Antioxidant and gastroprotective activities of *Andrographis paniculata* (Hempedu Bumi) in Sprague Dawley rats

S Q Wasman1*, A A Mahmood2, Lee Suan Chua3, Mohammed A Alshawsh2 & S Hamdan1

1Department of Biological Science, Faculty of Biosciences and Bioengineering, University of Teknologi Malaysia, 81310, UTM Skudai, Johor, Malaysia

2Department of Molecular Medicine, Faculty of Medicine, University of Malaya 50603, Kuala Lumpur, Malaysia

3Metabolites Profiling Laboratory, Chemical Engineering Pilot Plant, University Technology Malaysia, 81310 UTM Skudai, Johor, Malaysia

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Antioxidant and gastroprotective activities of aqueous and ethanolic extract of *Andrographis paniculata* leaves in rats have been reported. Sprague Dawley rats, 6 per group were used and rats in groups 1 to 6 were pretreated with (0.25% w/v) carboxymethyl cellulose (negative control, 5 ml/kg), 20 mg/kg omeprazole (positive control), (250 mg/kg and 500 mg/kg) of aqueous leaf extracts (APLAE) and (250 and 500 mg/kg) of ethanol leaf extracts (APLEE) respectively. Animals were orally administered with 95% ethanol (5 ml/kg) 60 min after their pretreatments. Rats were sacrificed 1 h after treatment and gastric contents were collected to measure pH and mucous weight. Stomach was analyzed for gross and histological changes. Ulcer control group showed extensive lesions of gastric mucosal layer, whereas rats pretreated with omeprazole, 250 and 500 mg/kg of APLAE showed significant and dose dependent reduction in gastric lesions with increased pH and mucus content of stomach. Rats pretreated with 250 or 500 mg/kg of APLEE showed significantly better inhibition of gastric mucosal lesions. Further, the *in vitro* antioxidant studies using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay showed that ethanol extracts have superior free radical scavenging activity with IC$_{50}$ value = 10.9 than aqueous extracts with IC$_{50}$ value = 24.65. Results of this study showed that pretreatment with ethonolic extract of *A. paniculata* ethanolic provided significant protection against gastric ulcer by regulating of pH, mucus production and antioxidant property.

**Keywords:** *Andrographis paniculata*, Antioxidant, Ethanol, Histology, Peptic ulcer

Gastric ulcer affects a considerable number of people worldwide and in the United States, approximately 500,000 persons are affected by gastric ulcer each year. Although, proton-pump inhibitors have become classic anti-ulcer therapy for treatment of peptic ulcers and other gastrointestinal infection, but there is still no definite cure for this disease. In addition, long term use of these drugs may be accompanied with ineffectiveness of different drug regimens and even resistance to drugs are emerging. Thus, there is an urgent need to find more safe and effective gastroprotective agents. In this regard medicinal plants are evaluated for their gastro-protective activity in animal studies.

*Andrographis paniculata* is a Medicinal plant, commonly known as king of bitters and locally named Hempedu Bumi in Malaysia. It comes from the family Acanthaceae. Traditionally, the leaves and roots have been used for different medicinal purposes in Asia and Europe. *A. paniculata* was reported to possess antimicrobial activity, antiviral properties, hepatoprotective and antioxidant, anti-diabetic, antihyperglycaemic activity, antiangiogenic activity, anti inflammatory property, and treatment of upper respiratory tract infections. Further, it was also reported to modulate natural killer cell activity and the immune response in mice, *in vitro* anticancer activities, cell cycle arrest and mitochondrial-mediated apoptosis in human leukemic HL-60 cells. Diterpenoides (6) and diterpenoidal (3) glycosides isolated from *A. paniculata* were found to be responsible for the above biological activities. In the present study antioxidant and gastroprotective activities of aqueous and ethanol extracts of *A. paniculata* on ethanol-induced gastric ulcer in rats have been reported.

*Correspondent author*
Telephone: +6-0177229378
Fax: +6003 79676600
E-mail: suhaylaqadir@yahoo.com
Materials and Methods

Plant materials—Air-dried herbs of *A. paniculata* leaf (Acanthceae) were obtained from Ethno Resources Sdn Bhd, Selangor Malaysia, and identified by comparison with the Voucher specimen no. 43261 deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya and Kuala Lumpur.

Chemicals and reagents—Ethanol, omeprazol (OMP) batch number 097793 date 10/2012, 2,2-diphenyl-1-picrylhydrazyl (DPPH), vitamin C, gallic acid, trolox, carboxymethyl cellulose (CMC), buffered formalin, paraffin wax, hematoxylen and eosin stain all the chemicals were purchased from Sigma-Aldrich, Germany.

Preparation of aqueous extract—Dried leaf was ground into powdered using electrical blender followed by extraction with sterile distilled water at the ratio of 1:20. The mixture was then heated and stirred on hotplate for 3 h (65°C) followed by cooling and filtration using filter paper and filter funnel. Then, distillated under reduced pressure in Eyela™(Sigma-Aldrich, USA) rotary evaporator until excess water was evaporated then subjected to lyophilization by a freeze-dryer to produce powdered forms of the extract, yielding 5.8 mg/100 g. The plant extract was then dissolved in sterilized distilled water and administered to rats in a dose of 250 and 500 mg/kg body weight, respectively.

Preparation of 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. Afterwards, 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.

Antioxidant activities—Antioxidant activity of the APLAE and APLEE were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical base on the electron transfer reaction between DPPH reagent and the plant extracts. Following the method described by Gorinstein with minor modification, stock solution (1mg/1ml) of the plant extracts and synthetic antioxidant standard (Vitamin C, Gallic acid and Trolox) were prepared and then diluted to get five different concentrations. A quantity of 5 µl of each plant extract and standard mixed with (195 µl of DPPH). The mixture was then incubated at 37°C for 30 min. The absorbance value was measured spectrophotometrically at 517 nm.

Experimental animals—Adult male healthy Sprague Dawley rats (200-225 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 randomly divided into 6 groups of 6 rats each. The rats were kept individually in a separate cage with wide-mesh wire-bottoms to avoid coprophagy and were kept in standard controlled room (25° ± 2°C and 12 h L and D cycle). Animals were supplied with standard laboratory food pellets, and water ad libitum.

Ethanol induced gastric ulcer—The method described by Mahmood 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. (Groups 3 and 4) rats received 250 and 500 mg/kg of APLAE 5 ml/kg. (Groups 5 and 6) received 250 and 500 mg/kg of APLEE 5 ml/kg 60 min after this pre-treatment, 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 and gastric content was collected by overdose of ketamine (100 mg/ml). Rat stomachs were rapidly excised and gastric content was collected and centrifuged to determine the pH, and mucus weight. 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.

Gastric lesion estimation—Each stomach was examined under a dissecting microscope (1.8x) with a square-grid eyepiece (big square: length × width = 10×10 mm 2 = 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. The inhibition percentage (I %) was calculated according to the following formula.
(I %) = [(UA control – UA treated) ÷ UA control] × 100%

Histological examination of gastric mucosa—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.

Statistical analysis—All values were reported as mean±SE. The statistical significance of differences between groups was assessed using one-way ANOVA. A value of $P < 0.05$ was considered significant.

Results

Evaluation of anti-ulcer activity using ethanol-induced ulcer—Pretreatment of CMC (negative control group) exhibited severe mucosal injury (Table 1 and Fig. 1a), whereas pre-treatment with OMP, 250 or 500 mg/kg of APLAE had significantly reduced area of gastric lesion formation compared to rats pretreated with CMC (Table 1, Fig. 1b, c and 1d). Gastric protection was more prominent with APLEE than APLAE with mild lesion after treatment with 250 mg/kg (Table 1 and Fig. 1e) and completely protective of gastric mucosal layer pretreatment with 500 mg/kg against ethanol induced peptic ulcer (Table 1 and Fig. 1f). It was also observed that APLAE, APLEE and OMP significantly increased $pH$ and mucus content in concentration dependent in comparing with ulcer control group rats (Table 1).

Histological examination revealed that ulcer control 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 and 250 mg/kg APLAE showed mild disruption to surface epithelium mucosa with mild edema and leucocytes infiltration of the submucosal layer (Fig. 2b, c). Protection of gastric mucosa was

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Pre-treatment (5 ml/kg dose)</th>
<th>Mucus production</th>
<th>$pH$ of gastric content</th>
<th>Ulcer area (mm$^2$) (Mean ± SEM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CMC (ulcer control)</td>
<td>0.24 ±0.06$^a$</td>
<td>3.60 ±0.67$^a$</td>
<td>980.0 ± 7.90$^a$</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>OMP (positive control (20 mg/kg))</td>
<td>0.57 ±0.07$^b$</td>
<td>6.84 ±0.70$^b$</td>
<td>121.67 ± 8.97$^b$</td>
<td>85.93</td>
</tr>
<tr>
<td>3</td>
<td>APLAE (250 mg/kg)</td>
<td>0.42 ± 0.05$^c$</td>
<td>4.10 ±0.31$^c$</td>
<td>540.0 ± 22.80$^c$</td>
<td>37.57</td>
</tr>
<tr>
<td>4</td>
<td>APLAE (500 mg/kg)</td>
<td>0.62 ± 0.03$^b$</td>
<td>6.30 ±0.65$^b$</td>
<td>57.0 ± 2.54$^d$</td>
<td>93.41</td>
</tr>
<tr>
<td>5</td>
<td>APLEE (250 mg/kg)</td>
<td>0.60 ±0.07$^b$</td>
<td>6.90 ±0.70$^b$</td>
<td>14.40 ± 1.14$^d$</td>
<td>98.53%</td>
</tr>
<tr>
<td>6</td>
<td>APLEE (500 mg/kg)</td>
<td>0.84 ±0.05$^d$</td>
<td>7.20 ±0.63$^b$</td>
<td>0.0 ± 0.0$^e$</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean with different superscripts are significantly different at $P < 0.05$

Fig. 1—Gross appearance of gastric mucosa lesion (→) in rats pre-treated with: (a)-CMC (ulcer control group); (b)-Omeprazole, 20 mg/kg (positive control group); (c)-APLAE, 250 mg/kg; (d)-APLAE, 500 mg/kg; (e)-APLEE, 250 mg/kg; (f)-APLEE, 500 mg/kg.
better as 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 APLAE and 250 mg/kg APLEE (Fig. 2d,e). While, rats pretreated with 500 mg/kg of APLEE showed complete protection of gastric mucosa with no edema and leucocytes infiltration (Fig. 2f).

**Evaluation of DPPH scavenging activities**—DPPH free radical scavenging activity of APLAE and APLEE was evaluated for the determination of their antioxidant properties. DPPH free radical scavenging assay of the positive control and plant extracts expressed as IC$_{50}$ values. Based on the IC$_{50}$ values calculated from the linearity curves APLEE showed the lowest concentration than APLAE (Table 2).

**Discussion**

Herbal products have gained powerful attention due to its effective roles in chemo-therapeutic agents and chronic disease prevention, including peptic ulcer. Their prolific effects are mainly due to their antioxidant and chelating properties. Results showed that the aqueous and ethanol extract of *A. paniculata* showed significant anti ulcer, cytoprotective properties and inhibited leukocyte infiltration of gastric wall.

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. The present study showed that high free radical scavenging activities of DPPH experiment of APLAE and APLEE may be responsible for its anti-ulcerogenic activities. *A. paniculata* leaf extract showed broad spectrum of biological activities and contain different active phytochemical. The most medicinally active phytochemical has been isolated and characterized are diterpinoide and diterpinoidal glycosides. They are responsible for anti-inflammatory activities and inhibit formation of oxygen derived free radicals such as superoxide, hydroxyl radicals, lipid peroxidation, and nitric oxide. This anti-inflammatory activity may also be a key factor in the prevention of gastric ulcer.

Inhibition of leucocytes infiltration and flattening of mucosal folds of gastric wall in rats pretreated with APLAE and APLEE were observed similarly, *Gynura procumbens* leaf extract exerted protective effect against mucosal lesions through inhibition of neutrophil infiltration in ulcerated gastric tissue. Flattening of mucosal folds suggests that gastroprotective effect of *Phyllanthus niruri* leaf extract may be due to a decrease in gastric motility. Gastric motility may play a role in development and

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC$_{50}$ value</th>
</tr>
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<tbody>
<tr>
<td>Ascorbic acid</td>
<td>5.6</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>2.1</td>
</tr>
<tr>
<td>Trolox</td>
<td>4.7</td>
</tr>
<tr>
<td>APLAE</td>
<td>24.65</td>
</tr>
<tr>
<td>APLEE</td>
<td>10.9</td>
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</table>

Table 2—Radical-scavenging activities of the *A. paniculata* leaf extract

Fig. 2—Histological section of gastric mucosa (→) in rats pretreated with: (a)- 5 ml/kg of CMC; (b)-5 ml/kg of 20 mg/kg omeprazole; (c)-5 ml/kg of 250 mg/kg APLAE; (d)-5 ml/kg of 500 mg/kg APLAE; (e)-5 ml/kg of 250 mg/kg APLEE; (f)-5 ml/kg of 500 mg/kg APLEE. [E: edema, LE: leucocytes infiltration, D: disruption to surface epithelium mucosa. H & E 10 x].
prevention of experimental gastric lesions. Relaxation of circular muscles may protect gastric mucosa through flattening of folds and increase mucosal area exposed to necrotizing agents and reduce volume of gastric irritants on rugal crest. Ethanol produces a marked contraction of circular muscles of rat fundic strip. Such contraction may lead to mucosal compression at the site of greatest mechanical stress, and crests of mucosal folds leading to necrosis and ulceration. Peptic ulcer is attributed to an imbalance between destructive factors, whether endogenous (acid, pepsin), or exogenous (ethanol, NSAIDs, smoking) and protective agents (mucus, bicarbonate secretion, prostaglandins, mucosal blood flow, nitric oxide).

Similarly, pretreatment with APLAE and APLEE exhibited a dose dependent increase of prostaglandins, mucosal blood flow, nitric oxide. Ethanol extracts showed significant inhibition of gastric mucus lesions compared to aqueous extracts. Furthermore, DPPH scavenging inhibition of gastric mucus lesions compared to mucus weight. Ethanol extracts showed significant prevention of experimental gastric lesions in rats, against experimentally-induced gastric lesions in rats, and their effects on estrous cyclicity of alloxan-induced diabetic rats, J Ethnopharmacol, 105 (2006) 196.

Acknowledgement

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