**</td>
<td>5.47 ± 0.12**</td>
</tr>
<tr>
<td>Ca²⁺ ATPase (µmol of inorganic phosphorus liberated/(min mg protein))</td>
<td>4.12 ± 0.17**</td>
<td>2.08 ± 0.38*</td>
<td>2.27 ± 0.38*</td>
<td>2.96 ± 0.08*</td>
<td>3.69 ± 0.07**</td>
<td>4.35 ± 0.06**</td>
</tr>
<tr>
<td>Mg²⁺ ATPase (µmol of inorganic phosphorus liberated/(min mg protein))</td>
<td>4.23 ± 0.17**</td>
<td>1.73 ± 0.38*</td>
<td>2.67 ± 0.38*</td>
<td>3.21 ± 0.08*</td>
<td>3.69 ± 0.07**</td>
<td>4.10 ± 0.06**</td>
</tr>
<tr>
<td>Ulcer Index</td>
<td>----</td>
<td>16.20±7.56</td>
<td>12.02±2.14*</td>
<td>9.15±1.36*</td>
<td>8.02±1.47**</td>
<td>6.02±1.47**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. Control group was compared with normal group. CJEE treated groups were compared with control group. * P < 0.05; ** P < 0.01
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