The gastro protective effects of *Cibotium barometz* hair on ethanol-induced gastric ulcer in Sprague-Dawley rats

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**Abstract**

**Background:** *Cibotium barometz* is a medical herb used traditionally in the Malaysian peninsula for several ailments, including gastric ulcer. The aim of this study was assessment the anti-ulcer effects of *C. barometz* hair on ethanol-induced stomach hemorrhagic abrasions in animals. Seven groups of Sprague Dawley (SD) rats were administered 10% Tween 20 in the normal control and ulcer control groups, and omeprazole 20 mg/kg and 62.5, 125, 250, and 500 mg/kg of *C. barometz* hair extract in the experimental groups. After 60 min, the normal control group of rats was orally administered 10% Tween 20, while absolute ethanol was orally administered to the groups of ulcer control, omeprazole and experimental groups. Stomachs of the rats were examined macroscopically and histologically. Homogenates of stomachs were used to evaluate endogenous antioxidant enzyme activities.

**Results:** Rats pre-fed with plant extract presented a significant decrease in the sore area, increased pH of gastric contents and preserved stomach wall mucus compared to the ulcer group. Histologically, rats pre-fed with *C. barometz* hair extract showed mild to moderate disruptions of the surface epithelium while animals pre-fed with absolute ethanol showed severe disruptions of the stomach epithelium with edema and leucocyte penetration of the submucosal layer. A Periodic acid Schiff (PAS) staining revealed that each rat pre-treated with the plant extract displayed an intense uptake of stomach epithelial glycoprotein magenta color compared to the ulcer control group. Immunohistochemical analysis revealed that rats pre-fed with the plant extract showed an up-regulation of the heat shock protein 70 (HSP70) and down-regulation of Bax proteins compared to ulcer control rats. Homogenates of the stomach tissue demonstrated significant increases in the endogenous antioxidant enzymatic activity and decreased lipid peroxidation (MDA) in rats pre-treated with *C. barometz* hair extract compared with the ulcer control rats. In acute toxicity, the liver and kidney revealed no hepatotoxic or nephrotoxic effects histologically.

**Conclusions:** The gastric cytoprotective action of *C. barometz* hair extract might be attributed to antioxidants, an increase in gastric pH, stomach mucus preservation, increased endogenous antioxidant enzymes, decreased lipid peroxidation, up-regulation of HSP70 and down-regulation of Bax proteins.
Background

Stomach ulcers are the common gastrointestinal and global disorders [1]. It occurs mainly due to the imbalance between the destructive and offensive factors of the mucosal barrier [2]. The destructive factors include stomach hydrochloric acid (HCl), mucosal hypoperfusion, free oxygen radicals, ethanol, Helicobacter pylori and excessive ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) that promote the gastric mucosal injury and contribute to gastric ulceration [3].

The prevention or treatment of gastric ulcers is a medical challenge [4]. Gastric ulcer therapy has major disadvantages, including limited efficacy of drugs against the gastric illness and severe side effects [5]. Therefore, medicinal plants may be a viable alternative therapy that has fewer side effects and contains a wide variety of antioxidants. Medicinal plants are also promising alternative medications for the development of new drugs to control gastrointestinal diseases, which has been reviewed extensively in the literature [2, 6–12].

Cibotium barometz hair (family Dicksoniaceae) is known traditionally as “golden hair dog fern”. Cibotium is a tropical native medicinal plant in the Malaysian Peninsula and parts of China. It is an anti-inflammatory plant and is used in contradiction of rheumatic and menstruation problems, as well as herniated discs and hyperostoeogeny [13, 14]. There are many phenolic compounds in Cibotium that are potent antioxidants and strong chelators. The rhizomes are comprised of approximately β-sitosterol, caffeic acid, daucosterol, alternariol, 5-hydroxymethyl-2-furancarbonaldehyde, (3R)-des-O-methyl lasiodipolidon, protocatechuic aldehyde, (24R)-stigmast-4-ene-3-one, onitin, 24-methyleneoclorotanol, protocatechuic acid, n-butyl-β-d-fructopyranoside, palmitic acid, 1-monopalmitin, d-glucose and 30% starch [15]. It has been confirmed that C. barometz obstructs osteoclast creation with no effects on cell viability [16]. The hair of Cibotium is a staple ingredient in the ointments that are applied in natural treatments, such as to stop bleeding [17]. The objective of our study was to evaluate the stomach-protective effect of C. barometz hair ethanol extracts on ethanol-induced stomach ulcers in rats.

Results

Antulcer study

Gross estimations

The results that pre-treated with C. barometz hair ethanol extract of male SD rats shown significant reduced of the ulcer area with compared to the ulcer groups (Table 1, Fig. 1) at four different doses in the pre-treated animals groups (62.5 mg/kg, 125 mg/kg, 250 mg/kg, 500 mg/kg) that were induced by ethanol 95%. However, the significant inhibition percentage of the ulcer area increased in rats pre-treated with C. barometz hair at doses of 62.5 mg/kg, 250 mg/kg, 125 mg/kg, and 500 mg/kg by 68.5%, 74.7%, 75.6%, and 78.5% respectively.

Gastric mucus content and acidity

The outcomes that shown in Table 1, the ulcerated SD rat group produced the lowest gastric mucosa mucus content, while animal groups pre-treated with G7 (500 mg/kg) and G6 (250 mg/kg) of C. barometz hair exhibited significant increasing in the mucus weight (g) with respect to G2 (ulcer control rats). However, pre-treatment with C. barometz hair (G4 to G7) produced a significant increase in the pH of the stomach contents compared to the ulcer control rats (G2).

Histological protocol of evaluation the stomach injuries

Hematoxylin and eosin and Periodic Acid Schiff (PAS) stainings

Histology revealed comprehensive damage to the stomach mucosa in the ulcer control rats. Moreover, the ulcerated rat control group had necrotic lesions in the deep gastric mucosa that demonstrated extensive leucocyte infiltration and edema of the submucosal layer, as shown in Fig. 2. Conversely, the animals pre-fed with C. barometz hair extract in the G4 - G7 groups presented relatively enhanced protection of the stomach mucosa with a depression in or lack of infiltration of leucocytes.

Table 1  Effect of the C. barometz hair extracts on the mucus weight, pH of stomach content, ulcer area, and % inhibition of ulcer area in the stomach

| Animal groups | No group | Pre-treatment 5 mL/kg | Mucus weights (g) | PH (acidity) | Ulcer area (mm²) | Inhibition % |
|---------------|----------|-----------------------|-------------------|--------------|-----------------|--------------|
| Normal control | G1       | 10% Tween20          | 2.28 ± 0.37       | 7.16 ± 0.94  | -               | -            |
| Ulcer control  | G2       | 10% Tween20          | 0.75 ± 0.37       | 2.76 ± 0.69  | 802.71 ± 87.32 | -            |
| Omeprazole     | G3       | 20 mg/kg             | 1.92 ± 0.15       | 5.71 ± 1.20  | 95.71 ± 50.02 | 88.1%        |
| C. barometz hair extract | G4       | (62.5 mg/kg)         | 1.86 ± 0.27       | 4.27 ± 0.91  | 253.20 ± 74.05 | 68.5%        |
|                | G5       | (125 mg/kg)          | 1.87 ± 0.12       | 4.52 ± 1.15  | 202.80 ± 32.01 | 74.7%        |
|                | G6       | (250 mg/kg)          | 2.00 ± 0.57       | 5.12 ± 1.35  | 195.60 ± 34.50 | 75.6%        |
|                | G7       | (500 mg/kg)          | 2.17 ± 0.51       | 4.56 ± 1.26  | 172.80 ± 53.10 | 78.5%        |

The values are expressed as the mean ± SEM. Indicates significance at p < 0.05 compared to the ulcerated group
Fig. 1 The effect of *C. barometz* hair on the macroscopic appearance of the stomach mucosa in alcohol-induced stomach mucosal injuries in male SD rats. G1 (Normal control group) exhibited no injuries to the gastric mucosa, G2 (Ulcer control group) had severe injuries to the stomach mucosa, G3 (Omeprazole) showed mild disruptions of the surface epithelium in the gastric mucosa. G4 (62.5 mg/kg), G5 (125 mg/kg), G6 (250 mg/kg), G7 (500 mg/kg) doses of *C. barometz* hair extract had moderate to mild disruptions of the surface epithelium in the gastric mucosa in a dose-dependent manner. Black arrow points to the hemorrhagic bands.

Fig. 2 The effect of *C. barometz* on the histology (H and E staining) of ethanol-induced stomach mucosa damage in male SD rats. G1 (Normal control group) had intact surface mucosal epithelium with no lesions; G2 (Ulcer control group) had a severe distraction of the surface epithelium and necrotic lesions; G3 (Omeprazole) had a mild distraction of the surface epithelium and reduction in the submucosal edema with inflammatory cells infiltration. The animals pretreated with *C. barometz* extract in G4 (62.5 mg/kg), G5 (125 mg/kg), G6 (250 mg/kg) and G7 (500 mg/kg) groups revealed a moderate to mild disruption of the surface epithelium, a reduction in submucosal edema and inflammatory cells infiltration in a dose-dependent manner as shown by lowering in or absence of the ulcer area (white arrow), submucosal edema and inflammatory cells infiltration (blue arrow). (Scale bar = 100 μm)
and edema (Fig. 2). C. barometz hair extracts revealed defending effects in a dose-dependent manner and showed remarkably improved protection of the stomach epithelium. The gastric mucosa in the pre-treated experimental groups, depending on the dose, exhibited a gradual increase in PAS staining intensity, indicated by the accumulation of the magenta color in the mucosal cell layer compared to the ulcer control group (Fig. 3). Additionally, this magenta staining was reduced and was not plentiful in the gastric mucosa of the ulcer group where the ulcer was induced with ethanol.

**Immunohistochemistry**

In the gastric mucosa, the expression of the HSP70 protein was down-regulated in the ulcerated group (G2), but significantly up-regulated in the animals pre-treated with omeprazole (G3) and with C. barometz hair extract (G4 to G7), as displayed in Fig. 4. Additionally, the immunohistochemical staining of the Bax proteins (Fig. 5) in the gastric mucosa revealed up-regulation in the ulcerated group while a significant down-regulation was demonstrated in rats pre-treated with C. barometz extract.

**Measurement of antioxidant enzymes and membrane lipid peroxidation (MDA) of stomach**

A significant reduction was observed in endogenous antioxidants enzymes (SOD, CAT and GPx) activities for the ulcer group of male SD rats. However, the rats pre-treated with C. barometz hair displayed an elevation of all antioxidant activities with respect to the (G2) ulcer group as shown in Figs. 6a, b, and c. The SOD enzyme activities were significantly higher at doses for G5 of C. barometz than G2 (Fig. 6a). The CAT enzyme activities illustrate the significant increases in G4, G5, G6 groups compared to G2 as shown in Fig. 6b. The GPx enzyme activities for gastric mucosal homogenates revealed significant increasing in the rats pre-fed with four doses of C. barometz hair ethanol extract with respect to G2 as shown in Fig. 6c. Additionally, the MDA level of C. barometz hair extract in G4, G5, G6 and G7 were significant lower than in the G2 ulcerated control group as seen in Fig. 6d.

**Acute toxicity test of C. barometz hair**

All SD rats that were treated with C. barometz hair ethanol extract demonstrated no mortality and toxic signs in the experiment. There were no signs of hepatotoxic or nephrotoxic effects, which were evaluated histologically and biochemically (Fig. 7). Additionally, there were no body weight variations or abnormal physiological or behavioral changes at 2 g/kg and 5 g/kg doses during the 14 days, compared to the control group that was given 10% Tween 20.

**Antioxidants activities of ethanol extract of C. barometz hair**

**Ferric reducing antioxidant power (FRAP) Test**

C. barometz hair antioxidant activity in vitro was measured totally using the FRAP test. Fig. 8 revealed the...
decline of ferric to ferrous ions that indicated a higher FRAP value for C. barometz hair (756.0 ± 0.038 μmol Fe (II)/g) than BHT (261.0 ± 0.015 μmol Fe (II)/g) and ascorbic acid (457.7 ± 0.009 μmol Fe (II)/g). However, the value was less than for quercetin (1544.3 ± 0.021 μmol Fe (II)/g) and gallic acid (1774.3 ± 0.003 μmol Fe (II)/g) standards.

The Scavenging of diphenyl - picrylhydrazyl radical activity (DPPH) test
C. barometz hair scavenging of DPPH free radicals was assessed using the DPPH test. Fig. 9 demonstrated the inhibition% of the DPPH free-radical scavenging activity of C. barometz hair that was 54.8% with an IC_{50} value of 45.6 ± 0.038 μg/mL. It was associated to the standards BHT, ascorbic acid, quercetin and gallic acid. The % inhibition of DPPH free-radicals scavenging activities of the standards was 51.63, 64.11, 87.52, and 55.47% with an IC_{50} value of 9.1 ± 0.01 μg/mL, 4.9 ± 0.02 μg/mL, 1.8 ± 0.001 μg/mL, 1.4 ± 0.02 μg/mL, respectively.

Discussion
Traditional medicine has become an important type of alternative medicine in many countries. Several medicinal plants have been used to protect the stomach from a number of ulcerative agents. One type of medicinal plant is C. barometz leaves, which are used to stop bleeding [13]. In this study, the results of acute toxicity experiment revealed no signs of toxicity or mortality in vivo and no variations in kidney and liver on
biochemical investigation. Additionally, no differences in body weights were observed for both sex SD rats. These findings corroborate previous studies [18–20]. Our data suggest that C. barometz hair extract has high free-radicals scavengings and antioxidant activities in vitro. Our results agree with a previous report that indicated that the consumption of medicinal plants that contain natural antioxidants can reduce free radicals and protect biological molecules from oxidative injury [21]. C. barometz hair is one of the medicinal plants that have medicinal properties and natural antioxidants [17]. C. barometz hair displays good free-radical scavenging activity and ferric-decreasing antioxidant power in a Fe³⁺-dependent hydroxyl-radical generation assay [22]. This outcome agrees with earlier studies of C. barometz, in which it showed a greater antioxidant activity [23]. Our data indicate that significant protection of stomach wall mucosa and decreases in ulcer area are observed in rats pre-fed with C. barometz extract. Our observation is consistent with the findings of several other studies [4, 12, 24].

We also observed a significant elevation of stomach mucus content and pH in rats pre-fed with C. barometz hair extract compared to category of the ulcer control rats. This shows the extracted plant of C. barometz is able to protect the mucosal layer of the stomach from destructive causes. These results are similar to those previously reported by others [25–30]. A severe distraction of the gastric mucosa layer, edema and leucocyte penetration of submucosal layers were apparent in the ulcer control rats compared with rats pre-fed with C. barometz hair extract. The rats treated with C. barometz hair extract displayed a mild distraction of stomach epithelium and reduced edema and inflammatory cell penetration of the submucosal layers. Similarly, many studies have also observed mild distraction of stomach mucosa, mild edema and penetration of inflammatory cells in the submucosal layers [31–35]. Additionally, the condensing of the submucosal layer was detected in the ulcerated group where there was edema and hemorrhagic abrasions in the mucosal layer, which is an indicator of ethanol damage. Absolute ethanol harshly damages the stomach glandular epithelium leading to augmented neutrophils access into the disrupted stomach mucosa. The presence of oxygen free radicals resulting from the penetration of neutrophils in the wounded gastric wall can damage stomach gastric mucosa in rats [34, 36]. Neutrophils are the main sources of inflammatory mediators and can release the potent reactive oxygen species, which are cytotoxic and can promote tissue injury [37]. Furthermore, neutrophils that congregate in the stomach mucosa have been exposed to irritation through the microcirculatory defect [38]. The present study illustrated strong staining of glycoprotein discharge of stomach glandular epithelium in rats pre-fed with C. barometz extract. Mucus released from the stomach epithelium performs one of the vital mechanism of stomach mucosal barrier resistance against absolute ethanol [39, 40]. Mucus and bicarbonate liberation may have a considerable role in ulcer development because the mucus/bicarbonate layers guards newly produced...
Fig. 8 FRAP test for total antioxidant activity evaluation of *C. barometz* hair extract with reference synthetic standards (BHT, ascorbic acid, quercetin, and gallic acid) were determined. All values are in triplicate and represented as the mean ± SEM.

Fig. 7 Histological slides of the liver and kidney sections for the acute toxicity experiment. Animals treated with 5 mL/kg of the vehicle (10% Tween 20) *a* and *b*. Animals treated with 2000 mg/kg (2 mL/kg) of the *C. barometz* hair extract *c* and *d*. Animals treated with 5000 mg/kg (5 mL/kg) of the *C. barometz* hair extract *e* and *f*. No significant changes were observed in the structures of the liver and kidney among the treated and control groups (hematoxylin and eosin stain; scale bar = 100 μm).
cells from acidic and peptic damage [9, 41, 42]. The amplified production of mucus in the present work was an indicator of local mucosal defense in the stomach and can be described as the possible cytoprotective mechanism. Numerous studies have suggested that the gastroprotective defense is due to increased mucosal resistance, and a decline in aggressive factors, primarily acid and pepsin [43–45].

The consequences of the present immunohistochemistry experiments indicate that rats pre-fed with *C. barometz* hair displayed an up-regulation of HSP70 proteins that contributed to the protection of stomach cells from heat shock, oxidative stress, and down-regulation of the Bax proteins. Earlier studies reported that there was up-regulation of HSP70 protein in animals fed with plant extracts [28, 34]. However, the down-regulation of Heat shock protein expression appeared in the ulcer control animals as a gastric injury marker. This is consistent with the results from other studies [20, 46]. Up-regulation of HSP70 protein in the stomach wall mucosa in animals pre-fed with *C. barometz* hair extract may stop the initiation of damage to the gastric epithelium upon exposure to absolute alcohol [23, 32]. The HSP70 protein protected mitochondria and delayed a stress-induced apoptotic programmer [47]. Our data suggest that Bax immunostaining confirmed that rats fed with *C. barometz* hair extract displayed increased expression of the Bax protein. Thus, these results demonstrate that *C. barometz* hair extract is a protective agent and prevents alcohol-induced damage to the rat stomach, which is associated with decreased expression of the Bax protein. Similarly, many amendments have been described by many investigators [18, 48]. Animals pre-fed with *C. barometz* hair extract displayed decreased expression of the Bax protein. On the other hand, up-regulation of Bax was observed in the ulcer control group. Bax is a key protein that is linked to apoptosis during mitochondrial damage and plays a significant role in the disruption of stomach mucosal integrity that is observed after ethanol administration [11, 12].

The results from the present work demonstrated that rats pre-fed with *C. barometz* displayed an increase in the activity of endogenous antioxidant enzymes (SOD, GPx, CAT) and a decrease in MDA levels in the stomach compared to the ulcer control rats. These findings are consistent with observations from other studies [7, 32]. Therefore, the elevation of SOD and GPx enzymatic activities leads to increased scavenging of superoxides, hydrogen peroxide, hydroxyl and lipid peroxyl radicals, resulting in a reduction in tissue damage [9, 49]. Additionally, increases in CAT enzymatic antioxidant activity rapidly converted the peroxyl radicals into biologically safe substance, such as water [1]. In contrast, the low levels of MDA reduced lipid peroxidation and ROS (reactive oxygen species) which are products of oxidative gastric damage [8]. Taken together, our observations suggest that *C. barometz* hair facilitates gastric mucosa protection due to its scavenging of free radicals and anti-inflammatory effects [5, 6].

**Conclusion**

Our study demonstrates that *C. barometz* hair has beneficial and dose-dependent anti-ulcer effects against ethanol-induced acute stomach hemorrhagic injuries in SD rats. The gastro-protective effects of this effective medicinal plant could be associated with free radicals scavenging activities, elevated levels of pH and gastric mucus glycoprotein, increases in the cellular endogenous enzymes activity of SOD, CAT and GPx antioxidants, and a reduction in MDA levels. Additionally, these
effects may be due to HSP70 up-regulation and Bax protein down-regulation. Thus, C. barometz hair is a promising gastro-protective agent that can potentially be used to treat gastric ulcers.

**Methods**

**Plant extraction**

*C. barometz* hair was identified and collected by the Herbarium of Rimba Ilmu, University of Malaya, Kuala Lumpur voucher No KLU 48648. One hundred grams of dried plants were soaked for five days in 900 ml of 95% ethanol and stirred daily in a laboratory glass bottle (1 L). Next, filter papers (Whatman No. 1) were used to filter the mixture and the ethanol (EtOH) extraction and distillation of *C. barometz* was accomplished under reduced pressures in a Buchi Rotary Evaporator R-215 (Chemoph-arm Sdn. Bhd., Switzerland). The *C. barometz* hair extract produced a 1.79% solution (dark-yellow; w/w). For acute toxicity, *C. barometz* extract were diluted in 10% Tween 20 and administered to experimental rats orally at doses of 2 g/kg and 5 g/kg [50]. For anti-ulcer activity against ethanol-induced stomach mucosal damage, it was diluted in 10% Tween 20 at doses of 62.5, 125, 250, and 500 mg/kg body weight for oral administration as previously described [46].

**Gastric ulcer test**

*Omeprazole*

Omeprazole was purchased from the University Malaya Medical Centre (UMMC) Pharmacy to use as a reference standard medication for gastric ulcers. For oral administration, omeprazole was dissolved in 10% Tween 20 and fed to animals at a dose of 20 mg/kg body weight (5 mL/kg) [18].

**Ethanol-induced gastric ulceration**

The healthy adult male SD rats (181-208 g) were obtained from the Experimental Animal House, Faculty of Medicine at the University of Malaya. The animals were distributed into seven groups (*n* = 6 per group). Each animal was fasted for 24 h and fasted from water for 2 h prior to the experiment. The animals were housed in wire-bottomed cages to prevent coprophagy. Group 1 (vehicle group) and Group 2 (ulcer group) were orally administered 10% Tween 20 (5 mL/kg). Group 3 (reference control group) was fed 20 mg/kg of omeprazole orally. Groups 4, 5, 6 and 7 were orally administered the doses of 62.5, 125, 250, and 500 mg/kg of *C. barometz* hair extract, respectively. After one hour, Group 1 rats were administered 10% Tween 20 (5 mL/kg), and groups 2-7 rats were given the absolute alcohol (5 mL/kg) (Golbabapour et al.,[14]). Then, all animals were an overdose anesthetized using xylazine and ketamine (150 and 15 mg/kg), and after one hour, a cervical dislocation was performed for direct excision of their stomachs.

**Measurement of the stomach juice acidity and mucus contents**

The stomach of each rat was untied alongside the greater curvature. The stomach contents were measured for the hydrogen ion concentrations using a pH meter titration with 0.1 N NaOH. The acid contents were expressed as meq/L. For each rat, the mucosa of stomach was scraped slightly using the histological section slides, and the mucus in each stomach was weighed using the electronic balance [24].

**Gross stomach injury evaluation**

Ulcers of the stomach mucosa appeared as extended hemorrhagic bands of abrasions parallel to the long axis in the gut. In each gut, the length and width of ulcers (mm) were measured using the planimeter (10 × 10 mm² = ulcer area) under a dissecting microscope (1.8x). The ulcer area was estimated by calculating number of the small squares, 2 mm × 2 mm, including the length and width of ulcer bands. For each stomach, the sum of the areas of all lesions was applied in the ulcer area (UA) calculations with the sum of small squares × 4 × 1.8 = UA (mm²), as previously reported by Rahim et al. [51]. The following equation in the calculation of the inhibition percentage was used in this study as previously described by Indran et al. [43]:

\[
\text{Inhibition\%} = \left[ \frac{\text{UA control} - \text{UA treated}}{\text{UA control}} \right] \times 100
\]

**Histological evaluation of stomach injuries**

*Hematoxylin and eosin staining*

For each rat, the stomach wall specimens were put in 10% buffered formalin for fixation, processed in machine of tissue-processing (Leica, Germany) and paraffin-embedded. For histological assessments, slides of the stomach sections were prepared at a 5 μm thickness and stained with staining of hematoxylin and eosin (H and E) [52].

*Mucosal glycoprotein staining*

To assess the mucus productions in the stomach, selected slides were put in the staining of Periodic Acid-Schiff (PAS), following the manufacturer’s instructions (Sigma Commercial Kits). The glandular portions of tissues were stained with this type of staining to visualize the production of mucus and changes in the basic and acidic glycoproteins. The light microscope (Nikon, Japan) was used to photograph then to observe any of the mucus produced (Nikon, Japan) [53].
Immunohistochemistry staining
Animal Research Kit (ARKTM) was used to detect the immunohistochemical localization proteins of HSP70 (1:100) and Bax (1: 200) on the study slides. Both antibodies were bought from Santa Cruz Biotechnology, Inc., California, USA. Briefly, the slides of each tissue were put in a hot air oven (60°C) for 25 min (Venticell, MMM, Einrichtungen, Germany). Each tissue slides was de-paraffinized by xylene and re-hydrated by graded alcoholism solutions. The antigens retrieval were achieved using the microwave boiling of each samples in sodium citrate buffer (10 mM). After that, the endogenous peroxidase enzymes were blocked via hydrogen peroxide (0.03%) containing sodium azides then each tissue sections was put gently in the rinsing buffer. The slides were incubated with HSP70 (1: 100) or Bax (1: 200) biotinylated first antibodies for 15 min. Next, they were rinsed with the rinse buffer to put them in the buffer bath. Each slide was kept in the humidified chambers. For 15 min, the sections of immunohistochemistry slides were incubated through streptavidin conjugated to horseradish peroxidase in PBS containing an anti-microbial agent (streptavidin–HRP). They were put again in the rinsing buffer then in the buffer bath before incubation with diaminobenzidine substrates chromagen for 5 min. the slides of this experiment were washed, counterstained with hematoxylin (5 s), dipped in 0.037 M/L of weak ammonium for 10 times. The washing through distilled water is important before the mounting of the slides with cover slips. Affirmative identification of this immunostaining was observed in the brown coloration of tissue sections under the light microscope. Protein expression levels were observed in the brown coloration of tissue sections under the light microscope. Protein expression levels were quantitated by the NIH image J software programs. The data are shown by mean ± SD and statistical significance was expressed as P < 0.05.

Antioxidants activity of gastric homogenate
Preparations of stomach homogenates
For each stomach tissue, the samples were washed comprehensively by the ice-cold potassium buffer phosphate (PBS). Each stomach homogenates (10% (w/v) was prepared with the ice-cold 50 mM (PBS) (pH 7.4) using the tissue homogenizer (Polytron, Heidolph RZR 1, Germany). They were centrifuged for 15 min at 10,000 rpm (4 °C) using refrigerated centrifuge Rotofix 32 (Hettich Zentrifugen, Germany). Next, the supernatants were used for the evaluation of antioxidants activity and lipid peroxidation levels.

Evaluation of antioxidant activities of gastric homogenate
The commercial kits of Cayman Chemical Company, Ann Arbor, USA were purchased for assessment the SOD, CAT and GPx activities of the stomach tissues. The manufacturer’s procedures were used for the determination of antioxidants activities in the gastric tissue supernatants of each sample.

Measurements of lipid peroxidation (MDA) levels of stomach homogenates
Lipoperoxidation of the mucus membrane in the gastric epithelium was determined by purchasing the commercial kit from [Cayman Chemical Company, Ann Arbor, USA.

Antioxidant activity in vitro
Ferric-reducing antioxidant power (FRAP) assessment
In brief, FRAP reagents were used using freshly preparing from acetic buffer (pH 3.6), 10 mM TPTZ [2,4,6-Tri(2-pyridyl)- s-triazine] solution in HCI (40 mM), and 20 mM Fe (III) chloride solution in proportions of 10:1:1 (v/v), correspondingly. Butylated hydroxytoluene (BHT), ascorbic acid, quercetin, and gallic acid were used as controls of the test. Ten microliters of each plant extract, standard and controls were put into 300 μL of the FRAP reagent (in triplicate) and left in a dark place for 4 min. Then the absorbance was recorded at 593 nm via a power wave × 340 ELISA Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). The standard curve was constructed linearly (R² = 0.998) between 100 and 1000 M FeSO₄. The outcomes were expressed as M Fe (II)/g dry weight of the extract.

Scavenging of diphenyl-picrylhydrazyl radical activity (DPPH) assay
A stock solution (1 mg/1 mL) of the extracted medicinal plant was prepared then diluted to produce five different concentrations (50, 25, 12.5, 6.25, 3.125, 1.56 μg/mL), and the antioxidant ascorbic acid was used as the standard. 5 μL of C. barontz and standard were mixed with 195 μL DPPH (40× dilution) in triplicate. After that, each mixture was incubated at 37 C. The absorbance value was measured for 2 h at 20 min intervals using a spectrophotometer of power wave × 340 on the ELISA Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) at 515 nm. The radical scavenging activity was estimated using this formula:

\[\% \text{ inhibition} = \{(\text{AB} - \text{AA})/\text{AB}\} \times 100\]

AB is the absorption of the blank sample; AA is the absorption of the tested samples. The inhibitory concentration 50% was determined besides the kinetics of scavenging reactions of DPPH. BHT, ascorbic acid, quercetin, and gallic acid were also verified against DPPH as positive controls [22].

Acute toxicity and experimental SD rats
Adult Sprague Dawley rats (male and female, 6–8 weeks old), were attained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethic no.PM/30/05/2012/NSIAW (R). The body weight of the rats was between 166–190 g. Each rat was fed standard
animal pellets and tap water. The acute toxicity test was performed to determine the nontoxic dose of C. barometz hair. Thirty-six SD rats (18 males and 18 females) were randomly separated equally into three categories each labeled as vehicle (10% Tween 20), 2 g/kg, and 5 g/kg of C. barometz hair preparation [25, 50, 54, 55]. The animals were fasted overnight before dosing (food but not water), and then the diet was withdrawn for an additional three to four hours after dosing. SD rats were observed for 30 min and 2, 4, 8, 24 and 48 h after feeding at the beginning of toxicological assessments. Mortality was documented over a period of 14 days. On the 15th day, the animals were injected with an overdose of anesthesia (xylazine with ketamine) then blood, kidneys and liver were obtained. Histology and serum biochemical parameters were assessed according to the OECD guidelines [56].

Statistical analysis
The statistically significant differences among the groups were measured by the SPSS statistical program software version 20. A one-way analysis of variance (ANOVA) was used with Tukey’s multiple comparison post hoc test. All data were reported as the mean ± SEM. A value of *P* < 0.05 was reflected significant.

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Availability of data and materials
All the data supporting your findings is contained within the manuscript.

Authors’ contributions
Conceived and designed the experiments: NSA MAA HMA SMN. Performed the experiments: NSA PH MH MFH AHSA SK. Analysed the data: NSA NA ANS SK. Contributed reagents/materials/analysis tools: NSA HK MH MFH. Wrote the manuscript: NSA MH. Revised and approved the manuscript: NAS SK SMN MAA.

Competing interests
The authors declared that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
All experimental protocols were approved by the ethics committee with Ethic No PM/30/05/2012/NSIAW (R) for animal experimentation of the Faculty of Medicine, University of Malaya, Malaysia.

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References
1. Lakoo D, Prasad SK, Krishnamurthy S, Hemalatha S. Gastroprotective activity of ethanolic root extract of Potentilla fulgens Wall. ex Hook. J Ethnopharmacol. 2013;146(2):505–14.
2. Chen H, Liao H, Liu Y, Zheng Y, Wu X, Su Z, et al. Protective effects of pogostone from Pogostemon Herba toxicity and gastroprotective activity in rats. Fitoterapia. 2015;100:110–7.
3. Vonkeman HE, Klok RM, Postma MJ, Brouwers JR, van de Laar MA. Direct medical costs of serious gastrointestinal ulcers among users of NSAIDs. Drugs Aging. 2007;24(8):681–90.
4. Jesus NZT, Falcão HS, Lima GPM, Caldas Filho MRD, Sales IRP, Gomes IF, et al. Hypothesis concerning activity (L) Port (Lamiaceae), a medicinal plant protects the stomach against several gastric ulcer models. J Ethnopharmacol. 2013;150(3):982–8.
5. Boligon AA, de Freitas RB, de Brum TF, Waczuk EP, de Freitas RB, de Brum TF, Waczuk EP, et al. Antilucerogeneic activity of Scutia buxifolia on gastric ulcers induced by ethanol in rats. Acta Pharmaceutica Sinica B. 2014;4(5):358–67.
6. Shaker E, Mahmoud H, Mnaa S. Anti-inflammatory and anti-ulcer activity of the extract from Allagi mauroorum (camelthorn). Food and Chemical Toxicology. 2010;48(10):2785–90. http://dx.doi.org/10.1016/j.fct.2010.07.007.
7. Abdulla MA, Noor S, Wong K-H Ali HM. Effect of culinary-medicinal lion’s mane mushroom, Hericium erinaceus (Bull., Fr.) Pers. (Aphyllo phoromycetideae), on ethanol-induced gastric ulcers in rats. Int J Med Mushrooms. 2008;10:4.
8. Bhatti S, Wahane VD, Kumar VL. Protective effect of Calotropis procera latex extracts on experimentally induced gastric ulcers in rat. J Ethnopharmacol. 2010;127(2):440–4.
9. Waisman S, Mobin A, Chua LS, Alwashw MA, Hamdan S. Antioxidant and gastroprotective activities of Andrographis paniculata (Hemipedu Bumi) in Sprague Dawley rats. Indian J Exp Biol. 2011;49(10):767–72.
10. Taha MM, Saliga MS, Ali HM, Abdulla MA, Abdelwahab SI, Hadi AHA. Gastroprotective activities of Tannerea diffusa Willd. ex Schult. revisited: Role of arbutin. J Ethnopharmacol. 2012;141(1):273–81.
11. Antonisamy P, Dhanasekaran M, Ignacimuthu S, Duraiyandiyani V, Balthazar JD, Agastian P, et al. Gastroprotective effect of epirole cedrane diterpene isolated from Tinospora cordifolia Mej (Guduchi) on indomethacin-induced gastric ulcer in rats. Phytomed. 2014;21(7):966–9.
12. Hajezaie M, Salehnen N, Kairim H, Zadehifard M, Shams K, Al Batran R, et al. Biochanin A, a gastroprotective agent in ethanol-induced gastric mucosal ulceration in rats. PLoS One. 2015;10(3):e0121529.
13. Siddahmed HMA, Hashim NM, Abdulla MA, Ali HM, Mohan S, Abdelwahab SI, et al. Anti-secretory, gastroprotective, antioxidant and anti-Helicobacter pylori activity of Zerumbone from Zingiber Zerumbet DC. J Ethnopharmacol. 2011;133(3):1083–8.
14. Zhao X, Wu Z-X, Zhang Y, Yan Y-B, He Q, Cao P-C, et al. Anti-osteoporosis activity of Citrobacter baronii extract on ovariectomy-induced bone loss in rats. J Ethnopharmacol. 2011;137(3):1083–8.
15. Wu R, Yang X-W. The constituents of Cibotium barometz and their permeability in the human Caco-2 monolayer cell model. J Ethnopharmacol. 2009;125(3):417–22.
16. Cuoong NX, Minh CV, Kiem PV, Huong HT, Ban NK, Nhiem NX, et al. Inhibitors of oestoclast formation from rhizomes of Cibotium barometz. J Nat Prod. 2009;72(9):1673–7.
17. Ong H, Nordiana M, Malay ethno-medico botany in Machang, Kelantan Malaysia. Fitosfera. 1999;70(5):502–13.
18. Ismail IF, Golkabapour S, Hassanardvish P, Hajezaie M, Abdul Majid N, Kadir FA, et al. Gastroprotective activity of Polyergus chinense aqueous leaf extract on ethanol-induced hemorrhagic mucosal lesions in rats. Evid Based Complementary Altern Med. 2012; 9. http://dx.doi.org/10.1155/2012/404012.
19. Adjei S, Amoateng P, Ossei-Safo D, Aboar Bo Nqussian B, Addo P, et al. Biochemical and haematological changes following an acute toxicity study of a hydro-ethanolic whole plant extract of Syneilechea nodiflora (L.) Gaertn in male Sprague-Dawley rats. J Med Biomed Sci. 2013;13(1):131–7.
20. Rouhollahi E, Moghadamtousi SZ, Hamdi OAA, Fadaeinasab M, Hajirezaie M, et al. Evaluation of acute toxicity and gastroprotective activity of Curcuma purpurascens B. rhizome against ethanol-induced gastric mucosal injury in rats. BMC Complement Altern Med. 2014;14:378.
21. Qader SW, Abdulla MA, Chua LS, Najim N, Zain MM, Hamdan S. Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. Molecules. 2011;16(4):3433–43.
22. Avah FM, Uzogweu PN, Oyugi JO, Rutherford J, Reonu P, Yao X-J, et al. Free radial scavenging activity and immunomodulatory effect of Stachytopheta angustifolia leaf extract. Food Chem. 2010;119(4):1409–16.
23. Laı H, Lim Y. Evaluation of antioxidant activities of the methanolic extracts of selected ferns in Malaysia. Wounds. 2011;10(11).
24. Halazi MF, Shaker RM, Bardi DA, Al-Wajeeh NS, Abiat A, Hassanandian P, et al. Gastroprotective activity of Etilh-4(a3, 5-di-tet-butyli-2-hydroxy benzylidenone) Amino benzoate against ethanol-induced gastric mucosal ulcer in rats. PLoS One. 2014;9(5):e95908.
25. AllRashdi AS, Salama SM, Alkkyumy SS, Abdullah MA, Hadi AHA, Abdelwahab SL, et al. Mechanisms of gastroprotective effects of ethanolic leaf extract of Jasminum sambac against HCl/ethanol induced gastric mucosal injury in rats. Evid Based Complement Alternat Med. 2012;2012:21.
26. da Silva LM, Allemand A, Mendes DAG, dos Santos AC, André E, de Souza AL-Wajeeh.
27. Nordin N, Salama SM, Golbabapour S, Hajeieze M, Hassanandian P, Kamailedeghan B, et al. Anti-ulcerogenic effect of methanolic extracts from Enicasanthellum pulchrum (King) heuden against ethanol-induced acute gastric lesion in animal models. PLoS One. 2014;9(11):e111925.
28. Amgalan MF, de Canaval NR, Barcelos RP, Dobrachinski F, Portella RDL, da Silva MA, Lugolenski TH, Dias GGM, da Luz SCA, Boligon AA, Ayadey MF, Millet LMA, Antunes SFA, Fachinotto R. Protective action of ethanol extract of Rosmarinus officinalis L. in gastric ulcer prevention induced by ethanol in rats. Food Chem Toxicol. 2013;55:48–55.
29. Misha V, Agrawal M, Onasanwo SA, Madhur G, Rastogi P, Pandey HP, Palit KD, Harra MA, et al. Protective mechanisms of Behnium tignonii Schum. J Ethnopharmacol. 2013;148(1):277–86.
30. Jain M, Mohan KV, Devi CSS. Gastroprotective effect of Cissus quadrangularis extract in rats with experimentally induced ulcer. Indian J Med Res. 2006;123:799–806.
31. Ibrahim IAA, Qader SW, Abdullah MA, Nimir AR, Abdelwahab SL, Al-Bayaty FH. Effects of Phellocarbom in crina leaf extract against ethanol-induced gastric mucosal injuries in Sprague-Dawley rats. Molecules. 2006;11(7):296–811.
32. Qader SW, Abdullah MA, Chua LS, Sinat HM, Hamdan S. Pharmacological mechanisms underlying gastroprotective activities of the fractions obtained from Polygonum minus in Sprague-Dawley rats. Int J Mol Sci. 2012;13:1481–96.
33. Al Batran R, Al-Bayaty F, Al-Obadii MMJ, Abdullah KAM, Hadi HA, Ali HM, et al. In vivo antioxidant and antulcerative effect of Panax ginseng extract against ethanol-induced acute gastric ulcer in rats. PLoS one. 2013;8(5):e64751.
34. Jarret, against ethanol-induced acute gastric ulcer in rats. Molecules. 2011;16:8654–69.
35. Salga MS, Ali HM, Abdulla MA, Abdelwahlab S, Hussain PD, Hadi AHA. Mechanistic studies of the anti-ulcerogenic activity and acute toxicity evaluation of Dichlorido-Copper (II)-4-(2,5-Bromobenzoylideneamino) ethytl Piperazini-Iium Phenate complex against ethanol-induced gastric injury in rats. Molecules. 2011;16:8654–69. doi:10.3390/molecules16108654.
36. Al-Bayaty F, Al-Obaidi MMJ, Abdualkader AM, Hadi HA, Ali HM, et al. Pyranoxycoartabiloxanthone A, a novel gastroprotective compound from Anticoma obtusus Jarrett, against ethanol-induced acute gastric ulcer in vivo. Phytomed. 2013;20(10):834–43.
37. Moghadamtousuz S, Rouhollah Z, Khamian M, Hadeiinassab M, Abdullah MA, Kadir HA. Gastroprotective activity of Annona muricata leaves against ethanol-induced gastric injury in rats via Hsp 70/Bax involvement. Drug Des Dev Ther. 2014;8:2099–111.
38. Golbabapour S, Hajeieze M, Hassanandian P, Abdul Majid N, Hadi AHA, Nordin N, et al. Acute toxicity and gastroprotective role of M. prunium in ethanol-induced gastric mucosal injuries in rats. BioMed Res Int. 2013:13. http://dx.doi.org/10.1155/2013/974185.
39. Hajrezaie M, Golbabapour S, Hassandarvish P, Gwaram NS, Hadi AHA, et al. Acute toxicity and gastroprotective studies with a newly synthesized Copper (II) Complex against thanol induced acute gastric ulcer in rats. J Ethnopharmacol. 2013;148(1):175–81.
40. Hor SY, Ahmad M, Fari E, Lim CP, Asuai MY, Zain MF. Acute and subchronical oral toxicity of Curcuma xanthorrhiza against ethanol-induced gastric lesions in Sprague-Dawley rats. J Ethnopharmacol. 2011;137(3):1057–76.
41. Rahim NA, Hassanandian P, Golabapour S, Ismail S, Tayabo S, Abdullah MA. Gastroprotective effect of ethanol extract of Curcuma xanthorrhiza leaf against ethanol-induced gastric mucosal lesions in Sprague-Dawley rats. BioMed Res Int. 2014; http://dx.doi.org/10.1155/2014/416409.
42. Hajeieze M, Golbabaour S, Hassanandian P, Gwaram NS, Hadi AHA, et al. Acute toxicity and gastroprotection studies of a new Schiff base derived Copper (II) Complex against thanol induced acute gastric lesions in rats. PLoS One. 2012;7(2):e51537.
43. Abdullah MA, Ali HM, Abdul-Aziz AK, et al. Evaluation of the anti-ulcer activities of Morus alba leaf extracts in experimentally-induced ulcerative gastric ulcer in rats. Biomed Res-India. 2009;20(1):35–9.
44. Thanabbhoom S, Jaricy K, Tharamee S, Ingkaninan K, Panthong A. Acute and subacute toxicity study of the ethanol extract from Loniceria japonica Thunb. J Ethnopharmacol. 2006;107(3):370–3.
45. Mahmood AA, Manord AA, Al-Bayaty F, Abdel-Wahab SM. Anti-ulcerogenic activity of Gynura procumbens leaf extract against experimentally-induced gastric lesions in rats. J Med Plants Res. 2010(4):685–91.
46. OECD. OECD Guideline for testing of chemicals. Env J Med. 2005.