

EXAMINE SOME MEDICINAL MALAYSIAN PLANTS FOR THEIR GASTRIC PROTECTION ABILITY IN SD-RATS AGAINST ETHANOL INDUCTION MODEL

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Abstract. Ethanol extracts of different traditional Malaysian plants (*Polygonum minus*, *Andrographis paniculata*, *Curcuma xanthorrhiza*, *Momordica charantia* and *Strobilanthes crispus*) were evaluated for their gastroprotective activities. In this experiment several groups of Sprague Dawley rats weighing 200-250gm were used. The first group was pre treated with (0.25% w/v) carboxymethyl cellulose (negative control, 5 ml/kg), the second group was pre treated with 20 mg/kg omeprazole (positive control) and the other groups were pretreated with the selected Malaysian medicinal plants in two different doses 250 and 500 mg/kg respectively. After 60 min all the rats were treated with 95% ethanol for ulcer induction. Rats were sacrificed 1 h after induction and the stomachs were analyzed for gross and histological changes. Negative control group showed extensive lesions of gastric mucosal layer, whereas rats pretreated with omeprazole and Malaysian plants showed significant and dose dependent reduction in gastric lesions. Amongst, the selected plants *Polygonum minus* potentially promote better inhibition percentage of ulcer area. So that we can conclude that *Polygonum minus* could be the selective plant as an antiulcerogenic agent for the treatment of gastric ulcer.

Keywords: Ulcer prevention; Malaysian medicinal plant; gross lesion; histopathology

Abstrak. Beberapa jenis ekstrak tumbuhan tradisional yang berlainan di Malaysia (*Polygonum minus*, *Andrographis paniculata*, *Curcuma xanthorrhiza*, *Momordica charantia* and *Strobilanthes crispus*) telah dinilai bagi aktiviti-aktiviti gastroprotective. Dalam ujikaji ini, beberapa kumpulan tikus Sprague Dawley seberat 200-250gm telah digunakan. Kumpulan yang pertama terlebih dahulu dirawat dengan selulosa (0.25% w / v), carboxymethyl (kawalan negatif, 5 ml / kg), manakala kumpulan kedua terlebih dahulu dirawat dengan 20 mg / kg omeprazole (kawalan positif) dan kumpulan-kumpulan lain terlebih dahulu dirawat dengan beberapa tumbuhan herba

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Malaysia yang terpilih dalam dua dos yang berbeza iaitu 250 dan 500 mg / kg. Selepas 60 minit, semua tikus-tikus tadi dirawat dengan 95% etanol untuk induksi ulser. Tikus dimatikan 1 jam selepas induksi dan organ perutnya telah diambil untuk dianalisis dari segi perubahan kasar dan histologi. Kumpulan kawalan negatif menunjukkan luka-luka yang banyak pada lapisan mukosa perut, manakala tikus yang dirawat dengan omeprazole dan tumbuhan Malaysia menunjukkan pengurangan luka di perut yang ketara dan ia berkait rapat dengan bilangan dos yang digunakan. Di antara tumbuh-tumbuhan yang dikaji, *Polygonum minus* berpotensi menggalakkan peratusan perencatan yang lebih baik di kawasan ulser. Oleh itu, *Polygonum minus* boleh dijadikan sebagai ejen antiulcerogenic untuk rawatan ulser gastrik.

Kata kunci: pencegahan ulser; tumbuhan perubatan Malaysia; luka kasar; histopathology

1.0 INTRODUCTION

Traditionally, medicinal plants have been used to treat a variety of diseases including peptic ulcer. Nowadays following the traditional belief, the demand of herbal plant increased in developing country. Medicinal plant have been postulated to have a huge number of secondary bioactive compounds such as alkaloid, flavenoid, terpinoid, tannins and others that contribute in the life saving [1]. Hence, the researchers attracted to use natural recourses from medicinal plant to investigate pharmacological aspects.

Malaysian plants have been widely used because of their prized aromas and tastes, which add variety and flavor to foodstuffs. Traditionally, many of these plants are also used to treat different human ailments. Several Malaysian medicinal plants such as *Melastoma malabathricum* [2], *Phyllanthus niruri* [3] and *Gynura procumbens* [4] have been reported to have gastroprotective properties.

In the present study, base on the traditional use, five Malaysian plants were selected to evaluate their gastroprotective properties. *Polygonum minus*, locally named 'kesum' in Malay, is a plant having a sweet and pleasant aroma. It comes from the family Polygonaceae [5]. Traditionally, *P. minus* (PMLEE) is used to treat stomach problems. Relatively few pharmacological studies have been conducted on *P. minus*, which have indicated antimicrobial and cytotoxic activity against HeLa (human cervical carcinoma) [6].

Andrographis paniculata (APLEE) is a medicinal plant, commonly known as king of bitters and named locally 'hempedu bumi' in Malaysia. Traditionally, its leaves and roots have been used for different medicinal purposes in Asia and Europe [7]. Pharmacological study has demonstrated that *A. paniculata* possesses

antimicrobial activity [8], antiviral properties [9], hepatoprotective and antioxidant [10], anti-diabetic, antihyperglycaemic [11] and antiangiogenic activity [12], in addition to anti inflammatory properties [13], and it is used in the treatment of upper respiratory tract infections [14].

Curcuma xanthorrhiza (temu lawak) (CXLEE) from the family Zingiberaceae has been traditionally used to treat stomach diseases, liver disorders, constipation, bloody diarrhea, dysentery, children's fevers, hemorrhoids, and skin eruptions [15]. Pharmacologically it has been reported that *Curcuma* has antimicrobial [16], and anti-cancer activities [17].

Strobilanthes crispus (SCLEE) from the Acanthaceae family is known as 'pecah kaca' or 'jin batu' in Malaysia as well as 'daun peach beling' in Jakarta. It is widely used in traditional medicine as an anti-diabetic, diuretic, antilytic and laxative [18]. *S. crispus* has been proved scientifically to have antihyperglysemic, hypolipidemic and antioxidant enzyme effects [19], anti-AIDS and anticancer activity [20], as well as to provide possible protection against lipid peroxidation and DNA damage induced by iron nitrilotriacetate [21].

Momordica charantia (MCLEE), known as bitter melon or bitter gourd (family: Cucurbitaceae), is an herb widely grown in tropical countries like Malaysia and India. It is commonly eaten as a vegetable and has been proven to have antibacterial [22], antimalarial [23], anti-ulcer [24], antidiabetic [25], and potential hypocholesterolemic and anti-oxidant activities [26]. This study was carried out to assess different Malaysian plants for their antiulcer property.

2.0 MATERIALS AND METHODS

2.1 Medicinal Malaysian Plants

Air-dried leaves of selected Malaysian plants, namely *Polygonum minus* (kesum), *Andrographis paniculata* (hempedu bumi), *Curcuma xanthorrhiza* (temulawak), *Momordica charantia* (bitter melon) and *Strobilanthes crispus* (pecah kaca), 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 and University of Malaya Kuala Lumpur.

2.2 Experimental Animals

Adult male healthy Sprague Dawley rats (150-200 g) were obtained from Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethics No. PM 07/10/2009 MAA (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. Animals were kept individually in a separate cage with wide-mesh wire-bottoms to avoid coprophagy and were kept in standard controlled room ($25 \pm 2^\circ\text{C}$ and 12 h L and D cycle). Animals were supplied with standard laboratory food pellets, and water *ad libitum*.

2.3 Ethanol Extract

Dried powdered leaves were extracted by drenching in ethanol (50 g/ 1000 ml, w/v) in a conical flask for 3 days at room temperature. The solvent was filtered followed by distillation under reduced pressure in Eyela™ rotary evaporator (Sigma-Aldrich, USA) until excess solvent was evaporated the resultant yield was 7.6 mg/100 g. The extract was suspended in CMC (0.25% w/v) and was administered to rats at doses of 250 and 500 mg/kg, respectively [4].

2.4 Antiulcer Evaluation

2.4.1 Ethanol Induction Model

Following the method described by Mahmood [4] was adopted. All rats were fasted for 48 h before the experiment but were allowed free access to drinking water up till 2 h before the experiment. All rats were treated by orogastric intubations. Negative control groups (Group 1) received CMC, 0.25% w/v, 5 ml/kg; (Group 2) the Positive control group received 20 mg/kg of omeprazole 5 ml/kg. (Group 3 -7) rats received 250 and 500 mg/kg of plant ethanol extract 5 ml/kg. After 60 min, all rats were administered with 95% ethanol 5 ml/kg and after an additional 60 min; the rats were all sacrificed by overdose of ketamine (100 mg/ml). Rat stomachs were rapidly excised [27], the stomach was then opened

along the greater curvature, washed with distilled water to record the ulcer area and then fixed in 10% buffered formalin for histological examination.

2.4.2 Gross Evaluation

Each stomach was examined under a dissecting microscope (1.8×) with a square-grid eyepiece (big square: length × width = 10×10 mm² = ulcer area). 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) as described by Mahmood [4]. The inhibition percentage (I %) was calculated according to the following formula

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

UA = ulcer area

2.4.3 Histopathological Examination

Stomachs were fixed in a buffered formalin (10%) solution for histological evaluation 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 & E evaluated for microscopical examination.

2.5 Statistical Analyses

The data was analyzed by Statistical Package Social Science (SPSS) version 17.0. One-way ANOVA was used to show the mean differences between all samples.

3.0 RESULTS AND DISCUSSIONS

The anti-ulcer activity of ethanol medicinal Malaysian plants extract in ethanol-induced gastric lesion model is reported in Table 3.1. The results of anti ulcer activity of negative control, positive control and plant extracts expressed as inhibition percentage in which the high inhibition percentage represents better cytoprotection impact. Based on the calculation of ulcer area, morphological and histological changes, ethanol extract significantly showed that elimination of ulcer area in dose dependent manner. Amongst the five medicinal Malaysian plants, *P. minus* and *A. paniculata* represented to be the best Malaysian plants for their antiulcer property.

Table 3.1 Effect of ethanol medicinal Malaysian plants extract on ulcer area and inhibition percentage of ethanol induced gastric ulcer rats values are mean \pm mean S.E (n= 6)

Pre-treatment (5 ml/kg dose)	Ulcer area (mm) ² (Mean \pm S.E.M)	Inhibition (%)
CMC (negative ulcer control)	978.3 \pm 32.4	-
OMP (positive control 20 mg/kg)	114.4 \pm 3.71	88.30
PMLEE (250 mg/kg)	9.000 \pm 1.75	99.00
PMLEE (500 mg/kg)	0.000 \pm 0.00	100.0
APLEE (250 mg/kg)	14.40 \pm 1.57	98.52
APLEE (500 mg/kg)	0.000 \pm 0.00	100.0
CXLEE (250 mg/kg)	91.10 \pm 9.29	90.68
CXLEE (500 mg/kg)	74.20 \pm 4.07	92.41
SCLEE (250 mg/kg)	116.4 \pm 9.08	88.10
SCLEE (500 mg/kg)	81.70 \pm 7.29	91.64
MCLEE (250 mg/kg)	134.2 \pm 10.8	86.28
MCLEE (500 mg/kg)	96.10 \pm 5.80	90.10

Macroscopical gross examination of the pretreatment of CMC (negative control group) exhibited severe mucosal injury (Table 3.1 and Fig.1(a)), whereas pretreatment with OMP, 250 or 500 mg/kg of the ethanol plants extract had significantly $P < 0.05$ reduced area of gastric lesion formation compared to rats pretreated with CMC. Gastric protection was more prominent with the pretreatment of PMLEE and APLEE 500 mg/kg, Table 3.1 and Fig. 1b and 1c, showed completely protective %100 of gastric mucosa against ethanol induced

peptic ulcer. While pretreatment of CXLEE, SCLEE and MCLEE 500 mg/kg exhibited mild lesion in which the inhibition percentage of ulcer area were %92.41, %91.64 and %90.1 respectively Table 3.1 and Fig. 1d, 1e and 1f.

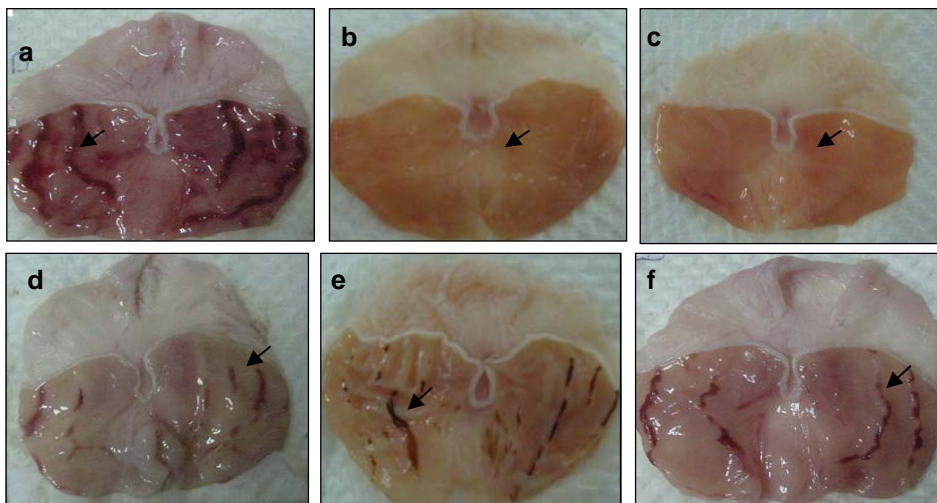


Figure 1 Gross appearance of gastric mucosa lesion in rats pre-treated with: (a)-CMC (ulcer control group); (b)-PMLEE, 500 mg/kg; (c)-APLEE, 500 mg/kg; (d)-CXLEE, 500 mg/kg; (e)-SCLEE, 500 mg/kg (e)-MCLEE, 500 mg/kg

It was also observed that histological examination of stomach of rats pretreated with CMC (negative control group) suffered markedly extensive damage to gastric mucosa layer with edema and leucocytes infiltration of submucosal layer (Fig. 2a). Rats pre-treated with 5 ml/kg of omeprazole, 20 mg/kg showed mild disruption to surface epithelium mucosa with mild edema and leucocytes infiltration of the submucosal layer. Protection of gastric mucosa was markedly seen by moderate disruption to the surface epithelium mucosa with moderate edema and leucocytes infiltration of the submucosal layer after pretreatment with 5 ml/kg of 500 mg/kg CXLAE, SCLAE and MCLAE (Fig. 2d,e and f). While, rats pretreated with 500 mg/kg of PMLAE APLEE showed complete protection of gastric mucosa with no edema and leucocytes infiltration (Fig. 2b and 2c) respectively.

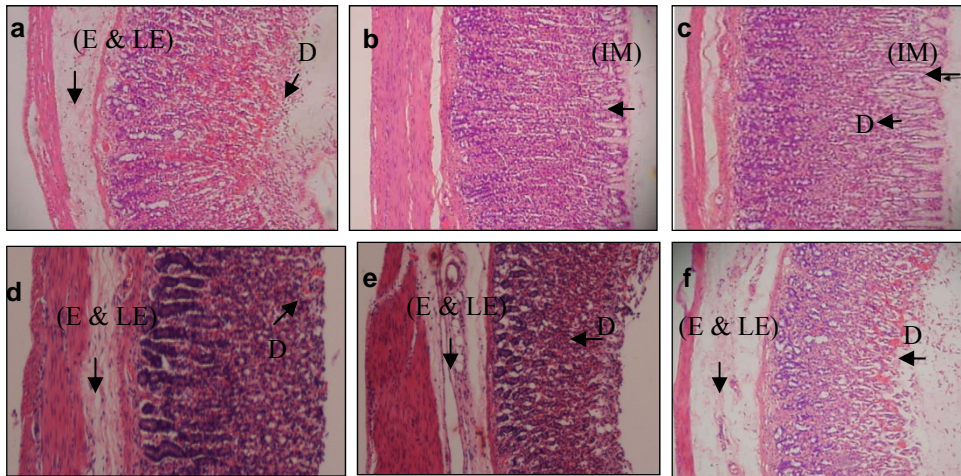


Figure 2 Histological section of gastric mucosa in rats pretreated with: (a)- 5 ml/kg of CMC; (b) 5 ml/kg of 500 mg/kg PMLEE; (c)-5 ml/kg of 500 mg/kg APLEE; (d)-5 ml/kg of 500 mg/kg CXLEE; (e)-5 ml/kg of 500 mg/kg SCLEE; (f)-5 ml/kg of 500 mg/kg MCLEE. [E: edema, LE: leucocytes infiltration, D: disruption to surface epithelium mucosa, IM: intact mucosa. H & E 10 ×]

Free radical effectively contributes in the pathogenesis of different diseases including peptic ulcer. On the other hand, antioxidant being postulated to act a powerful role to protect gastric mucosa against different alcerogenic factors [10]. In the current study, ethanol induction model was performed as it has been reported as a rapid and convenient model of gastric ulcer agent in experimental animals. As well as ethanol considered as a necrotizing factor that contribute to the formation of lesion in the gastric mucosa [28]. The mechanism underlying this lesion demonstrated to damage of the vascular and endothelium causing edema formation and lifting, or may be due to stagnate of gastric blood flow resulting to the appearance of hemorrhage, necrosis and tissues injury [29]. Several reports have been conducted that antiulcer significantly correlated with antioxidant. Umamaheswari and his collague reported that antioxidants is a factor that protect cells from damage caused by oxidative stress and enhancing the body's defense systems against degenerative diseases and are contributed in the mechanism of gastric ulcer [30].

The present study showed that ethanol extraction of all the plants reduced ulcer area compare to CMC group. Similarly with the results found by Deore and

co workers showed that aqueous and ethanol extract able to attenuate ulcer lesion significantly $p < 0.001$ 36.80% and 61.62% respectively on ethanol induced ulcerogenesis in rats. This activity may contribute to high antioxidant activity. As found in previous study [31] that ethanol extract of the five selected plants potent high free radical scavenging activities of DPPH, reduction capacity in FRAP assay and total phenolic content. Also it was presented that *P. minus* exhibited better antioxidant and TPC and completely eliminate gastric lesion. Hence it can be used as a natural antioxidant for the treatment of gastric ulcer. And further studies will be conducted on *P. minus* as an effective plant to identify the exact active compound underlying this high antioxidant activity and the mechanism behind antiulcer property.

4.0 CONCLUSION

Base on the present study we concluded that the ethanol extracts of the five Malaysian plants *Polygonum minus*, *Andrographis paniculata*, *Curcuma xanthorrhiza*, *Strobilanthes crispus* and *Momordica charantia* significantly attenuate ulcer area in ethanol induction model. Obviously, there was a marked difference between plant extract and negative control group in dose dependent maner. As previous study showed that *P. minus* exhibited high antioxidant and TPC; it can be used as a natural antioxidant contributed in antiulcer property. Further study needed to identify the exact active compound underlying this high antioxidant activity and gastroprotective property.

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