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Full Length Research Paper

Cytoprotective activities of *Polygonum minus* aqueous leaf extract on ethanol-induced gastric ulcer in rats

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Polygonum minus **is a medicinal plant commonly used in traditional medicine for the treatment of many aliments. The present study was performed to investigate anti-ulcer activity of *P. minus* aqueous leaf extract (PMALE) against ethanol-induced gastric ulcer in rats. Group 1 rats (ulcer control group) were pre-treated with vehicle (distilled water). Group 2 rats (reference group) were orally pretreated with 20 mg/kg omeprazole. Group 3 and 4 (experimental groups) were orally pre-treated with 250 and 500 mg/kg PMALE, respectively. After one hour later, all groups received absolute ethanol to generate gastric mucosal injury. After an additional hour, all the rats were sacrificed and the gastric juice was collected for determining of pH and mucous weight. The stomachs were examined for gastric ulcer areas. Grossly; the ulcer control group exhibited severe mucosal injury, whereas pre-treatment with either omeprazole or PMALE exhibited significant protection of gastric mucosal injury and increase in mucus production. Flattening of gastric mucosal folds was also observed in rats pretreated with PMALE. Histology, gastric wall of ulcer control group revealed severe damage of gastric mucosa, along with edema and leucocytes infiltration of the submucosal layer compared to rats pre-treated with either omeprazole or PMALE which showed marked gastric protection, reduction of edema and leucocytes infiltration of the submucosal layer. Acute toxicity study with a higher dose of 5 g/kg did not manifest any toxicological signs in rats. In conclusions, the present finding suggests that PMALE promotes ulcer protection as ascertained by the comparative decreases in ulcer areas, reduction of edema and leucocytes infiltration of the submucosal layer.**

Key words: *Polygonum minus* aqueous extract, cytoprotection, gastric ulcer, histology.

INTRODUCTION

Peptic ulcer disease has become one of the most common gastrointestinal disorders that involves the entire mucosal thickness and can ever penetrate the muscular mucosa (Falcao et al., 2008). Peptic ulcer occurs due to an imbalance between the offensive (acid, pepsin and helicobacter pylori) and defensive (mucin, prostaglandin and bicarbonate) factors (Lakshmi et al., 2009). Numerous plants have been evaluated as therapeutics for the treatment of a variety of diseases including peptic ulcer (Devi et al., 2008; Coelho et al., 2009). *Polygonum minus*, locally named 'kesum' in Malay, is a plant having a kind of sweet and nice aroma. It comes from the family of Polygonaceae (Burkill, 1966). Traditionally *P. minus* is use to treat stomach problems. Relatively, few farmacological studies have been conducted on *P. minus* such as anti microbial and cytotoxic activity against the HeLa (human cervical carcinoma) (Mackeen et al.,1997). Moreover, it is considered a potential source of natural antioxidants due to high content of gallic acid and total phenolic content and reducing power (Huda-Faujan et al., 2007; Azlim et al., 2010). The current study was

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undertaken to establish the antiulcer activity of *P. minus* aqueous leaf extract (PMALE) against ethanol induced gastric ulcer in rats.

MATERIALS AND METHODS

Preparation of plant extraction

P. minus 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 leaf was then ground into powdered using electrical blender followed by extraction with sterile distilled water. 50 g of blended powder were weighted and placed into 1000 ml flask. The sterile distilled water was added in ratio 1:20. After that, it was heated and stirred on hotplate for 3 h (85°C) followed by cooling and filtration using filter paper and filter funnel, followed by distillation under reduced pressure in Eyela™ rotary evaporator until excess water was evaporated. Aqueous extract was then subjected to lyophilization by a freeze-dryer to produce powdered forms of the extract. The extract was dissolved in sterile distilled water and administered to rats in a concentration of 250 and 500 mg/kg body weight, respectively.

Acute oral toxicity study

Adult healthy male and female *Sprague Dawley* rats (6-8 weeks old) were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethics No. PM 07/10/2009 MAA (a) (R). The rats weighed between 150 – 180 g. The animals were given standard rat pellets and tap water. The acute toxic study was used to determine a safe dose for the PMALE. Thirty six rats (18 males and 18 females) were assigned equally each into 3 groups labeled as vehicle (sterile distilled water); 2 and 5 g/kg of PMALE, respectively. The animals fasted over-night (food but not water) prior to dosing. Food was withheld for a further 3 - 4 h after dosing. The animals were observed for 30 min and 2, 4, 8, 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 acute toxicity LD50 was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all. The animals were sacrificed on the 15th day. Histological, hematological and serum biochemical parameters (Blood samples were collected into EDTA tubes for total and differential white blood cells (WBCs) count. Serum biochemistry parameters were analyzed for AST, ALT, ALP, total protein, albumin, albumin to globulin ratio (A:G), urea, creatinine and serum electrolytes (potassium, sodium and chloride) 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. 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

Sprague Dawley healthy adult male rats were obtained from the experimental animal house, Faculty of Medicine, University of Malaya, and Ethic No. PM/27/07/2009/MAA (R). The rats were divided randomly into 4 groups of 6 rats each. Each rat that weighted between 180 - 220 g was housed separately (one rat per cage). 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.

Ethanol induced gastric ulcer

Following the method described by Schmeda et al. (2005) all rats were treated by orogastric intubations. Ulcer control groups (Group 1) received 5 ml/kg sterile distilled water; Group 2 rats received 20 mg/ kg of omeprazole. Group 3 and 4 rats received 250 and 500 mg/kg of PMALE, 60 min after their pre-treatment, all rats were gavaged with absolute ethanol (5 ml/kg) (Hollander et al., 1985), and after an additional 60 min, the rats were all sacrificed by overdose of diethyl ether. Rat stomachs were rapidly excised and the gastric juice was collected for determination of pH and mucous production then the stomach opened along the greater curvature, washed with distilled water and fixed in 10% buffered formalin.

Examination of gastric lesion

Any ulcers would be found in the gastric mucosa, appearing as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Each gastric mucosa was thus examined for damage. The length (mm) and width (mm) of the ulcer on the gastric mucosa were measured by a planimeter (10 × 10 mm^2 = ulcer area) under dissecting microscope (1.8x). The area of each ulcer lesion was measured by counting the number of small squares, 2 × 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 mm^2) as described by Kauffman and Grossman (1978). The inhibition percentage (I %) was calculated by the following formula as described by Njar et al. (1995).

$$(I\ \%) = [(UA_{control} - UA_{treated}) \mid UA_{control}] \times 100.$$

Histological examination of gastric lesion

Stomachs were fixed in a 10% of buffered formalin solution for histopathological examination following the assessment of ulcer score. The fixed stomachs were embedded in paraffin wax to produce paraffin wax tissue sections then 5 µm sections stained with H and E evaluated for microscopically examination.

Statistical analysis

Value reported is expressed as mean ± SEM from 6 animals per group. Statistical analysis between the treatments was tested by using one-way analysis of variance (ANOVA), probability (p) values < 0.05 were considered significant.

RESULTS

Acute toxicity study

Animals treated with PMALE at a dose of 2 and 5 g/kg

Table 1. Observed ulcer area and inhibition percentage by PMALE in rats.

Animal group	Pre-treatment (5 ml/kg dose)	Mucus Production	pH of gastric content	Ulcer area $(mm)^2$ (Mean ± S.E.M)	Inhibition (%)
1	Distilled water (ulcer control)	0.34 ± 0.01^a	4.00 ± 0.06^a	865.00 ± 8.76^a	-
2	Omeprazole (20)	0.57 ± 0.01^b	6.84 ± 0.04^b	121.67 ± 0.99^b	85.93
3	PMALE (250)	0.46 ± 0.01^c	4.61 ± 0.01^c	188.17 ± 3.63^c	78.25
4	PMALE (500)	0.88 ± 0.01^u	5.5 ± 0.12^u	35.33 ± 1.61^u	95.92

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

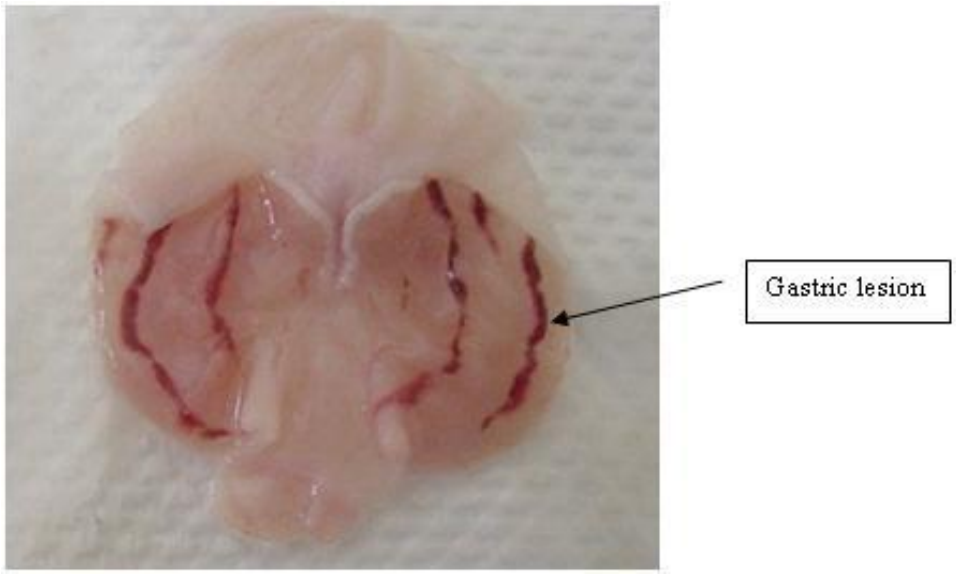

Figure 1a. Gross appearance of the gastric mucosa in a rat pre-treated with 5 ml/kg of sterile distilled water (ulcer control). Severe injuries are seen in the gastric mucosa.

were kept under observation for 14 days. All the animals remained alive and did not manifest any significant sign of toxicity at these doses. There were no abnormal signs, behavioral changes, body weight changes, or macroscopic finding at any time of observation. There was no mortality in the above-mentioned doses at the end of 14 days of observation. Histological examination of liver and kidney, hematology and serum biochemistry revealed no significant differences between the different groups. From these results it is concluded that the extract is quite safe even at these higher doses and has no acute toxicity and the oral lethal dose (LD50) for the male and female rats were greater than 5 g/kg body weight

Evaluation of gross lesion

The anti-ulcer activity of PMALE in ethanol-induced gastric lesion model is reported in Table 1 and Figure 1a, b, c and d. Results showed that pH and gastric mucous was significantly elevated at dose dependent in rats pre-treated with PMALE or omeprazole before being given absolute alcohol compared to rats pre-treated with only sterile distilled water (ulcer control group) (Figure 1a, b, c and d). Moreover, PMALE significantly suppressed areas of gastric ulcer formation and it was interesting to note that the flattening of gastric mucosal folds compared to rats pre-treated with sterile distilled water (Table 1 and Figures 1a, b, c, d and e). It was also observed that protection of gastric mucosa was more prominent in rats pre-treated with 500 mg/kg of PMALE (Table 1). The significant inhibition of gastric ulcer in rats pre-treated with PMALE was compared with Omeprazole which is a standard drug used for curing gastric ulcer.

Histological evaluation of gastric lesion

The present study demonstrated that rats treated with PMALE (250 and 500 mg/kg) or omeprazole showed

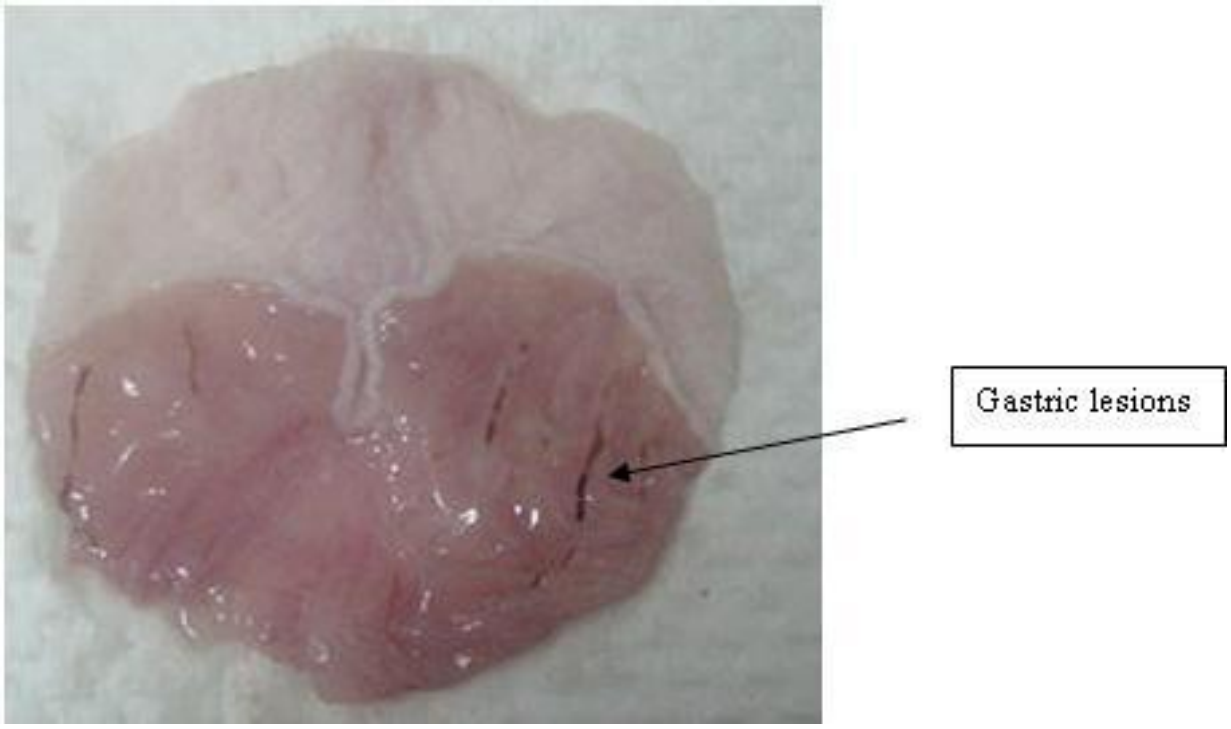

Figure 1b. Gross appearance of the gastric mucosa in a rat pre-treated with 5 ml/kg of omeprazole (20 mg/kg). Injuries to the gastric mucosa are very milder compared to the injuries seen in the ulcer control rat.

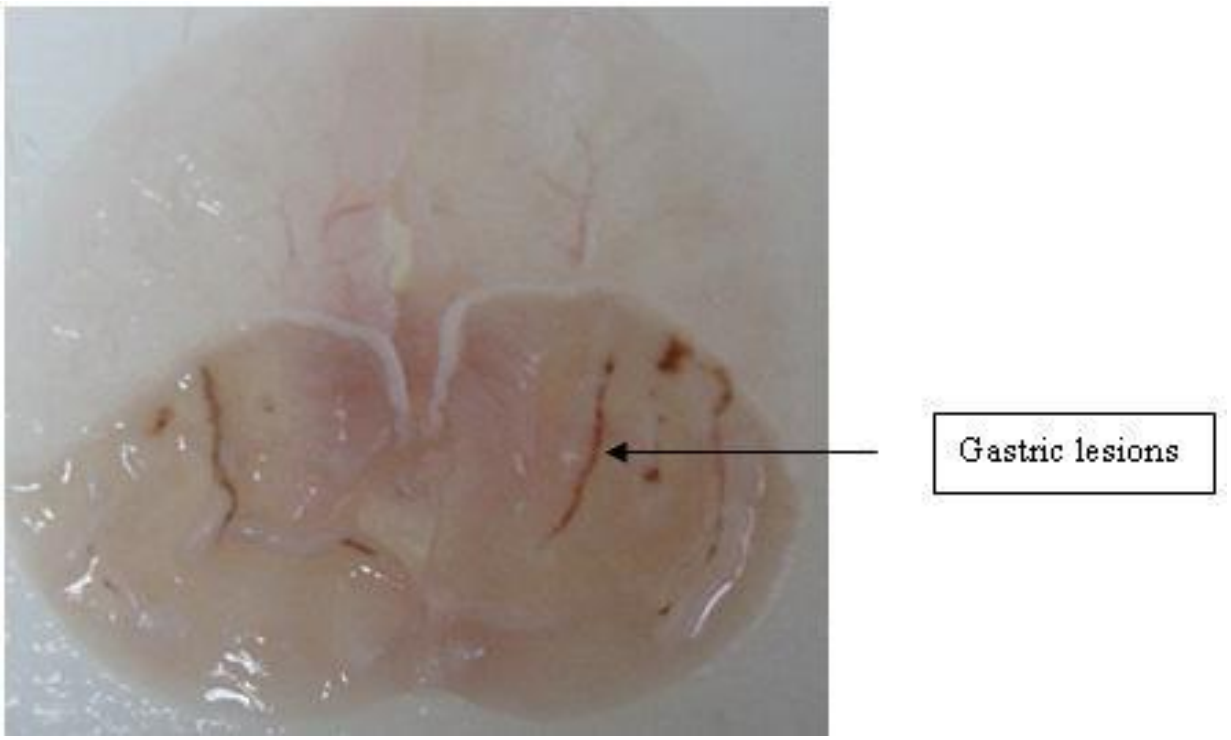

Figure 1c. Gross appearance of the gastric mucosa in a rat pre-treated with 5 ml/kg of PMALE (250 mg/kg). Mild injuries are seen in gastric mucosa.

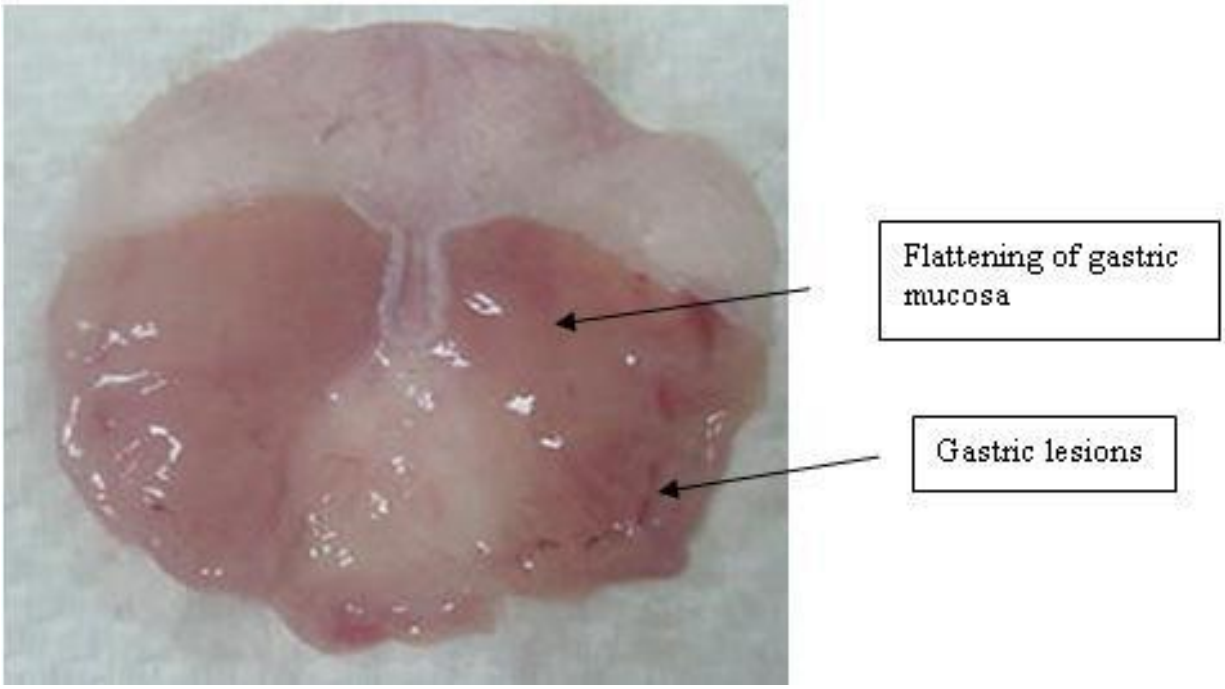

Figure 1d. Gross appearance of the gastric mucosa in a rat pre-treated with 5 ml/kg of PMALE (500 mg/kg). No injuries to the gastric mucosa are seen, and showed flattening of gastric mucosa.

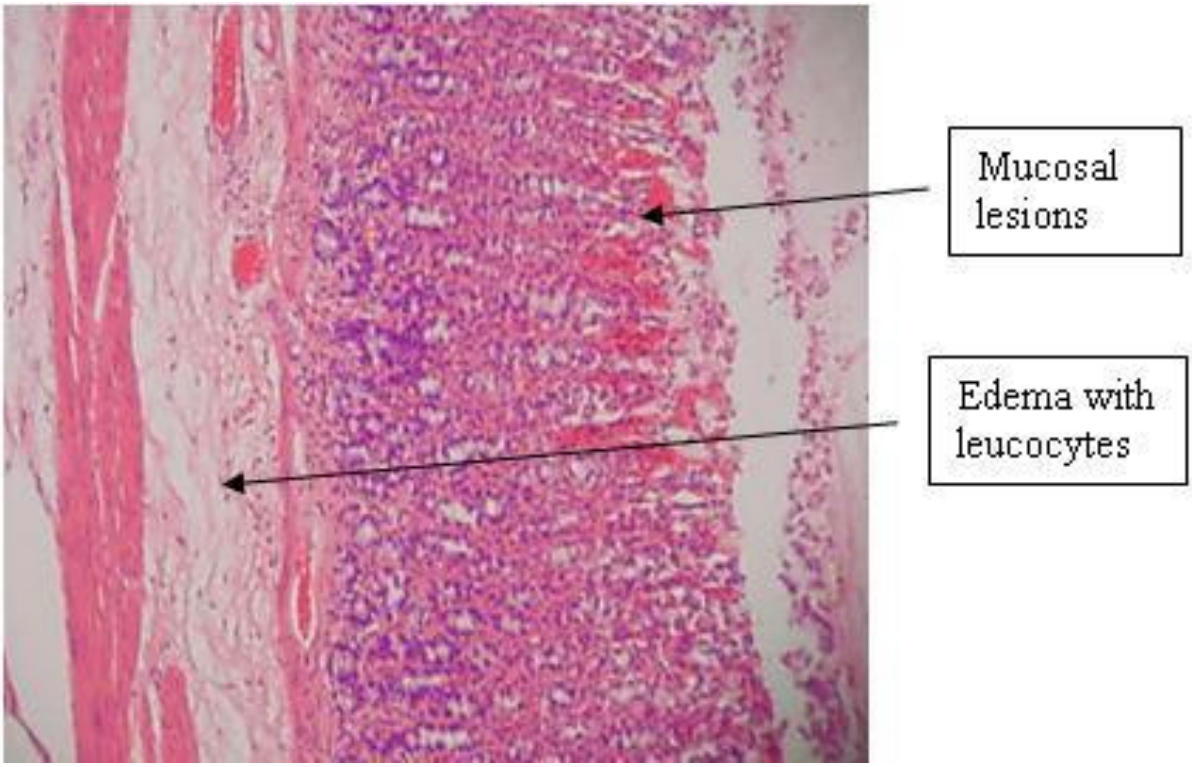

Figure 1e. Histological section of gastric mucosa in a rat pre-treated with 5 ml/kg of sterile distilled water only. There is severe disruption to the surface epithelium, and edema of the submucosa layer with leucocyte infiltration (H&E stain, 10x).

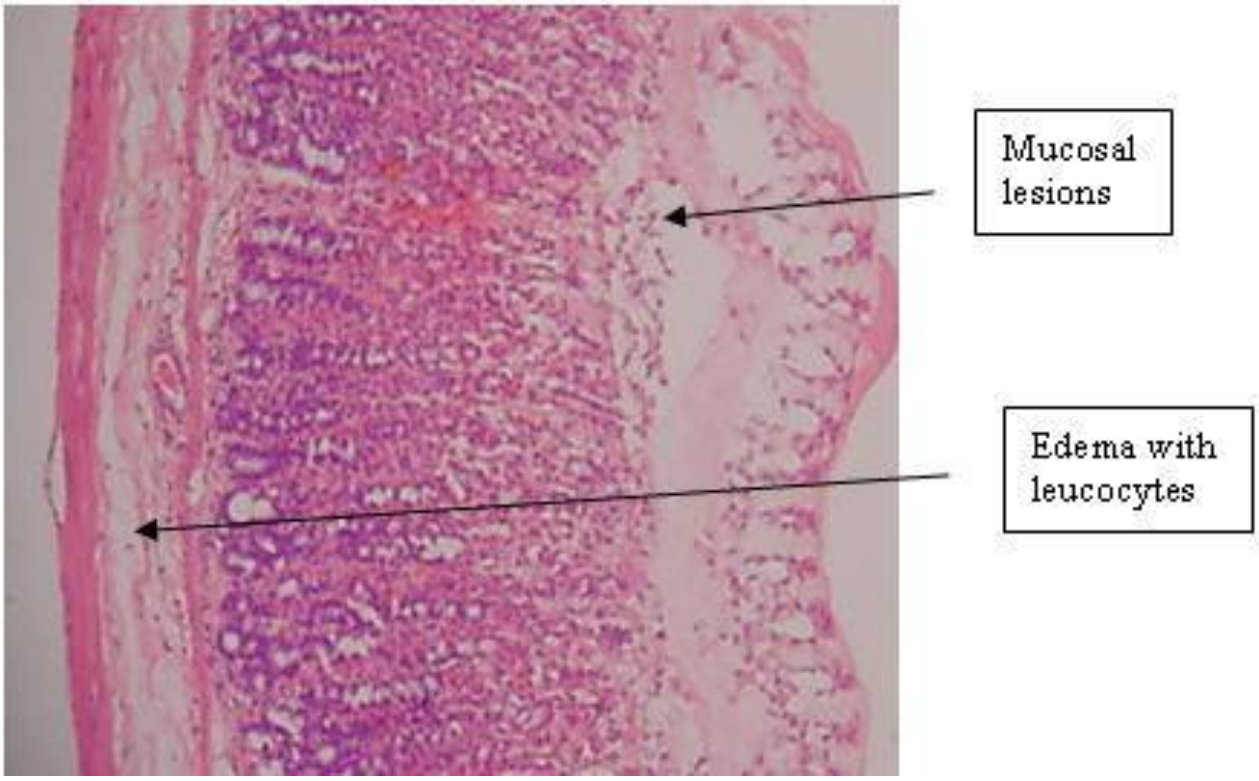

Figure 1f. Histological section of gastric mucosa in a rat pre-treated with 5 ml/kg of omeprazole (20 mg/kg). There is mild disruption to the surface epithelium with mild edema and leucocytes infiltration of the submucosal layer (H&E stain 10x).

marked prevention of gastric mucosal ulcer, lack of submucosal oedema and no leukocyte infiltration compared to animals treated with only distilled water (Figures 1e, f, g and h).

DISCUSSION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Although, in most of the cases the etiology of the ulcer is unknown, it is generally accepted that they are a result of an imbalance between the aggressive factors of acid and pepsin and maintenance of mucosal integrity through endogenous defensive mechanism. To regain the balance, different therapeutics including spice and plant extracts have been used (Goel and Sairam, 2002). Ethanol-induced gastric ulcers serve as a common ulcerogenic agent. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the stomach (Umamaheswari et al., 2007). There are various mechanisms involved in the ulcer production in different experimental models. Many experimental evidences have shown that antioxidants significantly strengthen the gastric walls and protect tissue from oxidative damage (Rajesh et al., 2009). Furthermore, gastric acid secretion now accepted to play an important role in the formation of gastric ulcer. On the other hand, substances which have the ability to suppress gastric acid secretion, such as proton pump inhibitors and histamine H_2 receptor antagonists are believed to accelerate the healing process of the gastric lesions or inhibit the formation of mucosal injury (Brzozowski et al., 2000).

The results of this study showed that the aqueous PMALE have significant anti-ulcer and cytoprotective properties in rats. Pretreatment with PMALE produced a dose-dependent revealed protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in rats. The results agree with the finding of Mahmood et al. (2010), who reported that *Gynura procumbens* leaf exerts a protective effect against mucosal lesions through inhibition of neutrophil infiltration in the ulcerated gastric tissue and Shimizu et al. (2000) demonstrated that the reduction of neutrophil infiltration into ulcerated gastric tissue promotes the healing of gastric ulcers in rats. Moreover, 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

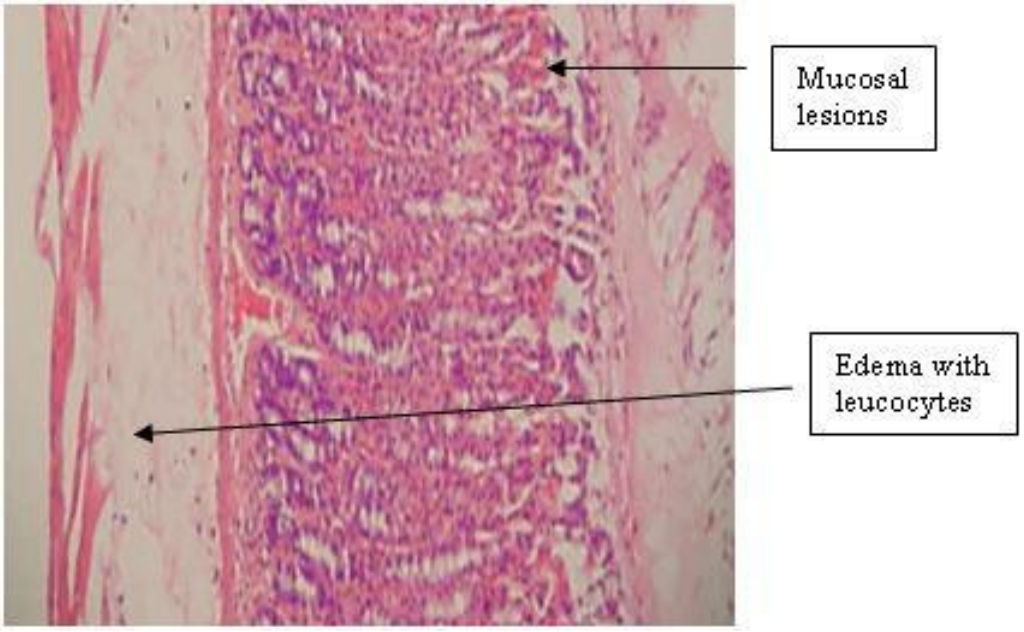

Figure 1g. Histological section of gastric mucosa in a rat pre-treated with 5 ml/kg of PMALE (250 mg/kg). There is mild disruption to the surface epithelium with edema and leucocytes infiltration of the submucosal layer (H&E stain 10x).

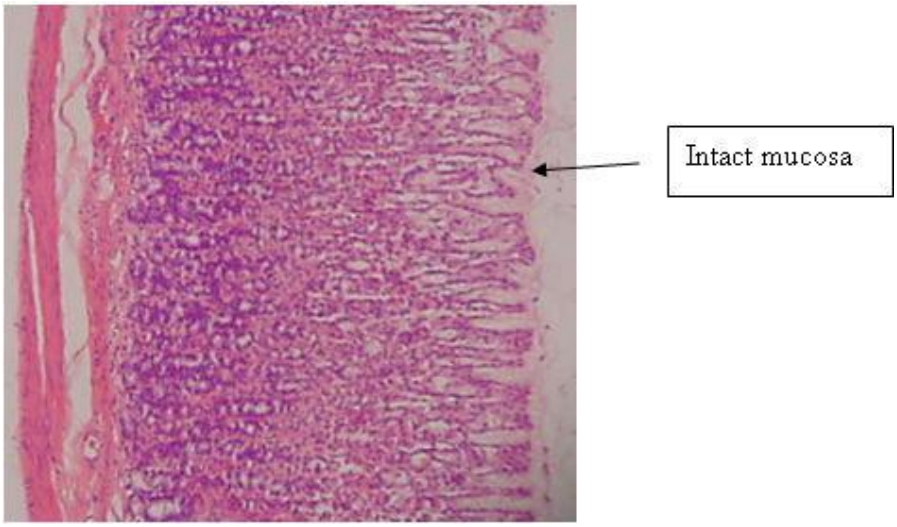

Figure 1h. Histological section of gastric mucosa in a rat pre-treated with 5 ml/kg of PMALE (500 mg/kg). There is no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer (H&E stain 10x).

against endogenous and exogenous ulcerogenic agents. The present study agrees with the previous results found by Huda-Faujan et al. (2007) that PMALE is among the Malaysian plant containing high antioxidant activity. Further, Banerjee et al. (2008) established that both malabaricones B and C possess significant healing property against indomethacin-induced stomach ulceration in mice. Their healing action could be attributed to their antioxidant activity along with the ability to modulate mucin secretion, prostaglandin synthesis and epithelial growth factor receptor expression.

The acute toxicity profile of PMALE could be considered favorable judging from the absence of adverse clinical manifestations in experimental animals

after 14 days of observation. Liver and kidney of the treated rats showed no significant change as compared to the control group. Hematology and clinical biochemistry values were within the range of the control animals tested. The highest dose of aqueous extract of PMALE which did not cause any toxicity was 5 g/kg body weight, suggesting that the PMALE is relatively non-toxic since in acute toxicity studies, the product is considered non-toxic if no deaths are registered after 14 days of observation and no clinical signs of toxicity are observed at doses at or below 5 g/kg.

In conclusion, the present study exhibited for the first time that PMALE was able to protect gastric mucosa from ethanol induced ulcer. 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 secretory activity.

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