Gastroprotective activities of *Turnera diffusa* Willd. ex Schult. revisited: Role of arbutin

Manal Mohamed Elhassan Taha a,⁎, Muhammad Saleh Salga a, Hapipah Mohd Ali a, Mahmood Ameen Abdulla b, Siddig Ibrahim Abdelwahab c, A. Hamid A. Hadi a

a Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
b Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia
c Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

**ARTICLE INFO**

_Article history:_
Received 15 November 2011
Accepted 10 February 2012
Available online xxx

_Keywords:_
Gastroprotective activities
*Turnera diffusa*_ Willd. ex Schult.
Arbutin
Ethanol
Aspirin
Cytokines

**ABSTRACT**

Ethnopharmacological relevance: *Turnera diffusa* Willd. ex Schult. has been used for the treatment of several human disorders including peptic ulcer.

**Objectives of the study:** The current study is an attempt to evaluate the anti-ulcerogenic activities of arbutin, a major constituent of *Turnera diffusa* on two ulcer models. The possible involvement of lipid peroxidation, nitric oxide, IL-6, IL-10, TNF-α and mucus barrier mechanism has been investigated.

**Materials and methods:** Effects of arbutin on ulcer index, gastric juice acidity, mucus content and histology, gross and histological gastric lesions, nitric oxide, cytokines levels (IL-6, IL-10 and TNF-α), and thiobarbituric acid reactive substances (TBARS), were evaluated in aspirin or ethanol-induced ulcer in vivo. Acute toxicity of arbutin was also examined in rodent model. MTT assay was used to assess the cytotoxicity of the compound on normal liver cells (WRL-68).

**Results:** Pre-treatment with arbutin or omeprazole protected the gastric mucosa as seen by reduction in ulcer area and mucosal content, reduced or absence of edema, inflammation and leucocytes infiltration on both models. Arbutin significantly (P<0.05) lowered the elevated TBARS level into gastric homogenate. Arbutin did not produce significant inhibition of NO. This natural compound has modulated the levels of interleukin-6, interleukin-10 and TNF-α. No in vitro or in vivo toxicities for arbutin were observed.

**Conclusion:** Thus it can be concluded that *Turnera diffusa* possesses anti-ulcer activity, which could be attributed to lipid peroxidation inhibitory, immuno modulatory and anti-oxidant mechanisms of arbutin but not to the intervention with nitric oxide inflammation pathway.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The pathogenesis of gastrointestinal problems caused by different aetiologies was observed to be associated with the alterations of various physiologic parameters such as reactive oxygen species (ROS), nitric oxide synthase, lipid peroxidation and secretion of excessive gastric acid. The number of ulcer patients rises synergistically with the number of available treatments (Carr et al., 2009; Umit et al., 2009). However, acute gastric inflammation has provided a means for investigating the chemotherapeutic activities of many compounds. Meanwhile, the search for more efficient compounds that can selectively bind to gastric ulcer is essential in chemotherapy (Salga et al., 2011).

It is known that ethanol is among many factors increasing risk of gastric ulcer formation such as stress, use of steroids and non-steroidal anti-inflammatory drugs. Ethanol is widely used to induce experimental gastric ulcer in animals. For the purpose of lesion formation, per-oxide administration of ethanol was utilised since it easily and rapidly penetrates into the gastric mucosa. By increasing mucosal permeability and release of vasoactive products, ethanol causes vascular damage, and gastric cell necrosis which, in turn, leads to ulcer formation. It is claimed that oxygen free radicals play a role in the pathogenesis of gastric damage caused by ethanol (Maity et al., 2009; Goswami et al., 2011; Salga et al., 2011). Oral administration of aspirin to rats is known to produce characteristic mucosal lesions due to the direct effect of the stomach epithelium. The mechanisms of aspirin on gastric epithelia are diverse; in contrast to injuring the mucosa, aspirin has been reported to induce various cytokines during gastric lesions, the pro-inflammatory cytokines, which in turn activates local fibroblasts, endothelial and epithelial cells (Choi et al., 2010).

*Turnera diffusa* Willd. ex Schult. (*Turneraceae*) is a native plant to America and Africa. This small shrub is traditionally used for the treatment of various diseases including sexual impotence.
neurasthenia, diabetes mellitus, urine retention, malaria, diarrhoea, peptic ulcer, and alcoholism. Regardless the intensive uses of Turnera diffusa as a traditional healing, few are the scientific studies that evaluate its bioactivities (Wagner et al., 1986; Arletti et al., 1999; Gracioso et al., 2002; Placente et al., 2002; Zhao et al., 2007). A group of researchers from different parts of the globe has only investigated the hypoglycaemic, aphrodisiac, anxiolytic and adaptogenic activities (Arletti et al., 1999; Placente et al., 2002; Zhao et al., 2007; Patel et al., 2011). On the other hand, a great debate has been raised regarding the mechanism, effectiveness and extracts’ polarity of Turnera diffusa as potential anti-ulcer treatment. Bezerra et al. (2011) demonstrated that the hydroalcoholic extract of Turnera diffusa failed to suppress gastric ulceration induced by stress (immobilization and cold). While Gracioso et al. (2002), observed a significant inhibition in the HCl/ethanol induced-gastric lesions of mice that received the hydroalcoholic and dichloromethanic extract of this plant (Gracioso et al., 2002; Placente et al., 2002). Ulcer induced with immobilization and cold animal model is employed to evaluate the stress response, while in the induction of ulcer by HCl/ethanol model the focus is the chemical defence of the cure. Phytochemical analysis revealed that the aerial parts of this plant contain flavone glycoside, flavonoids and arbutin. On the other hand, Placente et al. (2002) suggested that the antibiucerogenic activities of Turnera diffusa could be related to its flavonoid. The water extract of the aerial parts of Turnera diffusa was reported to contain 3.75% of arbutin (Placente et al., 2002). A recent study shows that arbutin, a major constituent of Turnera diffusa, is a long-lasting antioxidant compound (Takebayashi et al., 2010). Therefore, the current study was designed to test for the first time the anti-ulcerogenic properties of arbutin, a glycosylated hydroquinone from Turnera diffusa, on two different in gastroprotective models. Our research is the first to report the effect of arbutin against aspirin- and ethanol induced ulceration in rats; and clarifies the traditional uses of Turnera diffusa based on its major compound. The current study also provides a scientific base for the use of this active ingredient in the plant, in order to get an idea of the required dosage that should be used in traditional medicine.

2. Materials and methods

2.1. Reagents

Arbutin, Griess reagent, carboxymethylcellulose, sodium hydroxide and potassium hydroxide were purchased from Sigma Aldrich, Kuala Lumpur, Malaysia. Omeprazole was obtained from University of Malaya Medical Centre, Malaysia. All other chemicals and reagents were of analytical grade.

2.2. Study animals

Sprague Dawley adult male rats weighing about 220 ± 30 g were obtained from the Animal House, Faculty of Medicine, University of Malaya, Malaysia. Rats were distributed into ten groups of six rats each. The animals were fed with a standard pellet diet and tap water under controlled temperature conditions (23 ± 2 °C). All animals were given care for according to approved institutional guidelines on experiments as articulated by the National Academy of Sciences and guidelines published by the National Institute of Health, Malaysia.

2.3. Protocol for gastroprotectivity

Groups A, E and J rats were given food and water for 14 days, Groups B and F rats were treated with omeprazole dissolved in carboxymethylcellulose (CMC) and Groups C, D, G and H rats were orally administered with arbutin dissolved in carboxymethylcellulose (CMC) at 30 and 60 mg/kg b.w, respectively, for 14 days. Rats in Group I received arbutin alone for 14 days. The animals were fasted for 24 h on day 14. On day 15, group B and F, received omeprazole, Group C, D, G and H were given arbutin while Groups A, E and J received vehicle. One hour after this treatment, animals in Groups A-D received orally 95% ethanol at the dose of 5 ml/kg. At similar manner aspirin was orally fed to rats in Groups E–H. Group J remained normal. The animals were sacrificed 30 min later by cervical decapitation under anesthetized xylazin and ketamine. Serum was obtained for biochemical analyses.

2.4. Serum biochemical assays

Blood samples were analyzed via biochemical testing at University Malaya Medical Centre, Kuala Lumpur, Malaysia.

2.5. Measurement of gastric juice acidity

The stomach of each rodent was cut open/dissected along the greater curvature. Gastric contents were analyzed for hydrogen ion concentration by pH-meter titration with 0.1 N NaOH solution using digital pH meter. The acid content was expressed as mEq/l (Tan et al., 2000).

2.6. Histological evaluation of gastric lesions

Specimens of the gastric walls from each rodent were fixed in 10% buffered formalin and embedded in paraffin. Sections of the stomach were made at a thickness of 5 μm and stained with hematoxylin and eosin for histological evaluation.

2.7. Gross gastric lesions evaluation

The length (mm) and width (mm) of the ulcer on the gastric mucosa were measured by a planimeter [(10 mm × 10 mm = ulcer area) (Takebayashi et al., 2010)]. The area of each ulcer lesion was measured by counting the number of small squares, 2 mm × 2 mm, covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach were used for the calculation of the ulcer area (UA) wherein the sum of small squares × 4 × 1.8 = UA mm². The inhibition percentage (%) was calculated by the following formula as reported with slight modifications (Abdulla et al., 2009).

\[
\text{The inhibition percentage } (\%) = \frac{\text{UAcontrol} - \text{UAtreated}}{\text{UAcontrol}} \times 100%.
\]

2.8. Measurement of mucus production

The gastric mucosa of each rodent was gently scraped using a glass slide and the mucus obtained was weighed using a precision electronic balance.

2.9. Histochemistry of gastric endothelial mucus

To investigate the mechanism of arbutin in the protection against induced gastric injury, gastric tissues were stained histochemically to assess the mucus content. Formalin fixed and paraffin embedded gastric tissues were sectioned, dewaxed and stained using commercial PAS staining system kit (Sigma Aldrich, Malaysia) according to manufacturer’s directions.
2.10. Role of nitric oxide on the gastroprotective effect of arbutin

The level of nitric oxide in the gastric was evaluated as total nitrate/nitrite levels using the Griess assay (Tsikas et al., 1997). The stomach homogenates in 50 mM potassium phosphate buffer (pH 7.8) were centrifuged at 4000 rpm for 30 min at 4 °C. 50 μL of the Griess reagent (0.1% N-(1-naphthyl) ethylenediaminedihydrochloride, 1% sulphanilamide in 5% phosphoric acid) was added to 50 μL supernatant and mixed. After 10 min the absorbance was measured at 540 nm. The standard curves were obtained by using sodium nitrite. Results were expressed as micromoles nitrate/nitrite per gram of protein.

2.11. Thiobarbituric acid reactive substances assay

To evaluate the level at which arbutin protect against lipid peroxidation induced ulceration, the level of thiobarbituric acid-reactive substances (as indicators of lipid peroxidation) were assessed according to reported procedure (Hodges et al., 1999) with some modifications. The stomach homogenates were mixed with 0.125 ml of a solution containing 26 mM thiobarbituric acid, 0.26 M HCl, 15% trichloric acid and 0.02% butylated hydroxytoluene. The mixtures were heated at 96 °C for 15 min and centrifuged at 4000 rpm for 10 min. The supernatant was transferred to 96-well plate and the absorption was measured at 532 nm. Tetramethoxy propane absorption was used as the standard to estimate the concentration of malondialdehyde.

2.12. Cytokines evaluations

The levels of cytokines (IL-6, IL-10 and TNF-α) in the serum were evaluated using ELISA kits (Ray Biotech, USA) for rodents according to the manufacturer’s instruction. Primary antibodies were first coated on the well plate and after washing. Each well was then blocked to remove the non-specific binding. One hundred microliters of sample or cytokine standards were added to each well and then followed with biotin conjugated secondary antibodies. To obtain a color reaction, Streptavidin-HRP and substrate solution were added. The absorbance was measured at 450 nm with an ELISA reader (TECAN, Switzerland). Standard curves were plotted on each assay plate using recombinant IL-6, IL-10 and TNF-α in serial dilutions.

2.13. Gastric tolerability test

Arbutin treated rodents (Group I) were euthanized and their stomachs excised. The number of lesions was examined under an illuminated magnifier (×3) and assessed according to a modified scoring system of Adamli et al. (2002) (0: no lesions; 0.5: slight hyperaemia or ≤5 petechiae; 1: ≤5 erosions ≤5 mm in length; 1.5: ≤5 erosions ≤5 mm in length and many petechiae; 2: 6–10 erosions ≤5 mm in length; 2.5: 1–5 erosions >5 mm in length; 3: 5–10 erosions >5 mm in length; 3.5: >10 erosions >5 mm in length; 4: 1–3 erosions ≤5 mm in length and 0.5–1 mm in width; 4.5: 4–5 erosions ≤5 mm in length and 0.5–1 mm in width; 5: 1–3 erosions >5 mm in length and 0.5–1 mm in width; 6: 4 or 5 grade 5 lesions; 7: ≥6 grade 5 lesions; 8: complete lesion of the mucosa with haemorrhage).

2.14. In vitro and in vivo toxicity studies

2.14.1. Acute toxicity study in rodents

Adult male and female Sprague Dawley rats (6–8 weeks old; 150–180 g) were obtained from the Experimental Animal House [Ethics No. PM 07/05/2008 MAA (a)K], Faculty of Medicine, University of Malaya. The rodents were given standard pellets food and clean tap water. Thirty six rats were assigned into three groups of twelve rats each (six male and six female rats per group). The rats were divided into control, low dose and high dose groups. The rodents which underwent overnight fasts were dosed with arbutin at 1000 and 2000 mg/kg body weight, and continued fasting for 3–4 h after dosing. The rodents were kept under observation for any clinical, toxicological symptoms or mortality up to 14 days. The rodents were then euthanized on day 15 while all the relevant biochemical parameters analyzed.

2.14.2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell viability assay on human normal hepatic cells (WRL-68)

This colorimetric assay, is based on the conversion of the yellow tetrazolium bromide (MTT) to the purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells (Momsan, 1986), was used to determine any potential cytotoxicity. Human normal hepatic cells (WRL-68) were obtained from American Type Cell Collection (ATCC), maintained in a 37 °C incubator with 5% CO2 saturation and maintained in Dulbecco’s modified Eagle’s medium (DMEM). Medium were supplemented with 10% fetus calf serum (FCS), 100 units/ml penicillin, and 0.1 mg/ml streptomycin. For measurement of cell viability, cells were seeded at a density of 1 × 105 cells/ml in a 96-well plate and incubated for 24 h at 37 °C and 5% CO2. Cells were treated with CMN and incubated for 24 h. After 24 h, MTT solution at 2 mg/ml was added for 1 h. Absorbance was measured at 570 nm. Results were expressed as a percentage of control giving percentage cell viability after 24 h exposure to test agent. The potency of cell growth inhibition for CMN was expressed as an IC50 value, defined as the concentration that caused a 50% loss of cell growth. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells.

2.15. Statistical analysis

All values were reported as mean ± S.E.M. The statistical significance of differences between groups was assessed using one-way ANOVA. A value of P < 0.05 was considered significant.

3. Results

3.1. General observations

There was no difference in food and water consumptions between groups of rats during the entire period of this study. Arbutin did not produce any clinical signs in either ulcer or normal rats. There was also no difference in mean body weight gain among the rodent groups (Table 1).

3.2. In vitro and in vivo toxicity studies

The toxicity studies show no abnormal signs, behavioural changes, body weight changes or macroscopic findings 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 kidney and liver and serum biochemistry revealed no significant differences between control and treatment groups. Data for this experiment are not shown and its available upon request. On the other hand, this natural compound did not show any cytotoxic activities against normal liver cells (WRL-68).

3.3. Gastric acidity and mucus content

Oral administration of ulcerogenic substances in both models produced the lowest content of mucus. Arbutin significantly (P<0.05) and dose dependently increased the gastric mucus content, as compared to ulcer control groups in both models. Animals
Table 1: Protective effects of arbutin on ethanol- and aspirin-induced ulceration and mean body weights.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Pretreatment (5 ml/kg)</th>
<th>pH</th>
<th>Mucus</th>
<th>Ulcer index (FA)</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CMC (ethanol induced) control</td>
<td>2.84 ± 0.94</td>
<td>0.82 ± 0.15</td>
<td>934.2 ± 5.4</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>Omeprazole + ethanol</td>
<td>6.84 ± 0.72</td>
<td>1.57 ± 0.02</td>
<td>178 ± 6.16</td>
<td>79.7</td>
</tr>
<tr>
<td>C</td>
<td>Arbutin 30 mg/kg + ethanol</td>
<td>6.55 ± 0.81</td>
<td>1.44 ± 0.47</td>
<td>360 ± 10.4</td>
<td>63.4</td>
</tr>
<tr>
<td>D</td>
<td>Arbutin 60 mg/kg + ethanol</td>
<td>6.85 ± 0.47</td>
<td>2.08 ± 0.1</td>
<td>288 ± 10.6</td>
<td>70.7</td>
</tr>
<tr>
<td>E</td>
<td>CMC (aspirin induced) control</td>
<td>2.54 ± 0.91</td>
<td>0.80 ± 0.20</td>
<td>821 ± 10.2</td>
<td>NA</td>
</tr>
<tr>
<td>F</td>
<td>Omeprazole + aspirin</td>
<td>6.79 ± 0.11</td>
<td>0.57 ± 0.02</td>
<td>198 ± 9.6</td>
<td>75.7</td>
</tr>
<tr>
<td>G</td>
<td>Arbutin 30 mg/kg + aspirin</td>
<td>6.57 ± 0.40</td>
<td>1.94 ± 0.3</td>
<td>296 ± 8.4</td>
<td>63.8</td>
</tr>
<tr>
<td>H</td>
<td>Arbutin 60 mg/kg + aspirin</td>
<td>6.97 ± 0.30</td>
<td>2.83 ± 0.3</td>
<td>245 ± 13.8</td>
<td>69.1</td>
</tr>
<tr>
<td>I</td>
<td>Arbutin alone (60 mg/kg)</td>
<td>6.08 ± 0.47</td>
<td>0.67 ± 0.02</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>Normal control</td>
<td>7.05 ± 0.65</td>
<td>2.93 ± 0.2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NA, not applicable; UA, ulcer area; I, ulcer index.

Groups with different alphabets (a–c) are statistically significantly different at P < 0.05.

Pre-treated with arbutin and omeprazole significantly (P < 0.05) showed an increased pH of the gastric contents. Table 1 shows that there was a significant (P < 0.05) reduction in the index of ulcers at doses of 30 and 60 mg/kg body weight of the compound arbutin in both aspirin and ethanol-induced gastric ulceration (Fig. 1).

3.4. Biochemical, hematological, immunological parameters

Rodents with induced ulcers showed remarkable increases in liver enzymes (AST and ALT) and high density lipoprotein as shown in Table 2. However, when these rats were pre-treated with 30 and 60 mg/kg body weight of arbutin, the serum concentrations of these markers remained unchanged. There was no difference in concentrations of these markers between negative and arbutin-control rats. The serum C-reactive protein concentrations was observed to be markedly low (P < 0.05) in arbutin-treated than in non-treated rats (Table 2).

3.5. Histomorphological assessment

The histological analysis results showed that rats pre-treated with omeprazole (Fig. 2B) and arbutin (Fig. 2CD, G and H) had considerably reduced areas of gastric ulcer formation compared to rats pre-treated with only CMC (ulcer control groups, Fig. 2A and E). Moreover, flattening of gastric mucosal folds was observed in the treated rats. The rats pre-treated with only CMC showed comparatively more extensive damage to the gastric mucosa, oedema and leucocytes infiltration of the submucosal layer (Fig. 3A and E) whereby the rats pre-treated with omeprazole (Fig. 3B and F) and/or arbutin (Fig. 3C, D, G and H) had comparatively better protection of the gastric mucosa as seen by reduction in ulcer area, reduced or absence of submucosal edema and leucocytes infiltration. Arbutin has been shown to exert gastroprotective effects in a dose-dependent manner.

3.6. Effects of arbutin on lipid peroxidation

Rats treated with ethanol or aspirin showed marked (P < 0.05) higher stomach MDA levels than all groups (Table 3). The stomach MDA level was significantly (P < 0.05) lower in the rats pre-treated with arbutin than those not treated.

3.7. Nitric oxide level

The level of nitric oxide in the gastric was assessed using the Griess reagent and expressed as total nitrate/nitrite (Table 3). The fundus parts of the stomach of animals in Group A (ethanol-induced ulcer) and E (aspirin-induced ulcer) showed the lowest level of nitric oxide. Administration of omeprazole to the animals in Groups B and F and arbutin to the animals in Groups C, D, G and H revealed insignificant difference (P > 0.05) as compared to the animals in Group J (normal control rats).

3.8. Mucus staining

The PAS staining was used to detect the level of glycogen in control and ulcer-induced animals. Rats pretreated with omeprazole and/or arbutin had high tendency to restore the mucin in the glandular when compared with ethanol-induced ulcerated animals. This high tendency is evidenced by the accumulation of the magenta color in mucosal cells layer (Fig. 4). However, this magenta staining of PAS was not observed abundantly in stomach of the animals induced with ethanol or aspirin.

3.9. Effect of arbutin on serum cytokines

Serum cytokine level (Table 3) were evaluated to check the level of pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory (IL-10) in the rats exposed to ethanol or aspirin induced ulceration. The serum level of TNF-α and IL-6 were amplified in the ulcerated rats relative to the normal group. However, this rise was not observed in the rats pretreated with omeprazole and arbutin instead, an increase in the level of IL-10 was recorded showing anti-inflammatory activity of arbutin against necrotizing effects of ethanol.

3.10. Gastric tolerability

Arbutin when administered to the animals in Group I did not produce any significant gastric lesions. The changes were observed in the range of 0–1 according to the Adami’s scoring scale. Namely,
Table 2
Effects of arbutin on liver function tests and c-reactive protein.

<table>
<thead>
<tr>
<th>Animal groups†</th>
<th>Pretreatment (5 ml/kg)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CMC (ethanol induced) control</td>
<td>60.2 ± 1.8a</td>
<td>399 ± 6.6a</td>
<td>0.19 ± 0.2a</td>
</tr>
<tr>
<td>B</td>
<td>Omeprazole + ethanol</td>
<td>51.4 ± 1.8b</td>
<td>301 ± 5.3b</td>
<td>0.23 ± 0.1b</td>
</tr>
<tr>
<td>C</td>
<td>Arbutin 30 mg/kg + ethanol</td>
<td>48.1 ± 1.2b</td>
<td>312 ± 8.6b</td>
<td>0.23 ± 0.2b</td>
</tr>
<tr>
<td>D</td>
<td>Arbutin 60 mg/kg + ethanol</td>
<td>49.1 ± 1.6b</td>
<td>294 ± 5.4b</td>
<td>0.26 ± 0.1b</td>
</tr>
<tr>
<td>E</td>
<td>CMC (aspirin induced) control</td>
<td>65.1 ± 1.4a</td>
<td>401 ± 10.1a</td>
<td>0.18 ± 0.2a</td>
</tr>
<tr>
<td>F</td>
<td>Omeprazole + aspirin</td>
<td>51.4 ± 1.8b</td>
<td>298 ± 5.7b</td>
<td>0.25 ± 0.1b</td>
</tr>
<tr>
<td>G</td>
<td>Arbutin 30 mg/kg + aspirin</td>
<td>47.1 ± 2.2b</td>
<td>303 ± 6.8b</td>
<td>0.23 ± 0.2b</td>
</tr>
<tr>
<td>H</td>
<td>Arbutin 60 mg/kg + aspirin</td>
<td>47.3 ± 3.1b</td>
<td>281 ± 4.1b</td>
<td>0.26 ± 0.5b</td>
</tr>
<tr>
<td>I</td>
<td>Arbutin alone (60 mg/kg)</td>
<td>51.8 ± 1.6b</td>
<td>283 ± 10.1b</td>
<td>0.24 ± 0.3b</td>
</tr>
<tr>
<td>J</td>
<td>Normal control</td>
<td>48.8 ± 2.2b</td>
<td>289 ± 8.6b</td>
<td>0.28 ± 0.4b</td>
</tr>
</tbody>
</table>

* Groups with different alphabets (a–c) are statistically significantly different at P<0.05.

Fig. 2. Gross evaluation. Results showed that rats pre-treated with arbutin at doses of 30 and 60 mg/kg (D, C, G and H) and omeprazole (B and F) had considerably reduced areas of gastric ulcer formation compared to rats pre-treated with only CMC [ulcer control groups, A (ethanol) and E (aspirin)].
Fig. 3. Histopathological evaluation. Results showed that rats pre-treated with arbutin at doses of 30 and 60 mg/kg [(2D and 2 C for ethanol model) and (Fig. 2G and H for aspirin model)] and omeprazole (Fig. 2B and F, for ethanol and aspirin models, respectively) improved the histopathology of rat stomach compared to rats pre-treated with only CMC (ulcer control groups, Fig. 2A and E). Ulcer induced groups (Fig. 2A and E) showed severe disruption to the epithelium surface and deep mucosa, while white arrow indicates leukocyte infiltration and edema in the submucosa layer (H and E stain; 10×).
only slight hyperaemia or few petechiae were registered in rat stomach regardless of given dose.

4. Discussion

In this study, we used successfully aspirin and ethanol as inducers for gastro-ulceration in rodents, in order to assess the antulcerogenic properties of arbutin. This major glycosylated hydroquinone from Turnera diffusa significantly and dose-dependently protects the gastric mucosa in both models. Arbutin has been reported to possess antioxidant activity (Takebayashi et al., 2010; Tai et al., 2011). However, its antioxidant property has not been in vivo evaluated. Hence, this study assessed its activity using two animal models. The present study also revealed that arbutin did not show any significant changes in weight, toxic effects or ulcerogenic activity when administered orally to the normal rats. On the other hand, this natural compound did not show any in vitro cytotoxic activities against normal liver cells (WRL-68).

To define the side effect of arbutin on other organs, liver function tests were studied. In our study, ulcerogenic animals showed serious increase of serum AST and ALT measured as indicators of hepatic injury since elevated levels of these hepatic enzymes are indicators of chemically triggered tissue injury. Administration of arbutin normalized the levels of AST and ALT, which shows its action in preventing the acute tissue damage. In agreement with these findings, a previous report has also demonstrated that arbutin-containing plants could protect against galactosamine-induced liver injury in rats (Myagmar et al., 2004).

The defence ability of gastric mucosa relies exclusively on balance between the aggressive and the protective factors (El-Missiry et al., 2001; Carr et al., 2009). The compound arbutin has protected mucosal membrane, enhances mucus secretion, level of NO, IL-10 and inhibits acid secretion to fix the inflammation and mucosal erosion caused by ethanol or aspirin (Jahovic et al., 2007; Choi et al., 2010). Ethanol effectively reduces the level of NO in gastric mucosa and flow of gastric blood, thereby contributing to the development of hemorrhagic necrosis which consequently leads to the solubilisation of gastric mucus constituents. These actions result in an increased flow of Na+ and K+, increased pepsin secretion, loss of H+ ions and histamine into the lumen (Jahovic et al., 2007; Abdulla et al., 2009; Goswami et al., 2011). Arbutin did not affect activities of the inflammatory mediator NO, which was reported to inhibit neutrophil infiltration. The role of NO in the protective effect of

---

**Table 3**

Effects of arbutin on lipid peroxidation, nitric oxide, IL-6, IL-10 and tumor necrosis factor alpha on ulcerated rats.

<table>
<thead>
<tr>
<th>Animal groups†</th>
<th>Pretreatment (5 ml/kg)</th>
<th>Malondialdehyde (μmol/g tissue)</th>
<th>Nitric oxide (μmol)</th>
<th>Tumor necrosis factor alpha (pg/mg of stomach tissue)</th>
<th>Interleukin-6 (pg/mg of stomach tissue)</th>
<th>Interleukin-10 (pg/mg of stomach tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CMC (ethanol induced) control</td>
<td>31 ± 4.1b</td>
<td>5.2 ± 0.30b</td>
<td>595 ± 21b</td>
<td>82 ± 1.7b</td>
<td>84 ± 5.2b</td>
</tr>
<tr>
<td>B</td>
<td>Omeprazole + ethanol</td>
<td>15.5 ± 1.5b</td>
<td>9.2 ± 1.40b</td>
<td>115 ± 1.5b</td>
<td>10.4 ± 0.9b</td>
<td>249 ± 5.7b</td>
</tr>
<tr>
<td>C</td>
<td>Arbutin 30 mg/kg + ethanol</td>
<td>12 ± 1.23c</td>
<td>7.1 ± 0.90b</td>
<td>65 ± 2.5c</td>
<td>5.1 ± 0.5c</td>
<td>310 ± 4.9f</td>
</tr>
<tr>
<td>D</td>
<td>Arbutin 60 mg/kg + ethanol</td>
<td>9.1 ± 0.56c</td>
<td>9.5 ± 0.85c</td>
<td>10.5 ± 0.5d</td>
<td>3.5 ± 0.6d</td>
<td>389 ± 21c</td>
</tr>
<tr>
<td>E</td>
<td>CMC (aspirin induced) control</td>
<td>29.1 ± 2.0b</td>
<td>4.1 ± 1.20b</td>
<td>534 ± 16b</td>
<td>75 ± 4.1</td>
<td>78 ± 4.1</td>
</tr>
<tr>
<td>F</td>
<td>Omeprazole + aspirin</td>
<td>14.98 ± 2.67b</td>
<td>8.8 ± 1.2b</td>
<td>80 ± 1.5b</td>
<td>9.4 ± 1.9b</td>
<td>251 ± 0.45b</td>
</tr>
<tr>
<td>G</td>
<td>Arbutin 30 mg/kg + aspirin</td>
<td>15.5 ± 2.8b</td>
<td>7.2 ± 0.40b</td>
<td>15 ± 4.5d</td>
<td>6.6 ± 0.2e</td>
<td>298 ± 1.2d</td>
</tr>
<tr>
<td>H</td>
<td>Arbutin 60 mg/kg + aspirin</td>
<td>12 ± 0.20b</td>
<td>9.1 ± 0.90c</td>
<td>10.2 ± 0.4d</td>
<td>4.5 ± 0.6d</td>
<td>384 ± 3.6d</td>
</tr>
<tr>
<td>I</td>
<td>Arbutin alone (60 mg/kg)</td>
<td>10.1 ± 0.46c</td>
<td>8.5 ± 0.85c</td>
<td>10.5 ± 0.9d</td>
<td>4.3 ± 0.2d</td>
<td>387 ± 21.4d</td>
</tr>
<tr>
<td>J</td>
<td>Normal control</td>
<td>9.5 ± 1.5</td>
<td>9.6 ± 0.9</td>
<td>9 ± 2.5d</td>
<td>4.1 ± 0.1d</td>
<td>394 ± 10.7d</td>
</tr>
</tbody>
</table>

*Groups with different alphabets (a–d) are statistically significantly different at P<0.05.

---

Fig. 4. Effect of arbutin on gastric tissue glycogen-PAS staining in rats’ stomach subjected to ethanol- or aspirin induced ulcer after treatment: (A) ethanol-induced ulcer; (B) aspirin-induced ulcer; (C) normal stomach; (D) ethanol-induced ulcer treated with arbutin (60 mg/kg); (E) aspirin-induced ulcer treated with arbutin. H and E (100×).
potential anti-ulcer agents in ethanol-induced gastric ulcer mice was reported (Lundberg et al., 2008). Besides NO contribution, the current study also investigated the role of the effects of arbutin in the levels of cytokines (IL-6 and TNF-α and IL-10). Therefore, the current study, demonstrates the immunomodulatory activity of the compound.

Pre-administration of arbutin to ethanol or aspirin induced rats led to the flattening of mucosal folds which suggests the gastroprotective effect of this natural compound. Moreover, histopathological evaluation revealed the gastroprotective (Fig. 2) activities of arbutin by inhibition of leukocytes infiltration of gastric wall which promotes the ulcer healing through suppression of neutrophil infiltration and inflammation (Lundberg et al., 2008; Abdulla et al., 2009). Literature report has shown that potential anti-ulcer drugs can exert their protective effect against mucosal lesions through inhibition of neutrophil infiltration in the ulcerated gastric tissue. Furthermore, neutrophil accumulation in gastric mucosa has been shown to induce microcirculatory abnormalities (Watanabe et al., 1997). The present study also observed that arbutin was able to inhibit the level of pro-inflammatory mediator (TNF-α). Suppression of TNF-α production and neutrophil infiltration are very much associated. Similar results were reported on plant-based extracts against experimentally induced ulceration (Shimizu et al., 2000). This anti-inflammatory activity could also be a key factor in the prevention of gastric ulcer as reported earlier (Abdulla et al., 2010).

To further corroborate the mechanism of action of the compound arbutin, MDA level was evaluated and found that it is appreciably reduced, suggesting the facility of arbutin to prevent the formation of ROS and consequently the lipid peroxidation of gastric mucosa. The level of these harmful factors was measured using thiobarbituric acid reactive substances assay (TBARS) and found that this natural compound has considerably reduced the free radical formation and inhibited lipid peroxidation (Abdelwahab et al., 2011; Lavania et al., 2011). Takebayashi et al. (2010) reported that arbutin possesses considerable antioxidant activities when they used different chemically- and cell-based antioxidant assays.

In conclusion, arbutin notably and dose-dependently protected the gastric mucosa against ethanol- and aspirin-induced injury. Interestingly, arbutin did not interfere with the natural release of NO. The current findings revealed that experimentally induced ulceration has an effect on liver enzymes AST and ALT causing alcoholic hepatitis which leads to the immune response as observed by the rise in C-reactive proteins leading to a serious inflammation (Calabrese et al., 2004). This was further indicated by the rise in the level of interleukin IL-6 and tumor necrosis factor TNF-α. In contrast, the compound arbutin enhances the immune system by rising the secretion of interleukin IL-10 and HDL to reverse cholesterol transport, reserve NO and inhibit lipid peroxidation. However, this study warrants further research on arbutin as a possible therapeutic agent for gastric ulcer of various aetiologies.

Conflict of interest
None.

Acknowledgements
The authors would like to express their utmost gratitude and appreciation to University of Malaya (HIR grant: F00009-21001) for providing grant to conduct this study.

References


