Full Length Research Paper

Cytoprotective effects of *Bauhinia purpurea* leaf extract against ethanol-induced gastric mucosal ulcer in rats

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*Bauhinia purpurea* is a medicinal plant commonly used traditionally in the treatment of many ailments. The present study was undertaken to evaluate the protective effect of ethanolic extracts of *B. purpurea* ethanolic leaf extract (BPELE) against absolute ethanol-induced gastric mucosal damage in experimental rats. The rats were divided into four groups, respectively pre-treated orally with carboxymethyl cellulose solution (ulcer control groups), omeprazole 20 mg/kg (reference group), 250 and 500 mg/kg of BPELE (experimental groups) 1 h before oral administration of absolute ethanol to generate gastric mucosal damage. After an additional hour, the rats were sacrificed and the ulcer areas of the gastric walls were determined. The ulcer control group exhibited severe mucosal injury, whereas groups pre-treated with *B. purpurea* ethanolic leaf extract exhibited significant protection of gastric mucosal damage. These findings were also confirmed by histology of gastric wall. Significant increases in gastric mucus production and decrease in acidity of gastric content were observed in treated groups with BPELE compared to ulcer control group. These results concluded that the treatment with BPELE prior to absolute alcohol has significantly protect gastric mucosa as ascertained grossly by significant reduction of ulcer area, increases in gastric mucus production and decrease the acidity of gastric content and histology by comparatively decreases in gastric mucosal damage, reduction or absence of edema and leucocytes infiltration of submucosal layer compared to ulcer control group. BPELE was able to decrease the acidity and increase the mucosal defense in the gastric area thereby justifying its use as an antiulcerogenic agent.

Key words: *Bauhinia purpurea*, cytoprotection, gastric ulcer, mucosal defense, histology.

INTRODUCTION

Gastric ulcer is an illness that affects a considerable number of people worldwide. The pathogenesis of gastro-duodenal ulcers is influenced by various aggressive and defensive factors, such as mucus secretion, mucosal barrier, acid-pepsin secretion, blood flow, cellular regeneration and endogenous protective agents (Mizui et al., 1987). It has been shown that long term use of the anti-ulcer drugs may be associated with ineffectiveness of different drug regimens and even resistance to drugs are emerging (Al-Moffeh et al., 2007). Thus, there is an urgent need to identify more effective and safe anti-ulcer agents. A widespread search has been launched to identify new anti-ulcer therapies from natural sources. Herbs, medicinal plants, spices, vegetables and crude drug substances are considered to be a potential source to combat various diseases including gastric ulcer. In the scientific literature, a large number of medicinal plants with gastric anti-ulcer potential have been reported (Abdulla et al., 2010; Ketuly et al., 2011; Mahmood et al., 2010; Wasman et al., 2010).

*Bauhinia purpurea* (family *Leguminoseae*) known to the Malays as ‘pokok tapak kerbau’, *B. purpurea* is native to Southern China and India, and have been used to treat stomach tumors, wounds, glandular swellings, diarrhea and fever (Zakaria et al., 2007). Aqueous extract of *B. purpurea* leaves possess antinociceptive and anti-inflammatory activities and phytochemistry study has revealed the presence of flavonoids, triterpenes, tannins...
and steroids (Zakaria et al., 2007). Different parts of B. purpurea contained kaempferol, quercetin andisorhamnetin (Salatino et al., 1999) and Bauhiniaustinins 1-4 were found to exhibit anticaner activity against a minipanel of human cancer cell lines, including P388 lymphocytic leukemia cell line. Furthermore, Boonphong et al. (2007) demonstrated that some of the isolated compounds exerted antimycobacterial, antimalarial, antifungal, cytotoxic and anti-inflammatory activities. Panda and Kar (1999) have demonstrated the ability of B. purpurea bark extract to stimulate thyroid function in female mice via increasing serum triiodothyronine (T3) and thyroxine (T4) concentrations as well as increasing the hepatic glucose-6- phosphatase and antiperoxidative activities. B. purpurea bark and leaf extracts have the ability to ameliorate metformin-induced hypothyroidism in Type II diabetic mice (Jatwa and Kar, 2009). In another study, it was shown to exhibit wound healing effect on experimentally induced incision, incision, burn and dead space wound models in Sprague Dawley rats (Ananth et al., 2010). Thus far, there is no data available on gastroprotective activity of B. purpurea ethanolic leaf extract (BPELE). The present study was undertaken to evaluate anti-ulcerogenic properties of BPELE in rats.

MATERIALS AND METHODS

In this study, omeprazole was used as the reference anti-ulcer drug, and was obtained from the University of Malaya medical centre (UMMC) pharmacy. The drug was dissolved in carboxymethyl cellulose (0.5% w/v) (CMC) and administered orally to the rats in concentrations of 20 mg/kg body weight (5 ml/kg) according to the recommendation of Mahmood et al. (2010).

Plant specimen and extract preparation

B. purpurea leaves 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. The dried leaves were powdered using electrical blender. 100 g of the fine powder were soaked in 500 ml of 95% ethanol in conical flask for 3 days. After 3 days the mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and distilled under reduced pressure in an Eyela rotary evaporator (Sigma-Aldrich, USA). The dry extract was then dissolved in CMC (0.5% w/v) and administered orally to rats in concentrations of 25 mg/kg body weight (5 ml/kg) according to the recommendation of Abdulla et al., 2010).

Experimental animals for gastric ulcer

Sprague Dawley healthy adult male rats were obtained from the Experimental animal house, Faculty of medicine, University of Malaya, and Ethnic No. PM/27/07/2010/MAA (R). The rats were divided randomly into 4 groups of 6 rats each. Each rat that weighed between 200-225 g was placed individually in a separate cage (one rat per cage) with wide-mesh wire bottoms to prevent coprophagia during the experiment. The animals were maintained on standard pellet diet and tap water. The study was approved by the Ethics committee for animal experimentation, Faculty of medicine, University of Malaya, Malaysia. 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.

Gastric ulcer-induction by absolute ethanol

The rats fasted for 48 h before the experiment (Abdulla et al., 2010), but were allowed free access to drinking water up till 2 h before the experiment. Gastric ulcer was induced by orogastric intubation of absolute ethanol (5 ml/kg) according to the method described by Mahmood et al. (2010). Ulcer control groups were orally administered vehicle (CMC, 0.5% w/v, 5 ml/kg). The reference group received oral doses of 20 mg/kg omeprazole in CMC (5 ml/kg) as positive control. Experimental groups were orally administered BPELE in CMC solution (5 ml/kg) at doses of 250 and 500 mg/kg . 1 h after this pre-treatment, all groups of rats were administered with absolute ethanol (5 ml/kg) in order to induce gastric ulcers (Abdulla et al., 2010). The rats were euthanized 60 min later (Ketuly et al., 2011) under an overdose of xylazin and ketamine anesthesia and their stomachs were immediately excised.

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 weighed by using a precision electronic balance (Abdulla et al., 2010; Ketuly et al., 2011).

Measurement of acid content of gastric juice (pH)

Samples of gastric contents were analyzed for hydrogen ion concentration by pH metric titration with 0.1 N NaOH solutions using digital pH meter (Abdulla et al., 2010; Ketuly et al., 2011).

Gross gastric lesions evaluation

Ulcers of the gastric mucosa appear as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Gastric mucosa of each rat was thus, examined for damage. The length and width of the ulcer (mm) were measured by a planimeter (10 × 10 mm² = ulcer area) under dissecting microscope (1.8×). The ulcerated area was measured by counting the number of small squares, 2 mm × 2 mm, 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 area (UA) wherein the sum of small squares × 4 × 1.8 = UA (mm²) according to the recommendation of Mahmood et al. (2010). The inhibition (I%) was calculated by the following formula according to the recommendation of Wasman et al. (2010).

\[
(\%) = \left( \frac{UA_{\text{control}} - UA_{\text{treated}}}{UA_{\text{control}}} \right) \times 100\%.
\]

Histological evaluation of gastric lesions

Specimens of the gastric walls of each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 µm and stained with hematoxylin and eosin for histological...
Table 1. Effect of *B. purpurea* ethanolic leaf extract (BPELE) on ulcer area (UA) and inhibition percentage in rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Pre-treatment (5 ml/kg dose)</th>
<th>Mucus (g) production</th>
<th>pH of gastric content</th>
<th>Ulcer area (mm²)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CMC</td>
<td></td>
<td>0.36 ± 0.01a</td>
<td>4.05 ± 0.01a</td>
<td>931.27 ± 1.92a</td>
<td>-</td>
</tr>
<tr>
<td>2 Omeprazole</td>
<td>(20 mg/kg)</td>
<td>0.58 ± 0.01b</td>
<td>6.84 ± 0.02b</td>
<td>140.13 ± 1.01b</td>
<td>84.95</td>
</tr>
<tr>
<td>3 BPELE</td>
<td>(250 mg/kg)</td>
<td>0.54 ± 0.01b</td>
<td>5.42 ± 0.01c</td>
<td>201.80 ± 2.18c</td>
<td>78.33</td>
</tr>
<tr>
<td>4 BPELE</td>
<td>(500 mg/kg)</td>
<td>0.81 ± 0.05c</td>
<td>6.35 ± 0.04d</td>
<td>0.00 ± 0.00d</td>
<td>100</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard error mean. Means with different superscripts are significantly different. The mean difference is significant at the p>0.05 level.

Statistical analysis

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

RESULTS

pH of gastric content and mucus production

The acidity of gastric content in experimental animals pretreated with BPELE was decreased significantly compared to that of the ulcer control group (p<0.05). The mucus production of gastric mucosa also increases significantly (p<0.05) in animals pretreated with BPELE compared to the ulcer control group (Table 1).

Gross evaluation of gastric lesions

The anti-ulcer activity of BPELE in ethanol-induced gastric lesion model is shown in Table 1. Results showed that rats pre-treated with BPELE extracts before given absolute alcohol had significantly reduced areas of gastric ulcer formation compared to rats pre-treated with CMC (ulcer control group) (Figure 1) (p<0.05). Moreover, the BPELE significantly suppressed the formation of the ulcers and it was interesting to note the flattening of gastric mucosal folds in rats pretreated with 500 mg/kg BPELE (Table 1, Figure 1). Furthermore, ethanol-induced mucosal damage was significantly and dose dependently reduced in the size and severity by pretreatment of the animals with BPELE. The significant inhibition of gastric ulcer in pre-treatment with BPELE was comparable with omeprazole which is a standard drug used for curing gastric ulcer.

Histological evaluation of gastric lesions

Histological observation of ethanol induced gastric lesions in ulcer control group pre-treated with CMC only, showed comparatively extensive damage to the gastric mucosa, and oedema and leucocytes infiltration of the submucosal layer (Figure 2). Rats that received pre-treatment with BPELE had comparatively better protection of the gastric mucosa as seen by reduction in ulcer area, reduced or absent submucosal edema and leucocytes infiltration (Figure 2). The BPELE has been shown to exert the cytoprotective effects in a dose-dependent manner.

DISCUSSION

It is known that gastric lesions produced by ethanol administration appear as multiple-hemorrhagic red bands of different sizes along the glandular stomach. Ethanol is commonly used for inducing ulcer in experimental rats; it leads to intense gastric mucosal damage. Studies suggest that the ethanol-induced damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Szabo et al., 1995). Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus (Marhuenda et al., 1993). Exposure to ethanol increases the extension of cellular damage in a dose-dependent way (Mahmood et al., 2010). Omeprazole is a proton pump inhibitor which has been widely used as an acid inhibitor agent for the treatment of disorders related to gastric acid secretion for about 15 years (Li et al., 2004). Omeprazole has substituted evaluation (Abdulla et al., 2010; Ketuly et al., 2011).
Figure 2. Histological study of the absolute ethanol-induced gastric mucosal damage in rats. 2a, rats pre-treated with 5 ml/kg of CMC (ulcer control). There is severe disruption to the surface epithelium and necrotic lesions penetrate deeply into mucosa (orange arrow) and extensive edema of submucosa layer and leucocyte infiltration are present (white arrow); 2b, rats pre-treated with omeprazole (20 mg/kg). Mild disruption of the surface epithelium mucosa are present but deep mucosal damage is absent. There is edema and leucocytes infiltration of the submucosal layer; 2c, rat pre-treated with BPELE (250 mg/kg). Moderate disruption of surface epithelium are present but deep mucosal damage is absent. There is edema and leucocytes infiltration of the submucosal layer; 2d, rats pre-treated with BPELE (500 mg/kg). There is no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer (H and E stain 10×).

Benzimidazoles; it inhibits acid secretion by acting on the hydrogen-potassium exchanger (H⁺, K⁺-ATPase) for the apical plasma membrane of the gastric mucosa (Satoh et al., 1989). Omeprazole is highly selective for the proton pump and undergoes catalyzed conversion into active form within the acid forming space. The active inhibitors react with SH (thiol) group of the proton pump, resulting in inhibition of acid formation (Nagaya et al., 1991).

Results obtained in current study suggest that BPELE administered at the low and higher dose showed a protective action against ethanol-induced gastric mucosa damage as demonstrated by the reduction of the gastric UA and increased gastric mucous production and decrease the acidity of gastric content. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect reducing the secretion of bicarbonate and production of mucous (Marhuenda et al., 1993). The products of the 5-lipooxygenase pathway may also play a key role in the development of ulcer induced by irritant agents such as ethanol (Lange et al., 1985). BPELE prevented ethanol induced-gastric damage with mucous production increase. This may be explained with a correlation to a strengthening of the defence factors of gastric mucosa. It is evident that increased mucus production must have largely contributed to preventive effect of the BPELE. Similar findings exist in the literatures, where plant extracts have been shown to prevent gastric mucosal ulceration in rats (Ketuly et al., 2011; Wasmam et al., 2010). The mucus of the gastric wall is thought to play an important role as a defensive
factor against gastrointestinal damage (Wasman et al., 2010). Pretreatment with BPELE significantly decreases the acidity of the gastric content and increases the gastric mucus production. This suggests that gastro-protective effect of BPELE is mediated partly by preservation of gastric mucus production.

Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents (Trivedi and Rawal, 2001). Administration of antioxidants inhibits ethanol-induced gastric injury in rat (Ligumsky et al., 1995). BPELE possesses a broad spectrum of biological activities, and the plant extract has been shown to contain pharmaceutically active chemical constituents, such as flavonoids, saponins and terpenoids (Zakaria et al., 2007) and it is speculated that the gastroprotective effect exerted by BPELE could be attributed to its antioxidant property. Antioxidant property of the BPELE may possibly counteract oxidative damage caused by absolute ethanol toxicity. The observed anti-ulcerogenic activity may be due to its antioxidant effects and appears to strengthen the mucosal barrier, which is the first line of defense against endogenous and exogenous ulcerogenic agents. Previous studies have shown that flavonoids may be related to the antiulcer activity (Hiruma-Lima et al., 2006), and play a major role in the mechanism of gastroprotection (La Casa et al.,

Figure 2. Histological study of the absolute ethanol-induced gastric mucosal damage in rats. 2a, rats pre-treated with 5 ml/kg of CMC (ulcer control). There is severe disruption to the surface epithelium and necrotic lesions penetrate deeply into mucosa (orange arrow) and extensive edema of submucosa layer and leucocyte infiltration are present (White arrow); 2b, rats pre-treated with omeprazole (20 mg/kg). Mild disruption of the surface epithelium mucosa are present but deep mucosal damage is absent. There is edema and leucocytes infiltration of the submucosal layer; 2c, rat pre-treated with BPELE (250 mg/kg). Moderate disruption of surface epithelium are present but deep mucosal damage is absent. There is edema and leucocytes infiltration of the submucosal layer; 2d, rats pre-treated with BPELE (500 mg/kg). There is no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer (H and E stain 10×).
2000). It could be conceivable that the anti-ulcer activity of this plant could be linked to the flavonoids since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents (Saurez et al., 1996). It is well known that many flavonoids display anti-secretory and cytoprotective properties in different experimental models of gastric ulcer (Zayachkivska et al., 2005). Flavonoids possess anti-oxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion (Martin et al., 1994) and flavonoids can scavenge for the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol. These reactive intermediates are potentially implicated in ulcerogenicity (Lewis and Hanson, 1991).

The result of the present study also revealed protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in rats pretreated with BPELE. BPELE have been shown to contain anti-inflammatory activity (Zakaria et al., 2007) and it is speculated that the gastroprotective effect exerted by this plant extract could be attributed to its anti-inflammatory activity. This anti-inflammatory activity could also be a key factor in the prevention of gastric ulcer as reported by Swarnakar et al. (2005). Similarly, Abdulla et al. (2010) and Wasman et al. (2010) demonstrated that the reduction of neutrophil infiltration into ulcerated gastric tissue promotes the healing of gastric ulcers in rats. Mahmood et al. (2010) and Wasman et al. (2010) showed that oral administration of plant extract before ethanol administration significantly decreased neutrophil infiltration of gastric mucosa. Absolute alcohol would extensively damage the gastric mucosa leading to increased neutrophil infiltration into the gastric mucosa. Oxygenfree radicals derived from infiltrated neutrophils in ulcerated gastric tissues have inhibitory effect on gastric ulcers healing in rats (Suzuki et al., 1998). Neutrophils mediate lipid peroxidation through the production of superoxide anions (Zimmerman et al., 1997). Neutrophils are a major source of inflammatory mediators and can release potent reactive oxygen species such as superoxide, hydrogen peroxide and myeloperoxidase derived oxidants. These reactive oxygen species are highly cytotoxic and can induce tissue damage (Cheng and Koo, 2000). Furthermore, neutrophil accumulation in gastric mucosa has been shown to induce gastric ulceration (Abdulla et al., 2010; Ketuly et al., 2011; Wasman et al., 2010). Suppression of neutrophil infiltration during inflammation was found to enhance gastric ulcer healing (Mahmood et al., 2010; Tsukimi et al., 1996). In the present study, we observed flattening of the mucosal folds which suggests that gastroprotective effect of BPELE might be due to a decrease in gastric motility. It is reported that the changes in the gastric motility may play a role in the development and prevention of experimental gastric lesions (Abdulla et al., 2010; Ketuly et al., 2011). Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest (Mahmood et al., 2010; Wasman et al., 2010). Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration (Abdulla et al., 2010).

In conclusion, BPELE could significantly protect the gastric mucosa against ethanol-induced injury. Such protection was ascertained grossly by increase gastric mucus production and decrease the acidity of gastric content were significantly higher in treated groups compared to ulcer control group and also the reduction of ulcer areas in the gastric wall as well as histology by the reduction or inhibition of edema and leucocytes infiltration of submucosal layers. The data obtained confirm the traditional indications for this herb and present a new therapeutic option for the treatment of gastric ailments. The exact mechanism(s) underlying this anti-ulcerogenic effect remain unknown, but it seems that this extract contains pharmacologically active substances with potent antioxidant and anti-inflammatory activity which increase the mucus production and decrease the acidity of gastric content.

ACKNOWLEDGMENTS

This study was financially supported by the University of Malaya through University Malaya Research Grand 2009 (UMRG), RG102/09HTM

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