Gastroprotective and Antioxidant Activities of the Roots of *Hibiscus aculeatus* Roxb in Rats

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Abstract: Ethanol extract of *Hibiscus aculeatus* (HAE) was assessed in different acute and chronic gastric ulcer models in rats. HAE, 50-200 mg kg⁻¹ administered orally, twice daily for 5 days showed dose-dependent ulcer protective effect in pylorus ligation (9.21-52.63% protection, p<0.05), aspirin (23.95-56.25% protection, p<0.05), ethanol (13.55-58.47% protection, p<0.05), cold-restraint stress (18.34-72.92% protection, p<0.05 to p<0.001) and acetic acid (p<0.05 to p<0.001) induced acute and chronic ulcers. HAE also significantly (p<0.001) reduced ulcer incidence (40 and 10%) and severity (54.35 and 85.37% protection) of duodenal ulcer, induced by cysteamine. HAE offered protection (49.57 and 58.97%) against ethanol-induced depletion of gastric wall mucus. And also, HAE reduced the ulcer index with significant decrease in plasma corticosterone (21.15 and 33.51% protection, p<0.05), lipid peroxidation (16.66 and 38.88% protection, p<0.01 and p<0.001), superoxide dismutase (17.64 and 47.25% protection, p<0.05 and p<0.001) and an increase in catalase (25.84 and 83.14% protection, p<0.05 and p<0.001) activity respectively. Preliminary phytochemical screening of the HAE showed positive test for flavanoids, terpenoids, steroids, tannins and saponins. The results indicate that HAE possesses gastroprotective and antioxidant activity.

Keywords: *Hibiscus aculeatus*, gastroprotective, antioxidant

INTRODUCTION

*Hibiscus aculeatus* Roxb. (Malvaceae) is an annual or biennial suffrutescent trailing prickly herb found throughout India as undergrowth in forests up to 900 m (Warrier et al., 1995). The ethno medical use of *Hibiscus aculeatus* was recorded are diuretic, astringent, antiallergic, anti inflammatory and anthelmintic (Ambasta, 1986). Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood (Rao et al., 2000). Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage is caused by physical, chemical and psychological factors that leads to gastric ulceration in human and experimental animals (Rao et al., 1999). To the best of our knowledge there were no scientific reports available to support the ethno medical claims. Therefore, present study was designed to demonstrate the effect of *Hibiscus aculeatus* ethanol extract (HAE) on physical and chemical factors induced gastric ulceration in rats.

MATERIALS AND METHODS

Plant materials: *Hibiscus aculeatus* roots were collected from Kulasekharam, Thiruvananthapuram district of Kerala, India, in July 2002. The plant sample was authenticated by Dr. P. Jayaraman and Plant Anatomy Research Center, Chennai, Tamilnadu, India. A voucher specimen of the collected plant sample was also deposited in the Herbarium of Captain Srinivasa Murti Drug Research Institute (CCRAS), Tamilnadu, India (Voucher Specimen No. 10A6).

Preparation of extract: The roots of *Hibiscus aculeatus* was dried below 30°C for 6 days, coarsely powdered and extracted (1000 g) by cold maceration with ethanol for six days. The extract was separated by filtration and concentrated on rotary evaporator (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure to obtain 38 g of solid residue (yield 3.8%, w/w).
Phytochemical screening: Preliminary qualitative phytochemical screening (Rathi et al., 2003) of the roots of *Hibiscus aculeatus* gave the positive test for flavonoids, terpenoids, steroids, tannins and saponins.

**Animals:** Sprague-Dawley rats (140-180 g) were procured from the animal house of S.B. College of pharmacy, Sivakasi. They were kept in the departmental animal house at 26±2°C and relative humidity 44-56%, light and dark cycles of 10 and 14 h, respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*. All experiments were performed according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

**Experimental procedure:** HAE in doses of 50, 100 and 200 mg kg⁻¹ and H₂ receptor blocker, ranitidine, in the dose of 50 mg kg⁻¹ were administered orally twice daily at 10:00 and 16:00 h, respectively, for 5 days for acute and up to 5 or 10 days for chronic ulcer protective studies. Reduced Glutathione (RG) 150 mg kg⁻¹ was injected intraperitoneally twice: once before 20 h and another 1 h prior to subjecting the animals to cold-restraint stress and reported to exert its antioxidant defense mechanism (Das and Banerjee, 1993). Control group of animals received suspension of 1% carboxymethyl cellulose in distilled water (10 mL kg⁻¹).

**Aspirin (ASP)-induced ulcers:** ASP in dose of 200 mg kg⁻¹ was administered to the animals on the day of the experiment and ulcers were scored after 4 h (Goel et al., 1985). The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 mL of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. 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 after histological confirmation. The pooled group ulcer score was then calculated (Sanyal et al., 1982).

**Cold-restraint stress (CRS)-induced ulcers:** Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4–6°C. Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomach (Gupta et al., 1985).

**Pylorus ligated (PL)-induced ulcers:** Drugs were administered for a period of 5 days as described above and the rats were kept for 18 h fasting and care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbitone (35 mg kg⁻¹, i.p.), 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 (Rujhanawate et al., 2005). After 4 h, stomachs were dissected 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 mentioned in aspirin induced ulcers.

**Ethanol (EtOH)-induced ulcers:** The gastric ulcers were induced in rats by administrating absolute EtOH (5 mL kg⁻¹, 1 h) and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers (Holland et al., 1985). The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (square millimeters per rat).

**Acetic acid-induced chronic ulcer:** Induction of chronic gastric lesions was studied according to the method described by Okabe et al. (1971). A solution of 0.06 mL 50% acetic acid was instilled into the glass tube of 6 mm in diameter and allowed to remain 60 sec on the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end under anesthesia. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. HAE was given in the dose of 100 and 200 mg kg⁻¹ on day 1, orally, twice daily, 4 h after the application of acetic acid and continued either up to 5 or 10 days after induction of ulcer. The animals were sacrificed after 18 h of the last dose of drug either on sixth or eleventh day of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (square millimeters per rat) of ulcers.

**Cysteamine-induced duodenal ulcers:** Duodenal ulcers were induced by administrations of two doses of cysteamine hydrochloride, 400 mg kg⁻¹, p.o. in 10% aqueous solution at an interval of 4 h (Szabo, 1978). HAE at dose levels of 100 and 200 mg kg⁻¹, ranitidine
(50 mg kg\(^{-1}\), p.o.) were administered 30 min before each dose of cysteamine hydrochloride. All the animals were sacrificed 24 h after the first dose of cysteamine and duodena were excised carefully and opened along the antimesenteric side. The duodenal ulcers were scored for intensity, using a scale of 0-3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis and 3 = perforated or penetrated ulcer (into the pancreas or liver).

**Determination of gastric wall mucus**: The glandular segments from stomachs were removed, weighed and incubated in tubes containing 1% alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The alcian blue binding extract was centrifuged at 3000 rpm for 10 min and the absorbance of supernatant was measured at 498 nm. The quantity of alcian blue extracted (gram per gram of glandular tissue) was then calculated (Corne et al., 1974).

**Estimation of lipid peroxidation (LPO)**: The fundic part of the Cold-Restraint Stress (CRS)-induced ulcer stomach was homogenized (5%) in ice-cold 0.9% NaCl with a Potter-Elvehjem glass homogenizer for 30 sec. The homogenate was centrifuged at 800 x g for 10 min and the supernatant was again centrifuged at 12,000 x g for 15 min and the obtained mitochondrial fraction was used for the following estimations (Das and Banarjee, 1993). A volume of the homogenate (0.20 mL) was transferred to a vial and was mixed with 0.2 mL of a 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 mL of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.50 mL of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 mL with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged at 1000 x g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water (Jamall and Smith, 1985). 1,1,3,3-Tetraquoxypropan was used as standard for calibration of the curve and is expressed as nanomoles per milligram protein.

**Assay of antioxidant enzymes**: The fundic stomach was homogenized (5%) and mitochondrial fraction was prepared as described earlier. Decomposition of H\(_2\)O\(_2\) in the presence of catalase (CAT) was followed at 240 nm (Aebi, 1974). One unit (U) of catalase was defined as the amount of enzyme required to decompose 1 μmol of H\(_2\)O\(_2\) per minute, at 25°C and pH 7.0. Results are expressed as units of CAT activity per milligram of protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitroblue tetrazolium reaction system (Kakkar et al., 1984). One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25±2°C) and the results have been expressed as units of SOD activity per milligram of protein.

**Estimation of plasma corticosterone (PC)**: The animals were lightly anesthetized with ether and blood was collected from the supraclavicular plexus using the microcapillary technique in CRS-induced ulcer model. Three hundred microlitres of isooctane was added to 100 μL of plasma. After mixing and centrifugation, the isooctane was discarded. Six hundred microliter of chloroform was added to each tube and after extraction 400 μL of chloroform was transferred to another stoppered tube. To this 800 μL of acid-alcohol (50%) solution (2:1) was added. After 1 h, acid layer fluorescence was measured at 462 nm (excitation) and (emission) using a spectrophotometer and expressed as micrograms per deciliter (Glick et al., 1964).

**Statistical analysis**: All the data were presented as mean±SEM and analyzed by Wilcoxon Sum Rank Test (Padmanabha Pillai et al., 1982) and unpaired Student’s t-test for the possible significant interrelation between the various groups. Values of p<0.05 was considered statistically significant.

**RESULTS**

The effects of HAE at doses of 50-200 mg kg\(^{-1}\), twice a day for 5 days prevented the acute gastric ulcers in a dose related manner. The range of percentage protection were PL 9.21-52.63% (p<0.05), ASP 23.95-56.25% (p<0.05), EtOH 13.55-58.47% (p<0.05) and CRS 18.34-72.92% (p<0.05 to p<0.001), respectively. The percent protection of ranitidine ranged from 52.63-74.67% (p<0.05 to p<0.001), respectively, in various gastric ulcer models (Table 1). Secretion of mucus and bicarbonate by surface epithelial constitute a mucus-bicarbonate barrier, which is regarded as first line of defense against potential ulcerogens. The gastric wall mucus was significantly (p<0.001) enhanced by HAE and is regarded as a first line of defence against EthOH-induced gastric ulcers showing cytoprotective property (Table 2). In chronic ulcers induced by 50%
Table 1: Effect of *Hibiscus aculeatus* extract (HAE, twice daily for 5 days) on pylorus ligation (PL), aspirin (ASP), ethanol (EtOH) and cold-restraint stress (CRS) induced gastric ulcers in rats

<table>
<thead>
<tr>
<th>Treatment groups (mg kg⁻¹)</th>
<th>Ulcer index</th>
<th>ASP</th>
<th>EtOH (mm² per rat)</th>
<th>CRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.2±3.6</td>
<td></td>
<td>23.6±4.6</td>
<td>22.9±4.1</td>
</tr>
<tr>
<td>HAE 50</td>
<td>13.8±2.5</td>
<td></td>
<td>20.4±4.2</td>
<td>18.7±2.8</td>
</tr>
<tr>
<td>HAE 100</td>
<td>10.6±2.8</td>
<td></td>
<td>16.5±3.8</td>
<td>13.5±1.8*</td>
</tr>
<tr>
<td>HAE 200</td>
<td>7.2±1.3*</td>
<td></td>
<td>9.8±2.7*</td>
<td>6.2±2.1***</td>
</tr>
<tr>
<td>Ranitidine 50</td>
<td>5.6±1.7**</td>
<td>8.1±1.1*</td>
<td>7.9±2.1**</td>
<td>5.8±1.8***</td>
</tr>
</tbody>
</table>

Values are mean±SEM for six rats. *p<0.05 compared to respective control group, **p<0.01 compared to respective control group, ***p<0.001 compared to respective control group.

Table 2: Effect of *Hibiscus aculeatus* extract (HAE, twice daily for 5 days) on ethanol (EtOH)-induced gastric ulcers and gastric wall mucus in rats

<table>
<thead>
<tr>
<th>Treatment groups (mg kg⁻¹)</th>
<th>Ulcer index (mm² per rat)</th>
<th>Protection (%)</th>
<th>Gastric wall mucus (g/g wet glandular tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0±0.0</td>
<td>-</td>
<td>27.4±14.8</td>
</tr>
<tr>
<td>EtOH</td>
<td>23.4±3.8</td>
<td>-</td>
<td>162.4±10.4*</td>
</tr>
<tr>
<td>HAE 100</td>
<td>11.8±3.4</td>
<td>49.57</td>
<td>211.6±11.5</td>
</tr>
<tr>
<td>HAE 200</td>
<td>9.6±2.1**</td>
<td>58.97</td>
<td>256.7±12.2***</td>
</tr>
<tr>
<td>Ranitidine 50</td>
<td>0.1±1.8**</td>
<td>61.11</td>
<td>261.5±0.8**</td>
</tr>
</tbody>
</table>

Values are mean±SEM for six rats. *p<0.001 compared to respective control group, **p<0.001 compared to respective EtOH group.

Table 3: Effect of *Hibiscus aculeatus* extract (HAE, twice daily for 5 and 10 days) on acetic acid-induced chronic ulcers in rats

<table>
<thead>
<tr>
<th>Treatment groups (mg kg⁻¹)</th>
<th>Ucler index</th>
<th>Protection (%)</th>
<th>Five days treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.6±3.6</td>
<td>14.7±2.1</td>
<td>2.4±0.3***</td>
</tr>
<tr>
<td>HAE 100</td>
<td>18.2±2.2</td>
<td>8.2±1.6**</td>
<td>44.21</td>
</tr>
<tr>
<td>HAE 200</td>
<td>11.6±2.2**</td>
<td>50.85</td>
<td>83.67</td>
</tr>
</tbody>
</table>

Values are mean±SEM for six rats. **p<0.01 compared to respective control group, ***p<0.001 compared to respective control group.

Table 4: Effect of *Hibiscus aculeatus* extract (HAE) on cysteamine-induced duodenal ulcers in rats

<table>
<thead>
<tr>
<th>Treatment groups (mg kg⁻¹)</th>
<th>Ulcer incidence</th>
<th>Ulcer score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8/10</td>
<td>80</td>
</tr>
<tr>
<td>HAE 100</td>
<td>4/10</td>
<td>40</td>
</tr>
<tr>
<td>HAE 200</td>
<td>1/10</td>
<td>10</td>
</tr>
<tr>
<td>Ranitidine 50</td>
<td>1/10</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are mean±SEM for 10 rats. *p<0.001 compared to respective control group.

Table 5: Effect of *Hibiscus aculeatus* extract (HAE, twice daily for 5 days) on plasma corticosterone (PC), lipid peroxidation (LPO), catalase (CAT) and superoxide dismutase (SOD) activities in cold-restraint stress (CRS) induced ulcers

<table>
<thead>
<tr>
<th>Treatment groups (mg kg⁻¹)</th>
<th>Ulcer index</th>
<th>PC</th>
<th>LPO</th>
<th>CAT</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0±0.0</td>
<td>20.6±3.0</td>
<td>0.3±0.02</td>
<td>35.6±2.3</td>
<td>86.2±6.3</td>
</tr>
<tr>
<td>CRS</td>
<td>23.3±4.1**</td>
<td>36.4±5.1*</td>
<td>0.5±0.01**</td>
<td>17.8±1.8**</td>
<td>216.5±12.6**</td>
</tr>
<tr>
<td>HAE 100</td>
<td>13.6±2.3*</td>
<td>28.7±2.9</td>
<td>0.4±0.01**</td>
<td>22.4±1.4*</td>
<td>178.3±6.2*</td>
</tr>
<tr>
<td>HAE 200</td>
<td>8.7±2.1***</td>
<td>24.2±2.2</td>
<td>0.3±0.02***</td>
<td>32.6±1.8***</td>
<td>141.2±4.4***</td>
</tr>
<tr>
<td>Reduced glutathione 50</td>
<td>5.2±1.8**</td>
<td>22.5±1.8*</td>
<td>0.3±0.01***</td>
<td>33.2±1.8**</td>
<td>101.6±3.2***</td>
</tr>
</tbody>
</table>

Values are mean±SEM for six rats. *p<0.05 compared to respective control group, **p<0.01 compared to respective control group, ***p<0.001 compared to respective control group.

Acetic acid, HAE reduced ulcer index significantly with decreased perforations after 5 and 10 days treatment (Table 3). Cysteamine produced duodenal ulcers in 80% of the control rats. 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 to the duodenum. Treatment with HAE (100 and 200 mg kg⁻¹) produced a significant (p<0.001) and dose-dependent reduction in the severity and incidence of cysteamine induced duodenal ulcers. However, the H₂ receptor blocker ranitidine (50 mg kg⁻¹) also produces a significant protective effect (Table 4). Table 5 indicate the severity of ulcer index as well as enzyme activities. While studying the role played by the reactive oxygen species on CRS-induced gastric damage, lipid peroxidation and SOD were increased significantly of the ulcerated stomachs (p<0.001). Pretreatment with HAE and a general antioxidant, reduced glutathione,
significantly reduced the ulcer index, LPO, SOD levels and increased in CAT activity in comparison to the CRS ulcers (p<0.001). (Henke, 1979), stated that the central nervous system played an important role in stress ulceration and regulation of plasma corticosterone (Henke, 1979). HAE almost completely protected gastric ulceration by scavenging the free radicals that involved in the endocrinological plasma corticosterone.

DISCUSSION

The present study showed that the ethanol extract of Hibiscus aculeatus possess gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by various physical and chemical agents. Pylorus ligation-induced ulcers are due to autodigestion of the gastric mucosa and break down of the gastric mucosal barrier (Sairam et al., 2002). The incidence of ethanol-induced ulcers is predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products (Oates and Hakkinen, 1988) and reactive oxygen species resulting in the damage of rat gastric mucosa (Peskar et al., 1986). Ethanol-induced depletion of gastric wall mucus has been prevented by HAE. It implies that a concomitant increase in prostaglandins (Pihan et al., 1986), or sulphhydryl compounds (Szabo et al., 1981), contribute to protect the stomach from ethanol injury. A copious amount of gastric mucus is secreted during superficial mucosal damage and provides a favorable microenvironment in repair by restitution. Therefore, it is conceivable that the observed gastric ulcer protection of HAE provides a general evidence for the close relationship between these factors. Stress plays an important role in etiopathology of gastro-duodenal ulceration. Increase in gastric motility, vagal over activity (Cho et al., 1976), mast cell degranulation (Cho et al., 1979), decreased gastric mucosal blood flow (Hase and Moss, 1973) and decreased prostaglandin synthesis, are involved in genesis of stress induced ulcers. As etiopathogenesis of these ulcer models are different, mechanism of HAE should then include number of predisposing factors. On the other hand, the mucosal protection induced by non-prostanoid compounds was perhaps mediated through the mobilization of endogenous prostaglandins (Konturek et al., 1987). HAE significantly healed the penetrating ulcers induced by acetic acid after 5 and 10 days treatment.

Free radicals affect lipids by initiating peroxidation. Superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) are important ROS causing tissue damage (Fridovich, 1986). These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins (Halliwell and Gutteridge, 1985). Probably this effect was significantly reversed by prior administration of HAE providing a close relationship between free radical scavenging activity and the involvement of endocrinological (plasma corticosterone) responses. More work is required for the clear understanding of the mechanism of action with isolated active principles. However, in the present study the plant shows a potent antiulcer activity, which justifies the ethnomedical claims.

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REFERENCES

Glick, D., D.V. Redlich and S. Levine, 1964. Fluorimetric determination of corticosterone and cortisol in 0.02-0.05 mL of plasma or submilligram samples of adrenal tissue. Endocrinology, 74: 653-655.


