Full Length Research Paper

Evaluation of gastroprotective effects of Strobianthes crispus leaf extract on ethanol-induced gastric mucosal injury in rats

Mahmood A. A.¹, Atieh Abdollahi Fard²*, Harita H.³, Zahra A. Amin¹ and Salmah I.¹

¹Department of Molecular Medicine, Faculty of Medicine, University of Malaya.
²Institute of Biological Science, Faculty of Science, University of Malaya.
³Biology Department, School of Biology and Agricultural Sciences, Faculty of Applied Sciences, University Technology MARA, 40450 Shah Alam, Malaysia.

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The anti-ulcerogenic activity of Strobianthes crispus leaf extract was evaluated against ethanol-induced mucosal injury in rats. Five groups of Sprague Dawley rats were pre-treated respectively with: vehicle, distilled water (ulcer control), omeprazole (20 mg kg⁻¹, reference control), 250 mg/kg, 500 mg/kg and 1000 mg/kg S. crispus leaf extracts (experimental groups), 60 min prior to oral administration of absolute ethanol to generate gastric mucosal injury. Sixty minutes later, rats were sacrificed and gastric content, mucus and wall samples collected. Grossly, ulcer control rats exhibited severe injury to the gastric mucosa and decreased gastric mucus content pH of gastric content, whereas rats pre-treated with S. crispus leaf extracts resulted in significantly dose-dependent reduction of gastric lesion formation accompanied by significant increase in gastric mucus production and pH of gastric fluid. Gastric protection was more prominent in 1000 mg/kg of S. crispus-treated group. Histology, ulcer control rats showed the most severe and deepest gastric mucosal necrotic damage, with edema and leukocyte infiltration of the submucosal layer compared to experimental and reference control groups. Thus, our data suggest that the ulcer protective activity of S. crispus may be due to its defensive mucin secretion and increase in pH of gastric content, and less mucosal injury, no edema and leucocytes infiltration of submucosa. Furthermore, acute toxicity study has indicated no mortality with 5 g/kg dose of S. crispus in Sprague Dawley and did not produce any major clinical signs of toxicity.

Key words: Anti-ulcer, Strobianthes crispus leaf, acute toxicity, histology.

INTRODUCTION

Peptic ulcer disease is the most common gastrointestinal disorder in clinical practice. It is caused by disruptions of the gastric mucosal defence and repair systems. Medicinal plants have been shown to possess gastroprotective activity in animal studies (Malairajan et al., 2007; Rao et al., 2008). One of the herbs that have great potential is Strobianthes crispus. S. crispus (Acanthaceae) is native plant to tropical countries and the leaves have been used in traditional medicine in Malaysia. Traditionally, S. crispus leaves were boiled with water and has been used as anti-diabetic, diuretic, antilicytic, and laxative (Sunarto, 1977). In addition, the leaves of S. crispus has been shown to possess anti-AIDS and anti-leukemia (Kusumoto et al., 1992), high antioxidant activity (Norfarizan-Hanoon et al., 2009), anticarcinogenic (Asma et al., 2006b; Fadzelly et al., 2006a) and anti-hyperglycemic and hypolipidemic effect (Norfarizan-Hanoon et al., 2009). Rahmat et al. (2006) reported that the methanolic extract of this plant displayed strong cytotoxic effect on colon cancer (Caco-2), human breast cancer hormone non-dependent (MDA-MB-231) and liver cancer (HepG-2). This plant has many cystoliths of calcium carbonate and an infusion is mildly alkaline (Perry and Metzger, 1980). The leaves of this plant contained high amount of minerals (potassium, calcium, sodium, iron, and phosphorus). These leaves also contained high content of water-soluble vitamins (C,
B₁, B₂) and it also contains other composition such as catechins, alkaloids, caffeine, and tannins. Catechins of *S. crispus* leaves showed highest antioxidant activity compared to vitamin E (Ismail et al., 2000). Study of *S. crispus* tea showed that it contained considerably high amount of mineral content, phenolic content and displayed high antioxidant activity especially unfermented tea from old or matured leaves (Abu Bakar et al., 2004). An ester glycoside compound of caffeic acid, a verbascoside, was isolated from the leaves of *S. crispus* which is known to have analgesic effects internally, and antifungal and antibacterial effects when used externally (Soediro et al., 1983). Medicinal plant have been used to treat peptic ulcer in many developing countries (Arum and Asha, 2008; de Andrade et al., 2008), and these plants claimed effective by folk medicine require scientific investigation to ascertain their effectiveness, toxicology, and then provide alternative drugs and therapeutic strategies. However, there have been no reports about anti-ulcerogenic activities of *S. crispus* leaves. The current study was undertaken to evaluate the anti-ulcer activity of this leaf extract against ethanol-induced gastric ulcers in rats.

**MATERIALS AND METHODS**

**Omeprazole**

Omeprazole was obtained from the University of Malaya Medical Centre (UMMC) Pharmacy and was used as the reference anti-ulcer drug. Omeprazole is proton pump inhibitor drugs used for the treatment of peptic ulcers. Omeprazole blocks the enzymes in the wall of the stomach from producing acid. A decrease in production of stomach acid would thus allow the stomach to heal. In this study, the drug was administered orally to reference control group of rats in a dose of 20 mg/kg suspended in distilled water (5 ml/kg) (Pedemnera et al., 2006).

**Plant material**

Fresh leaf of *S. crispus* were obtained from Ethno Resources Sdn Bhd, Selangor Malaysia and identified by comparison with the voucher specimen deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur. Leaves were separated from the stalks, washed with distilled water, and air-dried in the shade for 7 to 10 days. The dried leaves were ground into powder using a Wiley mill (40 to 60 mesh) and then successively extracted with water by adding 400 g of powder to 8,000 ml of sterile distilled water (1:20) in a conical flask which was heated on a hotplate (80°C) for 3 h with constant stirring. The residue was subsequently removed by filtration using a filter funnel and distilled under reduced pressure in an Eyela rotary evaporator (Sigma-Aldrich, USA). The extract was freeze-dried and dissolved in distilled water and administered orally to three experimental groups of rats in doses of 250, 500 and 1000 mg/kg, body weight (5 ml/kg), respectively.

**Acute toxicity studies**

The acute toxic study was used to determine a safe dose for *S. crispus*. 48 healthy *Sprague Dawley* rats (24 males and 24 females) were obtained from the Experimental Animal House, Faculty of Medicine, University of Malaya, and were assigned equally into 4 groups labeled as vehicle (sterile distilled water); 1, 2 and 5 g/kg of *S. crispus* in vehicle, respectively. The animals were fasted overnight (food but not water) prior dosing. Food was withheld for a further 3 to 4 h after dosing. The animals were observed for 30 min and 2, 4, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. The animals were sacrificed on the 15th day. Hematological, serum biochemical and histological (liver and kidney) parameters were determined following standard methods (Bergmeyer, 1980; Tietz et al., 1983). The study was approved by the ethics committee for animal experimentation, Faculty of Medicine, University of Malaya, Malaysia and the Ethic No. PM/07/05/2009/MMA (a) (R). Throughout the experiments, all animals received human care according to the criteria outlined in the “Guide for the Care and Use of laboratory Animals” prepared by the National Academy of Sciences and published by the national Institute of health.

**Experimental animals and ethanol-induced ulcer**

Adult male *Sprague-Dawley* rats were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethics No. PM 28/9/2009 MAA (R)). The rats weighed between 180 to 200 g each. They were fasted for 48 h before the experiment, but were allowed free access to tap water up till 2 h before the experiment (Garg et al., 1993). During the fasting period, the rats were placed individually in separate cages with wide-mesh wire bottoms to prevent coprophagy. On the day of the experiment, the rats were randomly divided into 5 groups of 6 rats each. Group 1 rats were ulcer controls wherein each rat received distilled water only (5 ml/kg) orally; Group 2 rats received 20 mg/kg omeprazole (5 ml/kg) by oral route as well. Groups 3, 4 and 5 received 250, 500 and 1000 mg/kg *S. crispus* extracts (5 ml/kg) by oral route respectively; 60 min after the pre-treatments, all rats were gavaged with absolute ethanol of 5 ml/kg. After a one hour wait, all animals were sacrificed by overdoses of diethyl ether and dissected (Paiva et al., 1998). The rats’ stomachs were removed and gastric specimens were collected.

**Measurement of mucus production**

Gastric mucus production was measured in the rats that were subjected to absolute ethanol-induced gastric lesions. The gastric mucosa of each rat was obtained by gentle scraping the mucosa with a glass slide and the collected mucus were weighted by using a precision electronic balance (Tan et al., 2002).

**Measurement of acid content of gastric juice (pH)**

Samples of gastric contents (1 ml) were analyzed for hydrogen ion concentration by pH metric titration with 0.1 N NaOH solutions using digital pH meter (Tan et al., 2002).

**Gross evaluation of gastric lesions**

Each stomach was examined for damage. Ulcerations were found in the gastric mucosa, appearing as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Gastric ulcer was measured according to the method of Kauffman and Grossman (1978). The length (mm) and width (mm) of an ulcer on the gastric mucosa were measured by a planimeter (10 × 10 mm² = ulcer area) under a dissecting microscope (×1.8). The area of the ulcer lesion was measured by counting the number of small squares, 2 ×2 mm,
Table 1. Effect of *S. crispus* on ulcer area, inhibition percentage, mucus content and pH in ethanol-induced gastric mucosal injury in rats.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Pre-treatment (5 ml kg⁻¹)dose</th>
<th>Ulcer area (mm²) (Mean ± S.E.M)</th>
<th>Inhibition %</th>
<th>Mucus content (g)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water (Ulcer control)</td>
<td>950.00 ± 14.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>0.31 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Omeprazole (20 mg kg⁻¹)</td>
<td>323.33 ± 6.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.97%</td>
<td>0.52 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.0 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td><em>S. crispus</em> (250 mg kg⁻¹)</td>
<td>279.12 ± 9.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.62%</td>
<td>0.50 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.27 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td><em>S. crispus</em> (500 mg kg⁻¹)</td>
<td>150.00 ± 17.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.21%</td>
<td>0.61 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.63 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td><em>S. crispus</em> (1000 mg kg⁻¹)</td>
<td>10.00 ± 2.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>98.95%</td>
<td>0.67 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.96 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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All values are expressed as mean ± standard error mean. Means with different superscripts are significantly different, p < 0.05.

covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach was applied in the calculation of the ulcer are (UA) wherein the sum of small squares × 4 × 1.8 = UA mm². The inhibition percentage (%) was then calculated by the following formula with few modification (Njar et al., 1995):

(%) = [(UA<sub>control</sub> - UA<sub>treated</sub>) / UA<sub>control</sub>] × 100%

Histopathological studies of gastric lesions

Specimens of the gastric walls from each rat were washed with saline and fixed in 10% buffered formalin solution for histopathological studies. Sections of the gastric walls were made at a thickness of 5 µm, stained with hematoxylin and eosin, were assessed for histopathological changes such as congestion, edema, hemorrhage and necrosis (Shah and Khan, 1997). The microscopic slides were photographed.

Statistical analysis

All values were reported as mean ± SEM. The statistical significance of differences between groups was assessed using one-way ANOVA. A value of p < 0.05 was considered significant.

RESULTS

Acute toxicity study

Acute toxicity study is a study in which the animals were treated with the *S. crispus* extract at a dose of 1, 2 and 5 g/kg were kept under observation for 14 days. All the animals remained alive and did not manifest any significant visible of toxicity at these doses. Thus, clinical observations, blood biochemistry, hematology, and histopathology data did not show any significant differences between control and treated groups. We conclude that *S. crispus* orally administered to rats was safe and that no drug-related toxicity was detected even at the highest dose investigated.

Gross evaluation of gastric lesions, mucus and pH of gastric content

Rats orally administered with *S. crispus* leaf extracts or omeprazole as pre-treatment 60 min before administration of absolute alcohol showed significant (p < 0.05) increase production of mucus and increase the pH of gastric content, and significant reduction of the mean gastric ulcer area compared to rats pre-treated with distilled water (ulcer control) which clearly produced the expected characteristic zone of necrotizing mucosal lesions, reduction of mucus production and pH of gastric content (Table 1 and Figures 1, 2, 3 and 4). Moreover, flattening of gastric mucosal folds was also observed in rats pre-treated with 1000 mg/kg extract (Table 1; Figure 4). The plant extract significantly suppressed the formation of ulcers showing flattening of gastric mucosal folds. Of these, the rats that were pre-treated with 500 or 1000 mg/kg leaf extract had significantly (p < 0.05) reduced formation of gastric ulcers induced by ethanol compared to rats that were pre-treated with omeprazole or 250 mg/kg leaf extracts (Table 1). Rats pre-treated with 1000 mg/kg leaf extract had significantly (p < 0.05) reduced formation of gastric ulcers compared to rats pre-treated with 500 mg/kg leaf extracts (Table 1). Rats that were pre-treated with 500 or 1000 mg/kg leaf extract had significantly (p < 0.05) produced more mucus compared to rats pretreated with 250 mg/kg leaf extracts or rats pretreated with omeprazole (Table 1). The *S. crispus* leaf extracts were also shown to exert the cytoprotective effects in a dose-dependent manner.

Histological evaluation of gastric lesions

The cytoprotective effect was confirmed by histological examination. The rats pre-treated with distilled water (ulcer control) before administration of ulcer-inducing absolute ethanol showed markedly extensive damage to the gastric mucosa, with the lesions extending deep into the mucosal layer, and oedema and leucocytes...
Figure 1. Macroscopic appearance of the gastric mucosa in a rat pre-treated with only distilled water (ulcer control). Severe injuries are seen in gastric mucosa.

Figure 2. Macroscopic appearance of the gastric mucosa in a rat pre-treated with omeprazole (20 mg/kg). Injuries to gastric mucosa are milder.

infiltration of the submucosal layer (Figure 5). Rats pre-treated with 500 or 1000 mg/kg S. crispus leaf extracts had comparatively better gastric mucosal protection compared to rats pre-treated with omeprazole or 25 mg/kg plant extract as evidenced by the marked reduction in ulcer areas, and inhibition of oedema and leucocytes infiltration of the submucosal layer (Figures 6, 7 and 8).

DISCUSSION

Many medicinal plants are consumed as vegetable, used in food preparations and utilized for medicinal purposes. S. crispus is used as herbal tea and traditional medicinal preparations especially among the Indonesians and Malaysian population. Peptic ulcer results from an imbalance between aggressive factors (acid and pepsin)
production and the maintenance of mucosal integrity through the endogenous defense mechanism (Wallace and Granger, 1996). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion, or to stimulate the mucosal defense mechanism by increasing the mucus production protecting the surface epithelial cells (Goyal and Sairam, 2002).

Gastrointestinal injury is induced by various chemical agents (Desai et al., 1996). The induction of gastric

Figure 3. Macroscopic appearance of the gastric mucosa in a rat pre-treated with S. crispus extract (250 mg/kg). Injuries to gastric mucosa are milder.

Figure 4. Macroscopic appearance of the gastric mucosa in a rat pre-treated with S. crispus extract (1000 mg/kg). There were no gastric mucosal injuries and flattening of gastric mucosa was seen.
lesions by absolute ethanol administration is a popular method of screening plant extracts for anti-ulcer potency in experimental animal models. The cytoprotective properties of a plant extract is evaluated by assessing the
sizes of macroscopically and microscopically visible lesions that are induced in a gastric mucosa. The pathogenic effects of ethanol could be due to direct toxic effect, disturbances in gastric secretions, reducing the secretion of bicarbonates, alterations in permeability, gastric mucus depletion, creating stasis in gastric blood
flow, which contributes to the development of the hemorrhagic lesions of gastric mucosa and free-radical production (Salim, 1990; Holzer et al., 1991; Marhuenda et al., 1991). Oxygen-derived free radicals and oxidative stress of active oxygen have been reported to play an important role in the pathogenesis of acute gastric mucosal injuries induced by ethanol (Salim, 1990; Norteen et al., 1998).

Oxidative damage is considered to be a common factor in the pathogenesis of ulcer by different experimental and clinical models (Sairam et al., 2002). Antioxidants prevent the lesion formation caused by various ulcerogen and to alleviate oxidative stress by scavenging free radicals and protect biological macromolecules from their toxic effect and provide protection against cancer (Ames, 1983; Mizui et al., 1987). Antioxidant activity was observed in extract containing flavonoids ( Larson, 1998; Gonzalez and Di Stasi, 2002), therefore, the antiulcer activity of S. crispus extract on ethanol-induced gastric injury may be partially due to its relative antioxidant activity. Since S. crispus extract contained flavonoids and possess strong antioxidant properties, it may play a role in reduced gastric mucosal injuries in experimental animals (Ismail et al., 2000). Flavonoids possess anti-oxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion (Martin et al., 1994). Flavonoids can scavenge the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol. Ismail et al. (2000) also found the presence of tannin and alkaloids in the plant extract, thus giving rise of a wide range of putative pharmacological actions by S. crispus extract.

Our results were consistent with that presented by Jafri et al. (2001). They observed flattening of the gastric mucosal folds. The gastroprotective effect of extract might be due to decrease in gastric motility. Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds which will increase the surface mucosal area exposed to necrotizing agents and reduce the volume of gastric irritants on rugal crest (Jafri et al., 2001). Such action has been postulated to play a role in cytoprotective effect of prostaglandin (Takeuchi and Nobuhara, 1985). Ethanol produces a marked contraction of the circular muscles of stomach and can lead to mucosal compression at the crests of mucosal fold leading to necrosis and ulceration (Mersereau and Hindchey, 1982).

Administration of S. crispus extracts prevents gastric mucosa injury and accompanied by increase in mucus production and pH of gastric content, and inhibition of edema along with neutrophil infiltration of submucosal layer. The increased mucus production contributed to the preventive effect of the extract. These effects may be due to activation of defense factors such as protection of gastric mucosa and inhibition of offensive factors. Kobayashi et al. (2001) reported that teprenone exerts a protective effect against mucosal lesions through preservation of gastric mucus synthesis and secretion, and inhibition of neutrophil infiltration in the ulcerated gastric tissue. Shimizu et al. (2000) demonstrated that the reduction of neutrophil infiltration into ulcerated gastric tissue promotes the healing of gastric ulcers in rats, and Fujita et al. (1998) described that an increase in neutrophil infiltration into ulcerated gastric tissue delayed the healing of gastric ulcers in rats. Neutrophils mediate lipid peroxidation through the production of superoxide anions (Zimmerman et al., 1997). Oxygen free radicals derived from infiltrated neutrophils in ulcerated gastric tissues have inhibitory effect on gastric ulcers in rats (Suzuki et al., 1998).

Conclusion

Our results suggest that in rats given absolute ethanol gavages, pre-treatment with S. crispus leaf extract displayed gastroprotective activity. Grossly, significant suppressed ulcer areas and flattening of the gastric mucosal folds increase gastric mucus production and pH of gastric content. Histology comparatively decreased gastric mucosal injury and inhibited edema and leukocytes infiltration in submucosal layer of stomach.

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