

Gastroprotective Effects of *Melastoma malabathricum* Aqueous Leaf Extract against Ethanol-Induced Gastric Ulcer in Rats

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Abstract: The gastro-protective effects of the aqueous leaf extract of *Melastoma malabathricum* was investigated against ethanol-induced gastric mucosal injuries in rats. There were four groups of adult sprague dawley rats, consisting of six rats per group. Macroscopically, oral administration of absolute ethanol to rats that had been pre-treated with only distilled water significantly produced extensive lesions of the gastric mucosa. Oral pre-treatment with either 250 mg kg⁻¹ of *M. malabathricum* extract, 500 mg kg⁻¹ of *M. malabathricum* extract, or 20 mg kg⁻¹ of omeprazole 60 minutes before administration of absolute ethanol significantly reduced the formation of gastric mucosal injuries. The observed protection was significant and dose dependent in inhibiting ethanol-induced gastric ulcers. Microscopically, rats that had been pre-treated with only distilled water showed marked damage of the gastric mucosa, induced submucosal edema and leucocytes infiltration. However, animals that had been pre-treated with aqueous leaf extracts or omeprazole showed marked reduction of gastric mucosal damage, reduction of oedema and less leucocyte infiltration of submucosal layer. The results indicated that 500 mg kg⁻¹ aqueous leaf extract of *M. malabathricum* provided the best protection to the gastric mucosa in rats against ethanol-induced gastric ulcers.

Keywords: *Melastoma malabathricum*; gastric ulcer; Omeprazole; histology

INTRODUCTION

Melastoma malabathricum L. (Melastomataceae) is a common weed found throughout Malaysia. Different parts of the plants are used in folklore remedies for the treatment of various human ailments. The crude extract of *M. malabathricum* has been used in the treatment of diarrhoea as an astringent and is also used for post-partum treatment and for haemorrhoids^[1]. Some metabolites isolated from the *Melastoma* plant have also been reported to have antiviral and cytotoxic activity^[2], anti-helmethic and anti-spasmodic action^[3], anti-nociceptive, anti-inflammatory and anti-pyretic properties^[4], anti-oxidant and anti-cancer activity^[5] and anti-hypertensive activity^[6]. However, there is no data available regarding the anti-ulcerogenic activity of the *M. malabathricum* L. leaf extract. Therefore, the present study was undertaken to evaluate the anti-ulcerogenic property of the aqueous leaf extract of *M. malabathricum* in rats.

MATERIALS AND METHODS

Omeprazole: The reference anti-ulcer drug, omeprazole was obtained from the University Malaya Medical Centre (UMMC). The drug was dissolved in distilled water and administered orally to animals at a concentration of 20 mg kg⁻¹ (5 mL kg⁻¹).

Aqueous Extraction: *M. malabathricum* leaves were collected from different localities around the University of Malaya campus and identified by comparison with specimens available at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya. The leaves were washed with distilled water and then dried in an incubator at 50°C for 5-7 days. The dried leaves were ground to a powder before being extracted by maceration in distilled water (100 g 1500 mL⁻¹, w/v) for 5 days at 37°C. The extract was then filtered using a filter funnel and the excess water was evaporated under

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reduced pressure in an EYELA rotary evaporator. Once suitably evaporated, the extract was frozen at -80°C before being subjected to freeze drying to produce a dry extract. The dry extract was then stored at -20°C until use. The extract was dissolved in distilled water and administered to rats in a concentration of 250 and 500 mg kg^{-1} body weight (5 mL kg^{-1}) respectively.

Experimental animals: Adult male sprague-dawley rats were obtained from the Faculty of Medicine, University of Malaya's animal house (Ethic No. PM 28/09/2007 MAA (R)). Animals weighing 180-200 grams were deprived of food for 48 hours but they were allowed free access to tap water until 2 hours before the experiment. During the fasting period, the animals were placed individually in cages with wide-mesh wire bottoms to prevent coprophagy. On the day of experiment, the rats were randomly divided into 4 groups of 6 animals each.

Animal treatment: All rats were treated by orogastric intubations. Negative control rats (Group 1) received 5 mL kg^{-1} distilled water; Group 2 rats received 250 mg kg^{-1} of *M. malabathricum* extract (5 mL kg^{-1}); Group 3 rats received 500 mg kg^{-1} of *M. malabathricum* extract (5 mL kg^{-1}); and Group 4 rats, the positive control, received 20 mg kg^{-1} of omeprazole (5 mL kg^{-1}); Sixty minutes after this pre-treatment, all rats were gavaged with absolute ethanol (5 mL kg^{-1}) and after an additional 60 minutes, the rats were all sacrificed by overdose of diethyl ether. Rat stomachs were rapidly excised and opened along the greater curvature, washed with distilled water and fixed in 10% buffered formalin.

Gross gastric lesions evaluation: Each stomach was opened along the greater curvature and rinsed with distilled water to remove the gastric contents. The mucosa was examined under a dissecting microscope (1.8x) with a square-grid eyepiece (big square: Length X width = $10\text{ X }10\text{ mm}^2$ = Ulcer area) to assess the formation of hemorrhagic lesions. The length and width (area) of each lesion was determined and the sum of the area of all lesions, in mm^2 for each stomach was expressed as the ulcer area (mm^2). The ulcer area was calculated as described by Kauffman and Grossman^[7]. The inhibition percentage was calculated using the following formula^[8]:

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

Histological lesions evaluation: The gastric wall specimens were fixed in 10% buffered formalin and processed to produce paraffin wax tissue sections. The

stomach was assessed for damage by examining $5\text{ }\mu\text{m}$ sections stained with hematoxylin and eosin.

Statistical analysis: All values are reported as mean \pm SEM and the statistical significance of differences among groups were assessed using one-way ANOVA. A value of $p < 0.05$ was considered significant.

RESULTS

Gross examination: The present study demonstrated that oral administration with the extract (250 or 500 mg kg^{-1}) significantly prevented the formation of gastric mucosal ulcers and reduced ulcer formation in a dose dependent manner compared to animals administered with only distilled water (Table 1, Fig. 1 and 2). The present study also showed that the oral feeding of rats with 20 mg kg^{-1} omeprazole or 500 mg kg^{-1} extract resulted in higher protection for the gastric mucosa compared to rats administered with only 250 mg kg^{-1} of extract (Table 1). There were no significant differences between the cytoprotective abilities of the animals treated with 20 mg kg^{-1} omeprazole compared to the animals treated with 500 mg kg^{-1} extract.



Fig. 1: Severe macroscopic necrosis of gastric mucosa in negative control animals. Severe lesion of the corpus caused by absolute ethanol



Fig. 2: Mild macroscopic injury of gastric mucosa in (500 mg kg^{-1}) extract treated animals. Near complete inhibition of gastric lesion formation

Table 1: Tabulated results of gross examination of all oral treatment

Animal groups	Pre-treatment	Ulcer area (mm) ² (mean±SEM)	Inhibition (%)
1	Distilled water (Control)	845±52.17 ^a	-
2	<i>M. malabathricum</i> (250 mg kg ⁻¹)	210±8.17 ^b	75.15
3	<i>M. malabathricum</i> (500 mg kg ⁻¹)	70.00±8.27 ^c	91.72
4	Omeprazol (20 mg kg ⁻¹)	30±5.32 ^c	96.45

All values are expressed as mean and standard error mean. Means with different superscripts are significantly different (p<0.05)

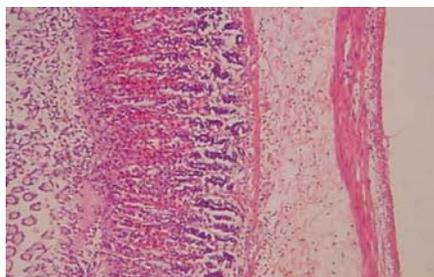


Fig. 3: Histological section of gastric mucosa in negative control animals (H and E stain, 40x). Severe disruption of the surface epithelium. Extensive edema of the submucosa and leucocytes infiltration

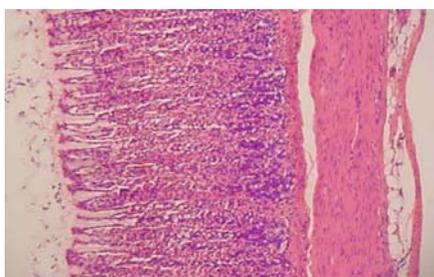


Fig. 4: Histological section of gastric mucosa in animals pre-treated with *M. malabathricum* extract (500 mg kg⁻¹) (H and E stain, 40x). Mild disruptions of surface epithelium. No presence of submucosal edema and leucocytes infiltration

Microscopic Examination: The present study demonstrated that rats treated with 20 mg kg⁻¹ omeprazole or extract (250 and 500 mg kg⁻¹) showed marked prevention of gastric mucosal ulcer, lack of submucosal oedema and no leukocyte infiltration compared to animals treated with only distilled water (Fig. 3 and 4).

DISCUSSION

The present study demonstrated that the oral administration of omeprazole or *M. malabathricum*

extract significantly protected the gastric mucosa in rats, compared to the rats administered with only distilled water. Oxidative stress plays a major role in the pathogenesis of various diseases including gastric ulcers. Oxidative stress can damage many biological molecules. The *M. malabathricum* flower extract possesses anti-nociceptive; anti-inflammatory and anti-pyretic activities and thus the ulcer prevention could be attributed to these properties and supports previous claims on its traditional uses to treat various ailments^[4]. *M. malabathricum* flower extract has been shown to contain anti-oxidant cytotoxic flavonoids which may mediate the protection of gastric mucosa^[5]. Anti-oxidants have been reported to play a significant role in preventing gastric ulcers. It has appeared that anti-oxidants may be an important contributory factor in the protection of gastric mucosa^[5]. Studies have shown that anti-oxidants significantly strengthen the gastric walls and protect tissue from oxidative damage^[9]. Anti-ulcerogenic properties of *M. malabathricum* could also be due to anti-viral and cytotoxic activities^[2]. *M. malabathricum* extracts exhibited significant anti-microbial activity and properties that support folkloric use in the treatment of some diseases. This probably explains the use of these plants by the indigenous people against a number of infections^[2]. Based on the results obtained, it can be concluded that *M. malabathricum* flower extract is potentially an anti-ulcerogenic agent that protects and strengthens the gastric mucosa. The exact mechanism of action is not known but it could be due to large quantity of mucus secreted by the gastric mucosa which acts as a mechanical barrier.

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