Anti-ulcer activity of the aqueous extract of *Melastoma malabathricum* L. leaf in rats

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Abstract: *Melastoma malabathricum* L. Smith (Melastomaceae) has been used in the Malay traditional culture to treat ulcer-based ailments. The objective of the present study was to investigate the potential anti-ulcer effect of aqueous extract of *M. malabathricum* leaves (AEMM) using ethanol- and indomethacin-induced gastric ulcer models in rats. Rats were divided into ten groups (n=6) and received DMSO (10%; negative group), ranitidine (100mg/kg; positive group) or AEMM (50, 250 and 500mg/kg) orally for 7 days and on the 8th day subjected to the respective gastric ulcer models. The stomachs were collected and subjected to macroscopic and microscopic analysis. At all groups tested, the AEMM exerted significant (p<0.05) anti-ulcer effect only against the ethanol-induced gastric ulcer model. The percentage of anti-ulcer for the 50-500mg/kg AEMM ranging between 50-82%, respectively. The macroscopic observations were supported by histological findings. In conclusion, AEMM exhibits potential anti-ulcer activity attributed to its previously proven high flavonoids content and antioxidant activity.

Keywords: *Melastoma malabathricum*; Melastomaceae; anti-ulcer activity; aqueous extract.

INTRODUCTION

Gastric ulcer is due to the disruptions of the gastric mucosal defense and repair systems that lead to erosion on the inside lining of the stomach (Gregory *et al*., 2009; Galani *et al*., 2010). Due to various adverse side effects associated with currently available anti-ulcer drugs (Gregory *et al*., 2009; Galani *et al*., 2010), current interest has been shifted towards natural products as a new source of gastroprotective agents (Gregory *et al*., 2009). *Melastoma malabathricum* L. Smith (family Melastomaceae), a small shrub that is native to the tropical and temperate countries, including Malaysia, is an attractive plant with various medicinal properties (Mohd. Joffry *et al*., 2011). Recognized as '*Senduduk*' in Malaysia, the leaves of *M. malabathricum*, particularly, are utilized to treat various ailments including ulcers and gastric ulcers (Mohd. Joffry *et al*., 2011). Despite various scientific reports on this plant's leaves pharmacological activities, no attempt has been made to study its anti-ulcer potential. Thus, the objective of the present study was to explore the anti-ulcer activity of aqueous extract of *M. malabathricum* leaves (AEMM) using various animal models.

MATERIALS AND METHODS

Plants' materials

The leaves of *M. malabathricum* were collected from their natural habitat in Serdang, Selangor, Malaysia between August-September 2010. A new voucher specimen, ACP 0017, has been deposited at the Herbarium of Institute of Bioscience, UPM.

Preparation of the AEMM

The procedure used for the preparation of AEMM was as described by Zakaria *et al*. (2011a). From approximately 80.0g of grinded dried leaves, the extraction yielded approximately 16.0g of dried AEMM (percentage yielded was ≈ 20%).

Experimental animal

Adult male Sprague-Dawley rats were cared as described by Zakaria *et al*. (2006) and the animal ethics approval was obtained from the Animal Ethics Committee, UPM with reference no: UPM/FPSK/PADS/BR-UUH/00383.

Chemical and drugs

Ranitidine, ethanol (Sigma Chemical, USA), diethyl ether, 10% buffer formaldehyde, xylene (Merck, Darmstadt, Germany), haematoxylin (Bio Optica, Milano, Italy), and eosin (VWR International, England) were used.

Acute toxicity study on AEMM

The acute toxicity model described by Mohamed *et al*., (2011) involving the use of a single dose administration of 5000mg/kg (p.o.) was adopted.

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Table 1: Effect of AEMM on ethanol-induced gastric ulcer model in rats

Pretreatment	Dose (mg/kg)	Ulcer Score	Ulcer Area (mm^2)	Percentage of Inhibition (%)
Normal	-	0	0±0	-
dH_2O	10	3.417±0.4167	27.17±4.715	-
Ranitidine	100	1.667±0.1054*	12.67±2.692*	53.37
AEMM	50	2.917±0.4167	13.17±4.423*	81.60
	250	2.083±0.3962*	7.00±2.352*	74.24
	500	1.667±0.1054*	5.00±1.693*	51.53

Values are expressed as means ± S.E.M; n=6 *p<0.05 was considered significant when compared with negative control group, NC

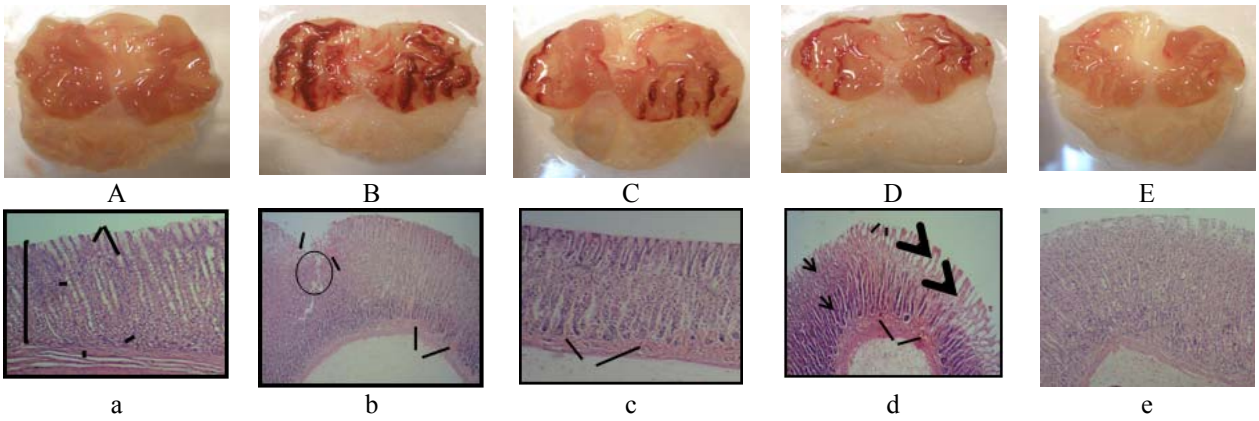

Fig. 1: Histological evaluation of antiulcer activity of AEMM against ETH-induced gastric lesions in rats.
(A): Stomach of a control normal rat; (B) Stomach of an ulcer-induced rat untreated; (C): Stomach of an ulcer-induced rat pre-treated with 100mg/kg ranitidine; (D): Stomach of an ulcer-induced rat pre-treated with 50mg/kg AEMM; (E): Stomach of an ulcer-induced rat pre-treated with 500mg/kg AEMM; The respective histopathological sections (H & E staining, 100x) are shown below; (a): Micrograph of stomach of the control rat shows normal gastric mucosa architecture (Notes: MS-mucus secretion; GP - intact epithelium with gastric pits; PC - parietal cells; CC-chief cells in region of mucosa; MM - muscularis mucosa); (b): Micrograph of stomach of dH_2O-pretreated rat shows severe effect of ethanol on stomach's mucosa as indicated by the presence of disrupted epithelial and glands, severe hemorrhagic erosion, edema in the upper mucosa layer and deep erosion at almost entire mucosal layer, numerous infiltration of red blood cells (RBCs) and neutrophils and cellular debris (Notes: H-hemorrhage; E-edema; DE-deep erosion); (c): Micrograph of stomach of ranitidine-pretreated rat shows intact epithelial and glands, moderate hemorrhage with RBCs, but not neutrophils, infiltrations, moderate edema and absence of erosion; (d): Micrograph of stomach of 50mg/kg AEMM-pretreated rat shows moderate epithelial and glands disruption, the presence of hemorrhage with RBCs, but not neutrophils, infiltrations, moderate edema and absence of erosion. There were no viable cells presented at mucosa layer (thick arrow) as compared to viable cell presented other part (thin arrow); (e): Micrograph of stomach of 500mg/kg AEMM-pretreated rat shows remarkable protection of the gastric mucosa with mild disruption of surface epithelium, preservation of normal glands structure, mild mucosal edema and absence of erosion.

Anti-ulcerogenic activity

Ethanol- and indomethacin-induced gastric ulcer models

The anti-ulcer activity of AEMM was evaluated according to the methods described by Zakaria *et al*. (2011b). The macroscopic (ulcer area and ulcer score) and histopathological evaluation of ulcer-induced stomachs was also carried out (Zakaria *et al*., 2011b).

STATISTICAL ANALYSIS

The results are presented as Mean ± Standard Error of Mean (S.E.M), and analyzes using the one-way analysis of variance (ANOVA) test with Dunnet post-hoc test with P<0.05 as the baseline for significance.

RESULTS

The AEMM exhibited a significant (P<0.05) anti-ulcer activity against ethanol-induced gastric ulcer model with the percentage of anti-ulcer recorded ranging between 51-82% in comparison to the 10% DMSO-treated groups. Interestingly, the 50mg/kg AEMM's anti-ulcer activity was equally effective to the 100mg/kg ranitidine (51.5% versus 53.4%, respectively). The ulcer area and ulcer score recorded after pre-treatment with AEMM or ranitidine were shown in table 1. These findings were supported by the histological observations (fig. 1).

Furthermore, AEMM, at all doses, failed to affect the indomethacin-induced gastric ulcer model (data not shown).

DISCUSSION

The AEMM has been proven to possess various pharmacological properties including anti-nociceptive, anti-inflammatory, anti-oxidant and anti-proliferative (Zakaria *et al*., 2006; Zakaria *et al*., 2011a). Only flavonoids could be detected from the extract that contained high amounts of total phenolic content (TPC) using phytochemical screening (Zakaria *et al*., 2011a). Thus, the observed anti-ulcer effect of AEMM may possibly be linked to its anti-oxidant and anti-inflammatory effects (Swarnakar *et al*., 2005; Abdulla *et al*., 2010) while overall effects of AEMM could be associated with its high TPC. Once in the gastric mucosa, ethanol enhances the synthesis of free radicals (e.g. hydroxyl radical and super oxide anion) as well as lipid peroxidation. These free radicals and other newly generated reactive metabolites acted with nearly all of the cell components leading to alteration in the structures and functions of cells. Moreover, these free radicals can also trigger certain mechanisms that will cause increase in cells' oxidative damage (Sheeba and Asha, 2006). Previous studies reported on the ability of ethanol to trigger extensive injury to mucosal capillaries, which, in turn, enhanced vascular permeability, formation of oedema and epithelial lifting. All of these ethanol-triggered changes lead to the gastric mucosal injury (see Zakaria *et al*., 2011b). On the other hand, the AEMM is suggests to attenuate ethanol-induced gastric ulcer by triggering the mechanism known as adaptive cytoprotection. This mechanism, which is considered as a local and nonspecific mechanism, takes places as a result of some compounds capacity to trigger prostaglandins synthesis (Bighetti *et al*., 2005). Prostaglandins will trigger the production of mucus and bicarbonates. In addition to the cytoprotection effect, AEMM may also reduce vascular permeability and, prevents the capillary endothelium injury and arachidonate metabolites release (Rachchh and Jain, 2008). The ability of AEMM to trigger prostaglandins synthesis seems to contradict its previously reported anti-inflammatory activity, which requires the inhibition of prostaglandins action or synthesis (Zakaria *et al*., 2006). These contrast observations were possibly the results of activation of different types of prostaglandins, namely prostaglandin E2 (PGE2) or, prostaglandin D2 (PGD2) and I2 (PGI2), respectively. PGE2 plays an important role in the synthesis of gastric mucus and AEMM is believed to trigger the synthesis of PGE2 leading to the observed anti-ulcer activity. On the other hand, PGI2 has been reported to possess anti-inflammatory activity and AEMM is thought to exert anti-inflammatory activity by triggering the synthesis or action of PGD2/PGI2. It is also worth discussing the failure of AEMM to reverse indomethacin-induced gastric ulcer formation. Indomethacin has been known to prevent endogenous prostaglandins synthesis, which lead to inhibition in the synthesis/secretion of endogenous gastric mucus in particular (Wilson, 1995). Based on these suggestions, it is believed that the failure of AEMM to attenuate indomethacin-induced gastric ulcer could possibly be due to the extract inability to activate endogenous prostaglandins synthesis (Wilson, 1995). Finally, the anti-ulcer of AEMM could be contributed by the presence of high TPC and, particularly, flavonoids (Vilegas *et al*., 1999). In conclusion, the anti-ulcer activity of AEMM might be attributed to its flavonoids-content and high TPC, and linked to its anti-oxidant and anti-inflammatory activities that require further extensive studies.

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