ANTIULCER ACTIVITY OF CRUDE ALCOHOLIC EXTRACTS OF BOUGAINVILLEA SPECTABILIS WILLD

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Received: 14 February 2006 Accepted: 14 April 2007

Abstract
The ethanol extract of Bougainvillea spectabilis (ALBS) leaves were studied for its antiulcer activity against aspirin plus pylorus ligation induced gastric ulcer, HCl /ethanol induced ulcer and water immersion stress induced ulcer in rats. The results were compared with ranitidine, sucralfate, omeprazole respectively, for each model suggesting a mechanism for the pharmacological action (antisecretory, cytoprotection, proton pump hypothesis were evaluated). The ethanol extract of Bougainvillea spectabilis showed reduction in gastric volume, free acidity, total acidity and the ulcer inhibition was found to be 100%. The plant extract showed significant cytoprotective effect 89.71% and the extract showed protection index 72% in water immersion stress induced ulcer.

Keywords:
Antiulcer, Bougainvillea spectabilis, Aspirin pyloric ligation ulceration, HCl/ethanol ulceration, Water immersion stress induced ulcer.

Introduction
Bougainvillea spectabilis (1) (Nyctaginaceae) is a climbing shrub with spikes grown as an ornamental plant in Gardens. Plants of Nyctaginaceae find use in traditional medicine as diuretics, anticancer, hypoglycemic, laxative, expectorant, stomachic, appetizer, alexiteric, seed- tonic and carminative (1). The various activity reported from this plant are hypoglycemic effect in normal as well as alloxon induced diabetic in albino mice (2), antimicrobial activity of phenolic and non-phenolic (3) protective effect of esculetin against paracetamol and CCl 4 – induced hepatic damage (4). The present study was designed to evaluate the antiulcerogenic activity of the crude ethanol extract of Bougainvillea spectabilis in different experimental models in rat and mice by aspirin plus pylorus ligation induced gastric ulcer in rats, HCl /ethanol induced ulcer in mice and water immersion stress induced ulcer in rats at 300 mg/kg to study the mechanism of pharmacological action (antisecretory, cytoprotection, proton pump hypothesis respectively).

Materials and methods
Plant material
The leaves of B. spectabilis was collected from Kancheepuram District during September 2000 and the plant was identified by Dr. P. Jeyaraman, plant anatomy Research center, Tambaram Chennai-45. Voucher specimen was deposited in the Asthagiri Herbal Research

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Foundation [AHRF 08] Chennai-59, for future reference.

Preparation of the extract
Air-dried powdered plant materials were extracted by maceration with ethanol for 72 h. Then the extract was concentrated using rotary vacuum to get the solid mass. The yield obtained was 10.1% and then suspended in 1% sodium carboxy methyl cellulose and used for antiulcer studies.

Animals used
Wistar albino rats of either sex / Swiss albino mice were obtained from Tamilnadu Veterinary College and Research Institute, Chennai. The animals were maintained in the polypropylene cages at 24±2°C and were feed with commercial pellet diet and water ad libitum. The project got clearance from Institutional animal ethical committee.

Drugs
The extracts, ranitiditine, sucralfate and omeprazole were suspended in 1% sodium carboxy methyl cellulose and used for antiulcer studies.

Acute toxicity studies
Acute toxicity studies were carried out using acute toxic class method as per OECD guidelines 425 (5). The test was carried out three using groups of Swiss albino mice by administering a dose 2000 mg/kg, of extract in 1% sodium carboxy methyl cellulose p.o. and observed for mortality and behavioral changes during 48 h. The results showed no clinical signs and mortality of the animal, and therefore an LD50 >5000mg/kg body weight may be assumed. We have reported the analgesic activity by three different doses (100, 300 and 500 mg/kg bodyweight) (6). The maximum activity found was at 300mg/kg, so the dose 300 mg/kg selected for the following studies.

Aspirin plus pyloric ligation induced gastric ulcer in rats
Wistar albino rats weighing 100-200g of either sex were divided into 3 groups, each group consists of 6 animals. All groups of animals received treatments as shown below along with 200 mg/kg of aspirin once daily for three days. Group 1 received 1.0 mL/kg p.o. 1% sodium carboxy methyl cellulose as vehicle control; group 2 received 50mg/kg, p.o. ranitidine as standard, group 3 received 300 mg/kg, p.o. ethanol extract of B. spectabilis. Ulceration in rats was induced as described by Goel et al (7). On the fourth day pylorus part was ligated following 36 h fasting (8). Four hours after the pyloric ligation the animals were sacrificed by decapitation. The stomach was opened and the ulcer index was determined (9), the gastric content was titrated against 0.01N NaOH to find out the free acidity and total acidity (10).

Ulcer lesion Index method
HCl/ethanol induced ulcer
Swiss albino mice weighing 24-30 g of either sex were divided into 3 groups, each group consists of 6 animals. Group 1 received 1.0 mL/kg p.o. 1% SCMC as vehicle control, group 2 received 100 mg/kg, p.o. sucralfate as standard control, group 3 received 300 mg/kg, p.o. ethanol extract of B. spectabilis. The experiment was performed as described by Yesilada et al (11). After 1h all the animals were treated with 0.2 mL of HCl / ethanol mixture p.o. (0.3M hydrochloric acid and ethanol 60%) to induce gastric ulcer. Animals were killed by cervical dislocation one hour after administration of HCl / ethanol mixture. Then the stomach was excised and lesion index was determined by measuring each lesion along its greater length measured in mm.

Water immersion stress induced ulcer in rats
Stress ulcers were induced by forcing them to swim in the glass cylinder (height 45 cm, diameter, 25 cm) containing water to the height of 35 cm maintained at 25°C for 3h
Antiulcer activity of crude alcoholic

(12). Animals were fasted for 24h prior to the experiment and divided into 3 groups each group consists of 6 animals. Group 1 received 1.0 mL/kg p.o. 1% SCMC as vehicle control, group 2 received 20 mg/kg, p.o omeprazole as standard control, group 3 received 300 mg/kg, p.o. ethanol extract of *B. spectabilis*. Than the drug treatment animals were allowed to swim in water for 3h. The stomach of each animal was removed and severity of gastric ulcer was assessed in terms of mean ulcer index as described by Alphine and Word (13).

**Statistical analysis**

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnett’s multiple comparisons using graph pad instat 3 and all the results obtained in the study were compared with the vehicle control group.

**Results**

In aspirin plus pyloric ligation induced gastric ulcer the ethanol extract of *B. spectabilis* showed significant reduction in gastric volume, free acidity, total acidity and ulcer score (Table 1). Photomicrograph of glandular portion of the stomach mucosa has showed in ulcerated mucosal layer in vehicle control and in case of standard drug ranitidine and ALBS showed normal mucosa with no ulcer (Fig. 1). In terms percentage ulcer inhibition the ethanol extract of *B. spectabilis* showed 100% activity as compared to control, where as in case of ranitidine ulcer inhibition was 95.37%. Oral administration of HCl/ethanol mixture at a dose of 2 mL/animal was sufficient to induce ulcer. The extracts at a dose of 300 mg/kg, p.o. showed significant reduction in ulcer lesion (89.71%) as compared with vehicle control group (Table 2). In case of water immersion induced stress in rats the reduction in ulcer score was also found to be significant (72.0%) as compared with vehicle control group (Table 3).

Table 1: Effect of alcoholic extract of *B. spectabilis* on gastric secretion, acidity, pH and ulcer score in aspirin plus pylorous ligated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume of gastric secretion mL/100g</th>
<th>Free acidity mEq/L/100g</th>
<th>Total acidity mEq/L/100g</th>
<th>pH</th>
<th>Ulcer score</th>
<th>% ulcer inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (1%SCMC)</td>
<td>2.633±0.042</td>
<td>225.00±6.124</td>
<td>555.00±7.500</td>
<td>2.200±0.163</td>
<td>3.600±0.200</td>
<td></td>
</tr>
<tr>
<td>Ranitidine 50mg</td>
<td>1.317±0.172</td>
<td>148.75±13.475**</td>
<td>492.50±20.736*</td>
<td>3.167±0.166</td>
<td>0.166±0.166**</td>
<td>95.37</td>
</tr>
<tr>
<td><em>B. spectabilis</em> 300mg</td>
<td>0.966±0.185**</td>
<td>55.00±6.021**</td>
<td>263.75±11.361**</td>
<td>3.000±0.258</td>
<td>0.00±0.00**</td>
<td>100</td>
</tr>
</tbody>
</table>

Each value is the mean± S.E.M of six determinations.
*P<0.05, **P<0.01 Dunnet test as compared to control.

Table 2: Effect of alcoholic extract of *B. spectabilis* against HCl/ethanol Induced gastric lesion in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose in mg/kg</th>
<th>Mean ± S.E.M</th>
<th>% Ulcer Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1% SCMC</td>
<td>22.667±3.509</td>
<td></td>
</tr>
<tr>
<td>Sucralfate</td>
<td>100 mg</td>
<td>1.167±0.5426*</td>
<td>94.85</td>
</tr>
<tr>
<td><em>ALBS</em></td>
<td>300 mg</td>
<td>2.333±0.494**</td>
<td>89.71</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E.M of 6 determinations.
*P <0.01 Dunnet test as compared to control value.
Table 3: Effect of alcoholic extract of *B. spectabilis* on water immersion stress induced ulcer in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose in mg/kg</th>
<th>Mean ulcer score ± standard error mean</th>
<th>% ulcer inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>1% SCMC</td>
<td>143.3 ± 12.01</td>
<td></td>
</tr>
<tr>
<td>OMZ</td>
<td>20 mg/kg</td>
<td>0.0 ± 0.0***</td>
<td>100</td>
</tr>
<tr>
<td>ALBS</td>
<td>300 mg/kg</td>
<td>40.0 ± 8.94*</td>
<td>72.0</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E.M of 6 determinations.

*P < 0.05, **P < 0.01, ***P < 0.001 dunnet test as compared to control value.

**Discussion**

Drugs in peptic ulcer such as H₂ blocker ranitidine, M₁ blockers pirenzepine, proton pump inhibitors omeprazole decrease the acid secretion while drugs like sucralfate promote mucosal defences. Although these drugs have a long history in treating ulcers, the clinical evaluation shows adverse effect and drug interaction during the course of treatment. Therefore it is necessary to look for an ideal anti-ulcer drug, especially from herbal drug, which may afford better protection and decrease the incidence of relapse. Hence, the ethanol extract of *B. spectabilis* was examined for their efficacy and for determination of their possible mechanism of action.

The method used here was: 1) aspirin plus pyloric ligation induced gastric ulcer model in rats, 2) HCl/ethanol induced gastric ulcer in mice, 3) water immersion stress induced ulcer in rats, each approach defining its own mechanism of antiulceration. The plant extracts were safe and free from toxicity since LD50 cut off for the extracts was found to be greater than 2000 mg/kg, p.o. as per OECD guidelines.

In aspirin plus pyloric ligation induced gastric ulcer model the ethanol extract of *B. spectabilis*...
_Bougainvillea spectabilis_ attenuated the gastric volume, free acidity, total acidity and ulcer index thus showing the antisecretory mechanism involved in the extracts for their antiulcerogenic activity. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. In case of vehicle control, aspirin induces gastric ulcers by causing back diffusion of H⁺ ions into the mucosal cells (14). This indicates that other mechanisms are also involved in ulcer formation. Moreover the disturbance of defensive factors like mucus secretion, bicarbonate secretion and mucosal blood flow has been reported to cause ulcers (15).

Ethanol induced ulcers are due to direct necrotizing effect of ethanol on gastric mucosa (16). So the protective effect of ethanol extract of _B. spectabilis_ against the gastric damage might be due to protection against 5-lipoxygenase or leukotriene pathway (17,18). The cytoprotective action possibly stimulates the prostaglandin synthesis, which in turn is involved in cytoprotection of the gastric mucosa.

Water immersion stress is one of the best models of stress in rats to induce ulcer. The model provides both emotional stress as well as physiological stress to the animal. In case of water immersion induced stress in rats the reduction in ulcer score was found to be significant. The results demonstrated that ethanol extract of _B. spectabilis_ produced antiulcerogenic effect in different ulcer models, thus suggesting that the extract possess antisecretory, cytoprotective and proton pump mechanism. Hence the _B. spectabilis_ can be used as antiulcerogenic agent in prolonged aspirin therapy.

References