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RETRACTED ARTICLE: The antiulcer effect of *Cibotium barometz* leaves in rats with experimentally induced acute gastric ulcer

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Abstract: *Cibotium barometz* is a pharmaceutical plant customarily used in traditional medicine in Malaysia for the treatment of different diseases such as gastric ulcer. The gastroprotective effect of *C. barometz* leaves against ethanol-induced gastric hemorrhagic abrasions in Sprague Dawley rats has been evaluated in terms of medicinal properties. Seven groups of rats (normal control and ulcerated control groups, omeprazole 20 mg/kg, 62.5, 125, 250, and 500 mg/kg of *C. barometz* correspondingly) were used in an antiulcer experiment and pretreated with 10% Tween 20. After 1 hour, the normal group was orally administered 10% Tween 20, whereas absolute alcohol was fed orally to ulcerated control, omeprazole, and experimental groups. Gastric's homogenate were assessed for endogenous enzymes activities. Stomachs were examined macroscopically and histologically. Grossly, the data demonstrated a significant decrease in the ulcer index of rats pretreated with plant extract in a dose-dependent manner with respect to the ulcerated group. Homogenates of the gastric tissue exhibited significantly increased endogenous enzymes activities in rats pretreated with *C. barometz* extract associated with the ulcerated control group. Histology of rats pretreated with *C. barometz* extract group using hematoxylin and eosin staining exhibited a moderate-to-mild disruption of the surface epithelium with reduction in submucosal edema and leucocyte infiltration in a dose-dependent manner. In addition, it showed heat shock protein70 protein up-expression and BCL2-associated X protein downexpression. These outcomes might be attributed to the gastroprotective and antioxidative effects of the plant.

Keywords: *Cibotium barometz* leaves, antioxidants, acute toxicity, antiulcer, histology

Introduction

Peptic ulcer is one of the widespread illnesses affecting humans. The most benign injuries in the stomach that are known as gastric ulcers affect many people around the world.[1] An imbalance between destructive factors and mucosal defence mechanisms in the mucosal epithelium causes gastric ulcer.[2] Many destructive factors enhance the occurrence of acute gastrointestinal disorders such as a higher secretion of acid–pepsin, a lower secretion of mucus and bicarbonate, severe psychological or physical stress, smoking, imbalanced bile salt secretion, *Helicobacter pylori* infection, ingestion of ethanol, aspirin, and other nonsteroidal anti-inflammatory drugs, and hereditary factors.[3,4] Several investigations have concerned the production of oxygen-derived free radicals in the pathogenesis of stomach ulcers.[5]

Additionally, the higher level of lipid peroxidation has a destroying effect on stomach glandular epithelial, which causes injury provoked by consuming ethanol.[6] Antioxidants are well known to restrain lipid peroxidation in addition to scavenging free radicals.[7]

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Therefore, there is a demand for medicinal drugs that are capable of scavenging these free radicals as well as create helpful outcomes against gastric sores. In living organisms, the initial row of protection against free radicals is the oxidative stress enzyme superoxide dismutase (SOD). SOD catalyses the dismutation of superoxide anions by changing them to hydrogen peroxides. The poisonous hydrogen peroxide is transformed into molecular oxygen and water by means of catalase or glutathione peroxidase.[8] Drugs such as antacids and proton pump inhibitors (omeprazole), which are employed in the management of gastric sores, are sometimes unsuccessful or not highly efficient. This might be due to a drug interaction or unpleasant side effects. A large number of researchers have reported on the numerous remedial plants applied in folk medicine as antiulcer mediators.[9–13] Plant extracts are attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers.[14–16]

Cibotium barometz (L.) J Sim (family Dicksoniaceae) is also known as Golden Hair Dog Fern. It is a tropical and subtropical plant.[17] This remedial plant is employed to prevent hemorrhage and for the management of rheumatism, polyuria along with leucorrhoea.[18] Earlier studies have shown that this plant possesses antioxidative, tyrosinase inhibiting as well as antibacterial actions.[19] *C. barometz* extract as well has the potential for inhibition of post-menopausal osteoporosis.[20] The beneficial results of *C. barometz* leaf extract on acute gastric cytoprotective properties are yet to be adequately investigated in trial studies. Consequently, the current study aimed to appraise the gastroprotective potential of the ethanolic extract of *C. barometz* against ethanol-induced stomach ulcers in rodents.

Materials and methods

Plant extraction

C. barometz leaves were collected and identified by the Herbarium of Rimba Ilmu, University of Malaya, Kuala Lumpur, voucher no. KL48648. The dried plant (100 g) was soaked in ethanol (95%, 900 mL) for 5 days. Buchi Rotary Evaporator R-215 (Chemoph-arm Sdn Bhd, Switzerland) was used to extract the solution.

For acute toxicity, *C. barometz* extract was dissolved in 10% Tween 20 and administered to experimental rats orally at doses of 2 g/kg and 5 g/kg. For antiulcer activity against ethanol-induced gastric mucosal injury, it was dissolved in 10% Tween 20 at doses of 62.5, 125, 250, and 500 mg/kg body weight for oral administration, in accordance with earlier reports.[21]

Antioxidant activity in vitro

Ferric-reducing antioxidant power assay

The ferric-reducing antioxidant power (FRAP) of the ethanol extract of *C. barometz* was assessed according to the method mentioned with minor modifications in Benzie et al's study.[22] The FRAP reagent was prepared freshly from acetate buffer (pH 3.6), 10 mM TPTZ [2,4,6-Tri(2-pyridyl)-s-triazine] solution in 40 mM HCl and 20 mM Fe (III) chloride solution in the proportion of 10:1:1 (v/v), respectively. Butylated hydroxytoluene (BHT), ascorbic acid, quercetin, and gallic acid were used as controls; 10 µL of plant extract, standard, and controls were added to 300 µL of the FRAP reagent (triplicate) and left in the dark for 4 minutes. Then the absorbance was recorded at 593 nm using a spectrophotometer of power wave ×340 ELISA Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). The standard curve was created linearly (R2 =0.998) between 100 and 1,000 M FeSO4.

Scavenging of diphenylpicrylhydrazyl radical activity assay

The antioxidant activity of the ethanol extract of *C. barometz* was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical based on the electron transfer reaction between DPPH reagent and the plant extract. The DPPH method designated by Gorinstein[23] with minimal modification was used. A stock solution (1 mg/1 mL) of the plant extract was prepared and then diluted to produce six different concentrations (50, 25, 12.5, 6.25, 3.125, 1.56 µg/mL) and an antioxidant standard (ascorbic acid) was used. Five microliters of each plant extract 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 hours at 20-minute intervals using a spectrophotometer of power wave ×340 ELISA Reader at 515 nm. The radical scavenging activity was calculated from according to the following equation:

$$\%\ \text{inhibition} = \frac{AB - AA}{AB} \times 100$$

AB is the absorption of the blank sample; AA is the absorption of the tested samples.

The inhibitory concentration of 50% was determined as well as the kinetics of DPPH scavenging reaction. BHT, ascorbic acid, quercetin, and gallic acid were also verified against DPPH as positive controls.

Acute toxicity test and experimental animals

Healthy adult Sprague Dawley (SD) rats, both male and female (6–8 weeks old), were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur. The animal ethics for this study was approved by the Ethics Council of Animal Experimental Unit (AEU) under the ethics No: PM/30/05/2012/NSIAW [R]. The body weight of the rats was between 162 and 190 g. The rats were given standard rat pellets and tap water. An acute toxicity study was carried out to determine a safe dose.[24] Histology and serum biochemical parameters were analyzed as described in detail previously. Throughout the experiment, all rats 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.[25,26]

Gastric ulcer

Omeprazole

In this trial, a reference antiulcer drug (omeprazole) was employed as well as obtained from the University Malaya Medical Centre Pharmacy. Omeprazole was dissolved in 10% Tween 20 and fed orally to the rats at a dose of 20 mg/kg body weight (5 mL/kg).[27]

Ethanol-induced gastric ulceration

The animals were distributed into seven groups of six each. Before the experiment, they were food fasted for 24 hours and water fasted for 2 hours. The rats were housed in wire-bottomed cages to prevent coprophagy. Groups 1 (vehicle group) and 2 (ulcerated group) were administered orally with 10% Tween 20 (5 mL/kg). Group 3 was given 20 mg/kg omeprazole orally, as the reference control group. Groups 4, 5, 6, and 7 were given oral doses of 62.5, 125, 250, and 500 mg/kg of *Cibotium barometz* ethanol extract, respectively. One hour later, group 1 was treated with 10% Tween 20 (5 mL/kg) and Groups 2–7 were given absolute ethanol (5 mL/kg).[13] All rats were anesthetized after 1 hour using xylazine and ketamine, followed by cervical dislocation and direct excision of their stomachs.[28]

Measurement of gastric juice acidity and mucus content

Each stomach was opened along the greater curvature. Gastric contents were analyzed for hydrogen ion concentration using pH meter titration with 0.1 N NaOH. The acid content and gastric mucosa were assessed to measure the gastric juice acidity.[29]

Gross gastric lesion evaluation

Ulcers of the gastric mucosa appeared as extended bands of hemorrhagic lesions parallel to the long axis of the gut. The gastric mucosa of each rat was examined for injuries. The length and width of the ulcer (mm) were measured with a plan meter (10×10 mm^2 = ulcer area [UA]) under a dissecting microscope (1.8×). The ulcerated area was determined by calculating the number of small squares 2×2 mm, covering the length and width of each ulcer band. The calculation of the areas of all lesions for each stomach was done and the inhibition percentage (I%) were calculated using the following formula according to the recommendation of AlRashdi.[30]

$$\text{Inhibition \%} = \frac{\text{UAcontrol} - \text{UAtreated}}{\text{UAcontrol}} \times 100\%.$$

Histological evaluation of gastric lesions

Hematoxylin and eosin staining

The gastric wall specimens were fixed in 10% buffered formalin, processed, and embedded in paraffin. Sections of the each were prepared at a thickness of 5 µm and stained with hematoxylin and eosin for histological and tissue architecture estimation.[31]

Mucosal glycoprotein staining

Mucosal glycoprotein production stained with periodic acid–Schiff (PAS) was assessed using PAS following the manufacturer's instructions (Sigma periodic acid–Schiff commercial kit). Staining with PAS was carried out for evaluation of the variations in glycoproteins (acidic and basic). A light microscope (Nikon, Tokyo, Japan) was used to photograph and observe the mucus produced.[32]

Immunohistochemistry staining

Each tissue section was heated at 60°C for 25 minutes in an oven. Then they were deparaffinized in xylene and rehydrated by graded alcohol. Antigen retrieval process proceeded in 10 mM sodium citrate buffer boiled in a microwave. The immunohistochemical staining was performed using an animal research commercial kit to detect the immune-staining localization of heat shock protein 70 (HSP70) (1:100) and BCL2-associated X protein (Bax; 1:50) proteins on tissue sections. The two proteins were purchased from Santa Cruz

Biotechnology, Inc., Santa Cruz, CA, USA. Positive findings of the immunohistochemical staining appear as brown stains under the light microscope.[31]

Antioxidant activity of gastric homogenate

Preparation of homogenate

The gastric tissue samples were washed thoroughly with ice-cold phosphate buffered saline (PBS). Homogenates (10% (w/v)) were then prepared with ice-cold 50 mM (PBS) (pH 7.4) using a homogenizer (Polytron, Heidolph RZR 1, Schwabach, Germany). The homogenates were centrifuged for 15 minutes at 10,000 rpm at 4°C using refrigerated centrifuge Rotofix 32 (Hettich Zentrifugen, Tuttlingen, Germany). The supernatant was used for examination of antioxidant activities and malondialdehyde (MDA) levels in vivo.[12]

Measurement of antioxidant activities of stomach homogenate

The SOD, catalase (CAT), and glutathione (GPx) activities of the gastric tissues homogenate were assessed using commercial kits (Cayman Chemical Co., Ann Arbor, MI, USA). The manufacturer's procedures were used for the determination of activities in the gastric tissue supernata of each sample.[32]

Measurements of lipid peroxidation (MDA) leve of stomach homogenate

Lipid peroxidation of the mucus membrane in the gastric tissue homogenate was measured using commercial kits (Cayman Chemical Co.).[33]

Statistical analysis

All values were evaluated as mean ± standard error of the mean. The statistical significance of differences between groups was measured using SPSS statistical program software version 20 through one-way analysis of variance with post hoc Tukey's multiple comparison test. A value of $P<0.05$ was considered significant.

Results

In vitro antioxidant activity of ethanol extract of *C. barometz* leaves

Ferric reducing antioxidant pow

The total antioxidant activity o the ethanol xtract of *C. barometz* was measured sing the RAP te Figure 1 shows the reduction of fe to ferrous ion which indicates a greater FRAP value for *C. arom* leaves (915.7±0.071 µmol Fe (II)/g) than BHT (26 ±0.009 mol Fe (II)/g) and ascorbic acid 7±0.005 µ e (II)/g). However, the value is lower than t of quercetin (1,544.3±0.012 µmol Fe (II) d gallic ac (1,774.3±0.002 µmol Fe (II)/g) stan ards.

Scav nging of D PH assay

The sca ging the DPPH free radicals by *C. barometz* leaf ol extract was evaluated using a DPPH assay. Figure 2 illustrates the inhibition percentage of the DPPH free radical scavenging activity of *C. barometz* leaf ethanol extract, which as 87.5% with an IC_{50} value of 30.1±0.05 µg/mL. This was compared to the standards BHT, ascorbic acid, quercetin, and gallic acid. The % inhibition of DPPH free-radical scavenging activity of the standards was 51.63%, 64.11%, 87.52%, and

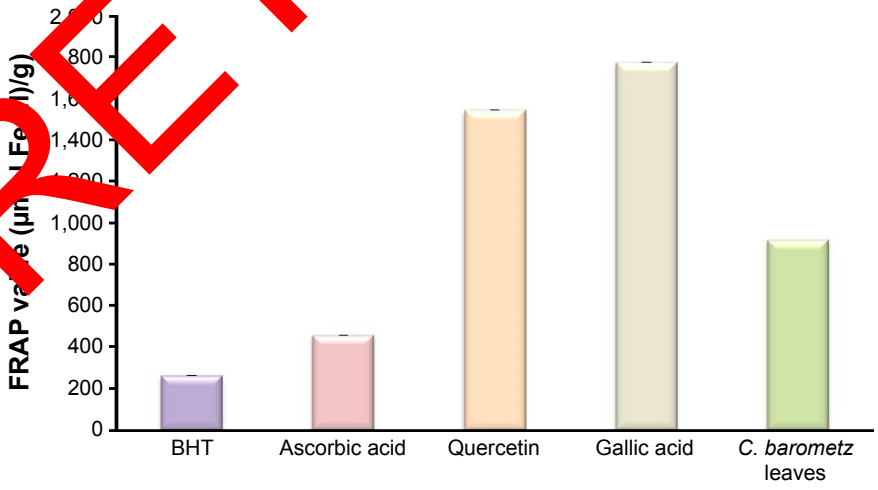

Figure 1 FRAP analysis.
Notes: FRAP assay for total antioxidant activity evaluation of *Cibotium barometz* ethanolic extract with synthetic reference standards (BHT, ascorbic acid, quercetin, and gallic acid) were determined. All values are represented as mean ± standard error of the mean.
Abbreviations: BHT, butylated hydroxytoluene; FRAP, ferric-reducing antioxidant power.

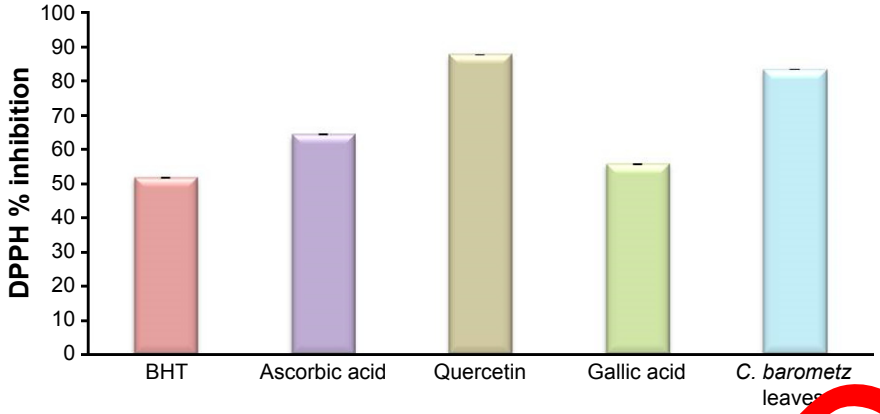

Figure 2 DPPH analysis.
Notes: DPPH assay for free radical scavenging activity (inhibition %) of *Cibotium barometz* leaves ethanolic extract with synthetic reference standards (BHT, ascorbic acid, quercetin, and gallic acid). The data are represented as mean ± standard error of the mean.
Abbreviations: BHT, butylated hydroxytoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

55.47% with an IC_{50} value of 9.1±0.15 µg/mL, 4.9±0.11 µg/mL, 1.8±0.04 µg/mL, 1.4±0.13 µg/mL, respectively.

Toxicity test of *C. barometz* leaves in experimental animals

None of the animals that were fed with *C. barometz* leaf ethanol extract displayed any mortality or toxic symptoms during the experimental study. There were no body weight changes or abnormal physiological or behavioral variations at dosages of 2 and 5 g/kg following extract administration (Tables 1 and 2). The histological analysis and biochemical evaluation on the liver and kidney and their weights were normal in comparison to the control group (Figure 3, Tables 3–8). Subsequently, male and female SD rats did not exhibit any significant signs of toxicity at the above dosages.

Antiulcer study

Gross evaluation

Results revealed that pretreatment of SD rats with *C. barometz* leaf ethanol extract significantly diminished the UA compared to the ulcerated group (Table 9, Figure 4). The inhibition percentage of the UA in rats pre-fed with *C. barometz* leaf ethanol extract was increased in a dose-dependent manner.

Gastric mucus content and acidity

As the results show in Table 9, the ulcerated SD rat group produced the lowest gastric mucosa mucus content, although animal groups pretreated with G7 (500 mg/kg) and G6 (250 mg/kg) of *C. barometz* leaf ethanol extract showed a significant increase in the mucus weight (g) with respect to G2 (ulcerated group). Pretreatment with *C. barometz* leaf ethanol extract (G4 to G7) produced a significant increase in the pH of the gastric contents compared to the ulcerated group G2.

Measurement of gastric antioxidant enzymes and membrane lipid peroxidation (MDA)

The ulcer control rats revealed a major reduction in antioxidant (SOD, CAT, and GPx) endogenous enzyme activities. Rats pretreated with *C. barometz* leaf ethanol extract demonstrated an elevation of all antioxidant activities with respect to the ulcer control rats, as shown in Figure 5A–C. The SOD enzyme activities in Figure 5A were significantly higher at doses of 250 and 500 mg/kg of *C. barometz* leaf ethanol extract than ulcer control rats. Rats fed with *C. barometz* leaf ethanol extract showed significant increase in CAT activity compared to ulcer control rats Figure 5B. The GPx enzyme activities in Figure 5C for gastric mucosal homogenates reveal

Table 1 Effects of *Cibotium barometz* leaves extract on kidney biochemical parameters in female rats

Dose female	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	CO_2 (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Vehicle (10% Tween 20)	146.67±0.56	4.65±0.22	106.01±0.77	24.80±0.98	21.17±0.60	7.12±1.25	36.33±3.62
C. barometz (2 g/kg)	147.00±0.52	4.8±0.09	106.67±0.71	23.47±0.58	21.83±0.79	9.70±0.98	38.83±3.49
C. barometz (5 g/kg)	146.00±0.93	4.82±0.2	107.83±0.79	21.97±1.31	20.67±0.76	6.07±0.39	36.53±3.03

Notes: Values expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at $P<0.05$.

Table 2 Effects of *C. barometz* leaves extract on liver biochemical parameters in female rats

Dose female	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (μmol/L)	CB (μmol/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle (10% Tween 20)	80.00±2.83	13.33±1.23	66.67±1.91	1.00±0.00	1±0.00	95.67±11.48	39.00±1.59	195.50±12.45	6.00±0.86
C. barometz (2 g/kg)	80.50±3.16	13.67±0.92	67.50±2.26	1.00±0.00	1±0.00	102.83±10.28	41.33±1.09	206.50±14.86	5.33±0.95
C. barometz (5 g/kg)	77.80±2.18	12.50±0.92	64.67±1.36	1.00±0.00	1±0.00	88.50±13.87	37.33±1.91	194.17±11.79	4.51±0.81

Notes: Values expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at $P<0.05$.
Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; *C. barometz*, *Cibotium barometz*; CB, conjugated bilirubin; CO_2, carbon dioxide; GGT, G-glutamyltransferase; TB, total bilirubin.

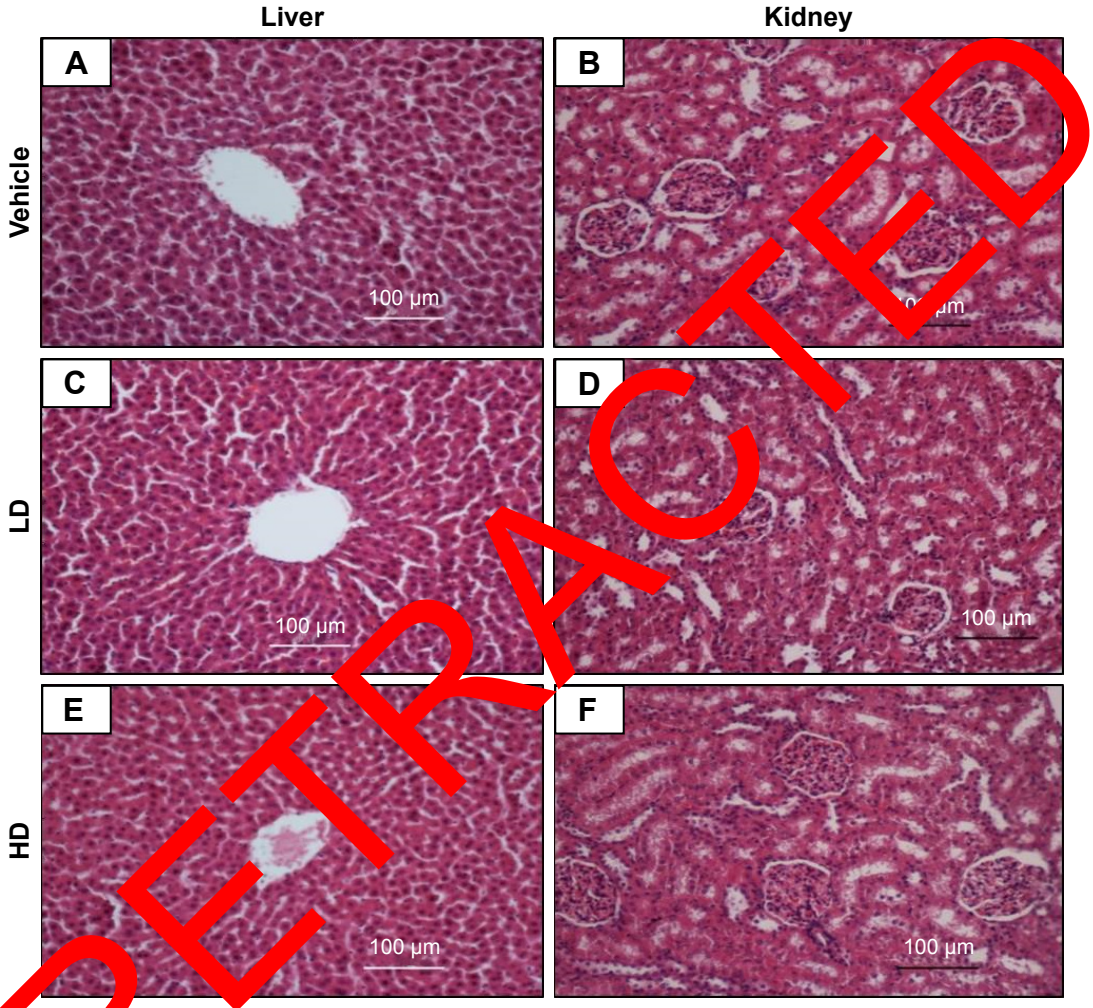

Figure 3 Histolog[illegible] sec[illegible]ns of the [illegible]and kidney for the acute toxicity experiment.
Notes: Rats treated [illegible]5 mL/kg of the vehicle (10% Tween 20) (**A** and **B**). Rats treated with 2 g/kg (2 mL/kg) of the *Cibotium barometz* extract (**C** and **D**). Rats treated with 5 g/kg (5 mL/kg) of [illegible]*C. barometz* extract (**E** and **F**). No significant changes in the structures of livers and kidneys between the treated and control groups (hematoxylin and eosin stain).

Table 3 Effects of *C. barometz* leaves extract on lipid profile biochemical parameters in female rats

Dose female	Triglyceride (mmol/L)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)
Vehicle (10% Tween 20)	0.35±0.04	0.48±0.54	1.73±0.14
C. barometz (2 g/kg)	0.30±0.04	0.34±0.72	1.47±0.18
C. barometz (5 g/kg)	0.38±0.07	0.55±0.83	1.59±0.20

Notes: Values expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at $P<0.05$.
Abbreviations: *C. barometz*, *Cibotium barometz*; HDL, high density lipoprotein.

Table 4 Effects of *C. barometz* leaves extract on kidney biochemical parameters in male rats

Dose male	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	CO_2 (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (μmol/L)
Vehicle (10% Tween 20)	145.33±0.88	5.22±0.05	104.67±0.49	26.15±0.28	20.33±0.21	5.03±0.22	31.83±2.77
C. barometz (2 g/kg)	145.67±0.84	5.03±0.15	105.17±1.11	26.07±0.70	19.50±0.43	5.17±0.24	30.50±1.52
C. barometz (5 g/kg)	142.83±0.4	5.40±0.08	101.50±0.34	27.75±0.29	19.00±0.52	5.05±0.15	30.33±1.74

Notes: Values expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at $P<0.05$ as compared to vehicle groups.
Abbreviation: *C. barometz*, *Cibotium barometz*.

Table 5 Effects of *C. barometz* leaves extract on liver biochemical parameters in male rats

Dose male	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (μmol/L)	CB (μmol/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle (10% Tween 20)	67.67±1.05	11.33±0.33	56.33±0.95	1.00±0.00	1.00±0.00	238.00±21.27	57.[illegible]1.28	220.33[illegible]1.30	1.67±1.69
C. barometz (2 g/kg)	69.00±1.37	11.38±0.61	57.67±1.12	1.00±0.00	1.00±0.00	190.17±18[illegible]	57.83±[illegible]	216.87[illegible]9.82	2.50±1.15
C. barometz (5 g/kg)	68.33±0.88	11.50±0.50	56.83±0.87	1.00±0.00	1.00±0.00	192.17[illegible]5.25	58.00±1.81	[illegible]0±6.45	2.40±1.12

Notes: Values expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at $P<0.05$ compared to vehicle groups (10% Tween 20).
Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; Abbreviation, *C. barometz*, *Cibotium barometz*; CB, conjugated bilirubin; CO_2, carbon dioxide; GGT, G-glutamyltransferase; TB, total bilirubin.

Table 6 Effects of *C. barometz* leaves ethanol extract on lipid profile biochemical parameters in male rats

Dose male	Triglyceride (mmol/L)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)
Vehicle (10% Tween 20)	0.57±0.04	1.73[illegible].05	1.58±0.07
C. barometz (2 g/kg)	0.50±0.07	1.57±0.04	1.40±0.05
C. barometz (5 g/kg)	0.40±0.04	1.62±0.09	1.54±0.09

Notes: Values expressed as mean ± standard error of the mean. There [illegible]o significant changes between groups. Significant value at $P<0.05$ as compared to vehicle groups.
Abbreviations: *C. barometz*, *Cibotium barometz*; HDL, high density lipoprotein.

Table 7 Effects of *C. barometz* leaves ethanol extract on the body weight of the male and female SD rats at both HD (5 g/kg) and LD (2 g/kg) treatment compared to vehicle group 10% Tween 20 (6 SD rats/group)

C. barometz extract	Body weight (g)					
	Male			Female		
	1st day	7th day	14th day	1st day	7th day	14th day
Vehicle (10% Tween 20)	176.5±[illegible]	225.7±2.6	230.7±3.4	180.3±6.10	194.3±7.2	199.0±10.2
LD (2 g/kg)	169.[illegible]±4.6	214.8±2.4	224.7±2.8	181.3±5.8	190.7±4.7	196.2±8.9
HD (5 g/kg)	[illegible]7.7±3.7	210.7±3.9	221.3±2.5	172.0±7.1	187.2±54.2	192.3±3.0

Notes: $P<0.05$. Data are presented as mean ± standard deviation.
Abbreviations: HD, high dose; LD, low dose; SD, Sprague Dawley.

Table 8 Effects of *C. barometz* leaves extract on the liver and kidney weights of male and female rats (6 Sprague Dawley rats/group) at both LD (2 g/kg) and HD (5 g/kg) treatment compared to vehicle group (10% Tween 20)

C. barometz extract	Liver weight (g)		Kidney weight (g)	
	Male	Female	Male	Female
Vehicle (10% Tween 20)	6.97±0.1	6.3±0.3	1.69±0.1	1.6±0.1
LD (2 g/kg)	6.57±0.3	6.5±0.2	1.59±0.1	1.68±0.1
HD (5 g/kg)	6.23±0.2	5.27±0.1	1.85±0.1	1.37±0.0

Notes: Significant value at $P<0.05$. Data are presented by mean ± standard error of the mean.
Abbreviations: *C. barometz*, *Cibotium barometz*; HD, high dose; LD, low dose.

Table 9 Effect of the *C. barometz* leaves extracts on the mucus weight, pH of gastric content, ulcer area, % inhibition of ulcer area in stomach

Animal groups	Group	Pretreatment 5 mL/kg	Mucus weight (g)	pH (acidity)	Ulcer area (mm^2)	Inhibition %
Normal control	G1	10% Tween 20	2.27±0.13*	7.15±0.37*	–	–
Ulcer control	G2	10% Tween 20	0.74±0.13	2.75±0.26	804.63±36.66	–
Omeprazole	G3	20 mg/kg	1.90±0.05*	5.74±0.51*	96.03±20.46*	88.02
C. barometz leaves extract	G4	62.5 mg/kg	0.80±0.03	4.56±0.49*	256.80±32.61*	67.96
	G5	125 mg/kg	1.32±0.09	4.47±0.16*	163.20±22.82*	79.64
	G6	250 mg/kg	1.41±0.18*	5.86±0.42*	150.00±21.39*	81.29
	G7	500 mg/kg	1.78±0.17*	5.20±0.47*	126.0±5.50*	84.28

Notes: The values are expressed as mean ± standard error of the mean. *Indicates significance at $P<0.05$ compared to ulcerated group.
Abbreviation: *C. barometz*, *Cibotium barometz*.

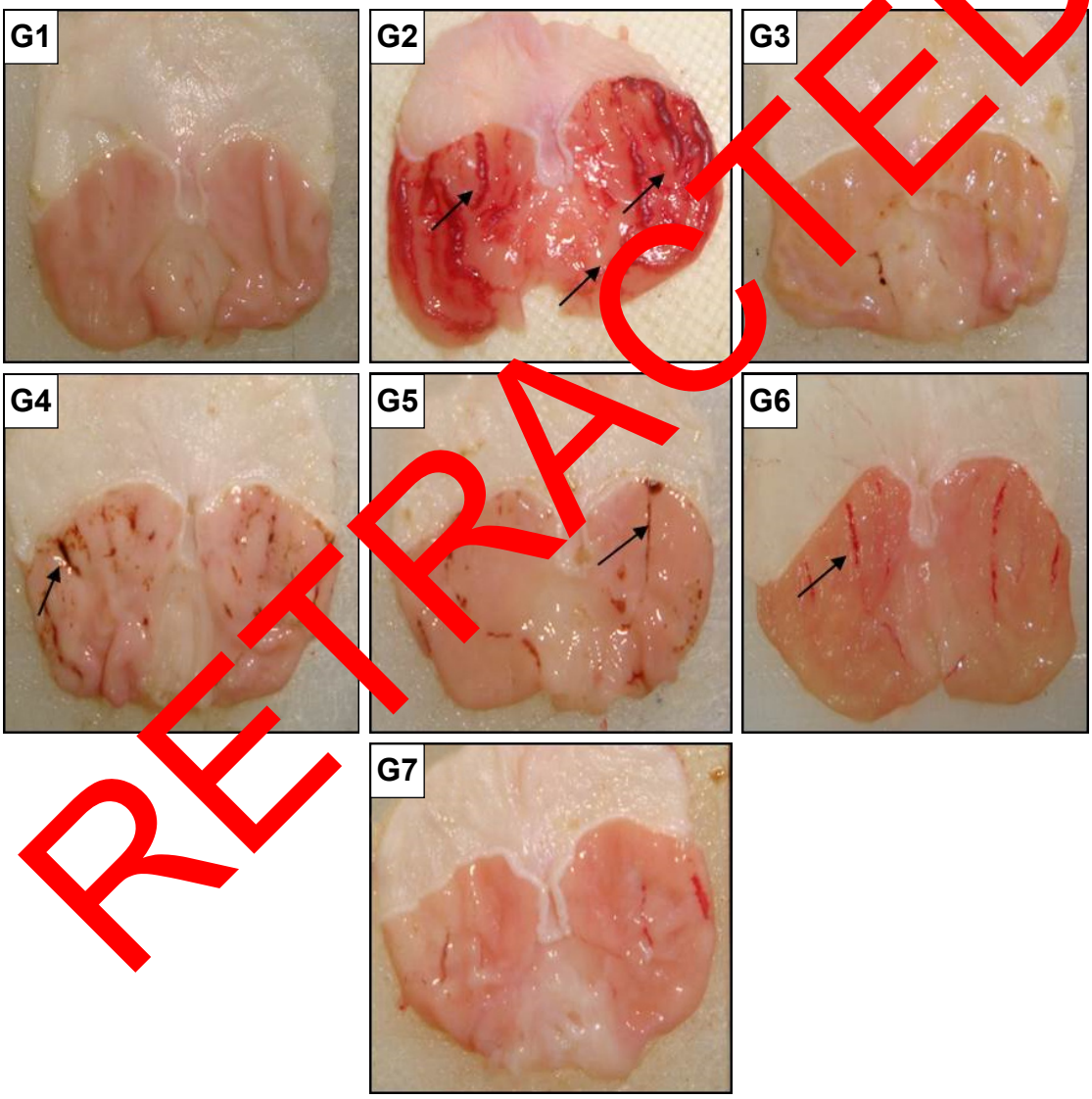

Figure 4 The effect of *Cibotium barometz* on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in male SD rats.
Notes: G1 (normal control group) showed no injuries to the gastric mucosa, G2 (ulcer control group) had severe injuries to the gastric mucosa, G3 (omeprazole) showed mild disruptions of the surface epithelium in the gastric mucosa. G4, G5, G6, and G7 given 62.5, 125, 250, and 500 mg/kg, respectively, doses of *C. barometz* extract showed moderate-to-mild disruptions of the surface epithelium of the gastric mucosa in a dose-dependent manner. Black arrows point to the hemorrhagic bands.
Abbreviation: SD, Sprague Dawley.

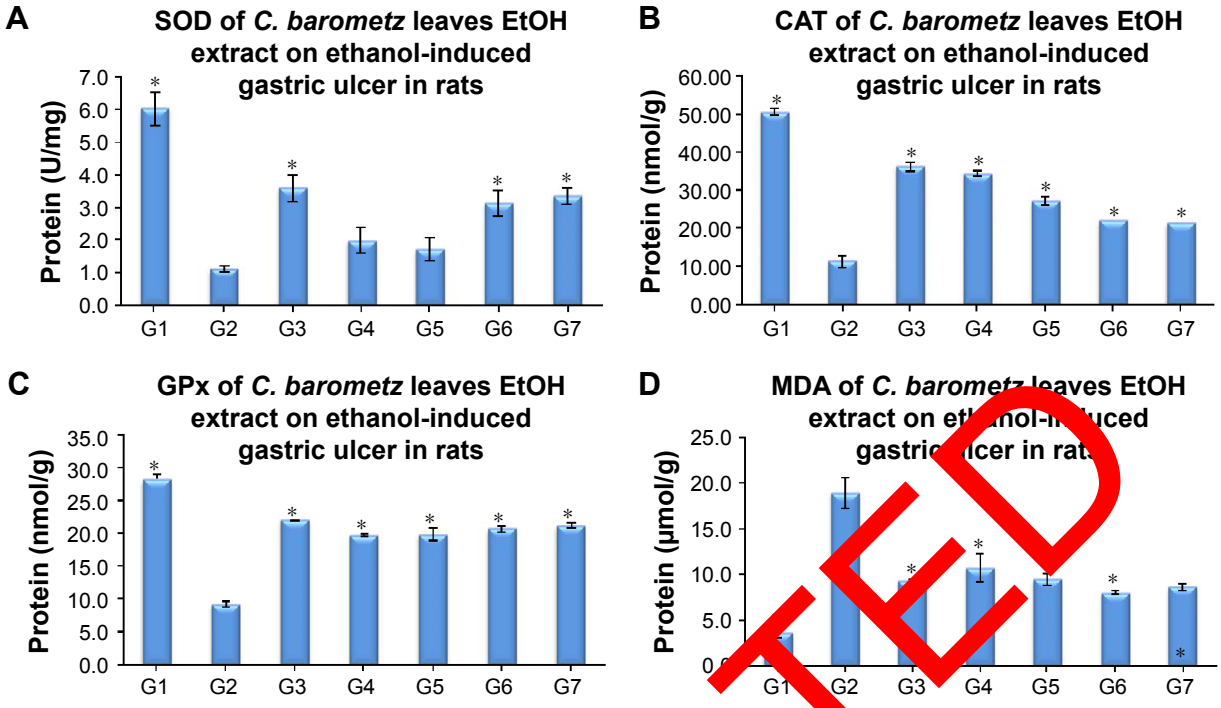

Figure 5 Effect of *Cibotium barometz* ethanol extract on gastric tissue homogenate of SOD (**A**), CAT (**B**), GPx (**C**), and MDA (**D**) assays.
Notes: G1 (normal control group), G2 (ulcerated control group), G3 (omeprazole), G4 (62.5 mg/kg), G5 (125 mg/kg), G6 (250 mg/kg), and G7 (500 mg/kg) of *C. barometz* ethanol extract. All values (in triplicate) are expressed as mean ± standard error of the mean. *Significant at $P<0.05$ compared to ulcerated group. SOD, CAT, and GPx, antioxidant activities were higher in G1, G3, and G4–G7 than G2, although MDA of G2 was higher than the other groups.
Abbreviations: *C. barometz*, *Cibotium barometz*; CAT, catalase; GPx, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.

a significant increase in the rats pretreated with *C. barometz* leaf ethanol extract and omeprazole with respect to G2. On the other hand, the MDA enzyme activities of *C. barometz* leaf ethanol extract in G4–G7 groups were significantly lower than in the G2 ulcerated control group as seen in Figure 5D.

Histological evaluation of gastric lesions

Hematoxylin and eosin staining and PAS staining

Histological observation demonstrated comprehensive damage to the gastric mucosa in the ulcerated control group of animals. Furthermore, the ulcerated rat control group had necrotic lesions in the deep gastric mucosa, which showed extensive leucocyte infiltration and edema of the submucosal layer, as illustrated in Figure 6. Otherwise, the animals pretreated with *C. barometz* leaf ethanol extract in the G4–G7 groups displayed relatively enhanced protection of the gastric mucosa with a depression or absence of infiltration of leucocytes and edema (Figure 6). *C. barometz* extract demonstrated protective effects in a dose-dependent manner and revealed remarkably better protection of the gastric mucosa. The gastric mucosa in the pretreated experimental groups, depending on the dose, showed a gradual increase in PAS staining intensity by the accumulation of the magenta color the mucosal cell layer compared to the ulcerated group (Figure 7). Nevertheless, this magenta staining decreased and was observed to be not plentiful in the gastric mucosa of the ulcerated group where the ulcer was induced with ethanol.

Immunohistochemistry

In the gastric mucosa, as shown in Figure 8, the expression of the HSP70 protein was downregulated in the ulcerated control group G2 but upregulated in the animals pretreated with omeprazole (G3) or with *C. barometz* leaf ethanol extract (G4 to G7). Furthermore, the immunohistochemical staining of Bax protein in the gastric mucosa elucidated upregulation in the ulcerated group while downregulation was manifested in rats pretreated with *C. barometz* leaf ethanol extract (Figure 9).

Discussion

The results of the present work clearly demonstrated that oral feeding of *C. barometz* extract did not manifest any signs or symptoms of toxicity and this observation is consistent with the outcome of other studies using different herbal plant extracts.[34–37] The data established that *C. barometz* extract has potent antioxidant activities and free radical

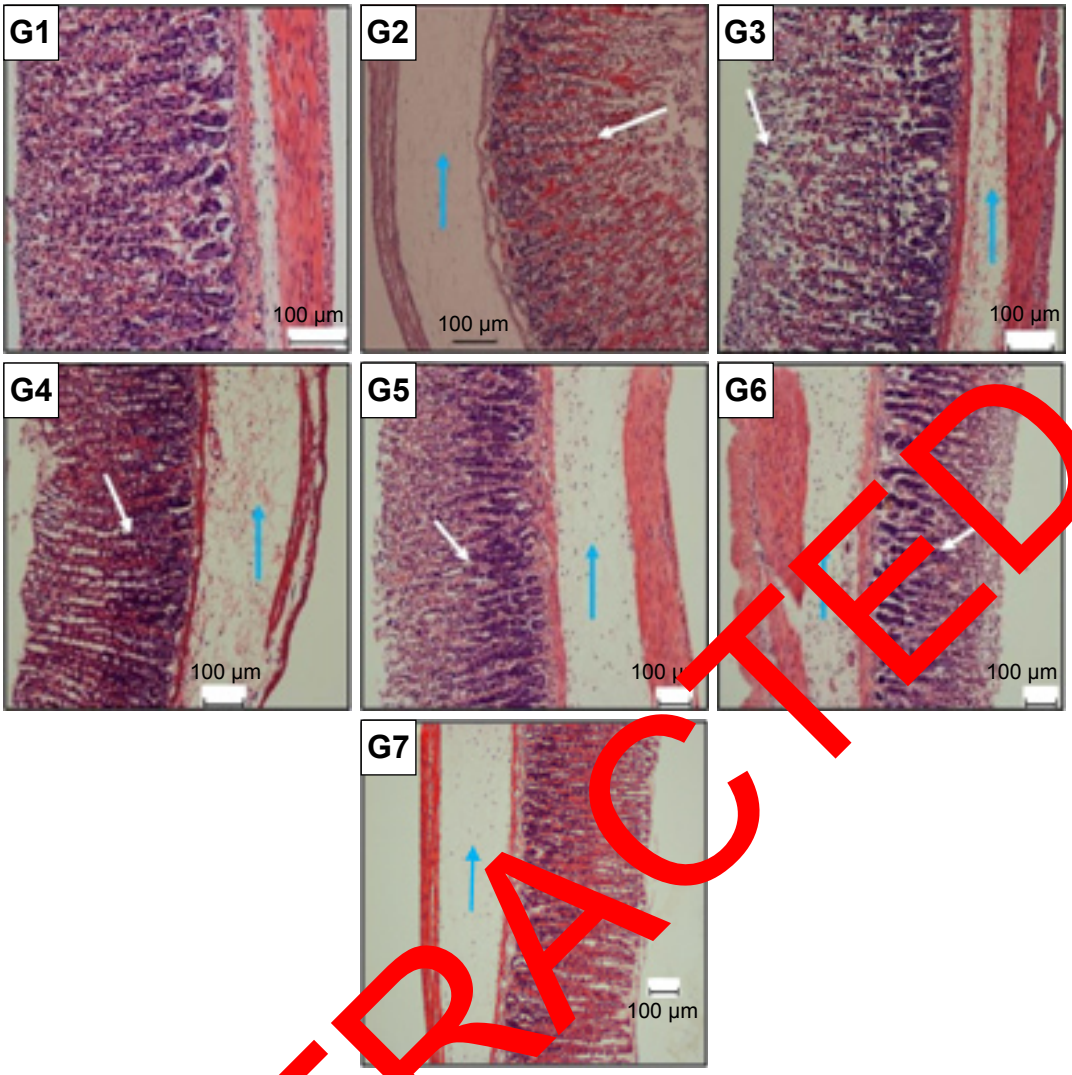

Figure 6 The effect of *Cibotium barometz* on the ...logy (hematoxyl... eosin staining) of ethanol-induced gastric mucosa damage in male Sprague Dawley rats.
Notes: G1 (normal control group) had inta... surface ...cosal epitheliu... no lesion; G2 (ulcerated control group) had a severe disruption of the surface epithelium and necrotic lesions; G3 (omeprazole) had a mild disruption o... surface epithelium and reduction in submucosal edema with leucocyte infiltration. The animals pretreated with *C. barometz* extract in the G4 (62.5 mg/kg), G5 (125 mg/kg), ... (250 mg/kg), and G7 (500 mg/kg) groups revealed a moderate-to-mild disruption of the surface epithelium, reduction in submucosal edema, and ...ucocyte infiltration in a do... dependent manner as shown by the reduction in or absence of the ulcer area in the treated groups (white arrows), submucosal edema and ...cocyte infi...tion (blue arrows).

scavenging e...cts in ...itro. Si...ly, the herbal medicine has antioxi...nt effic... and causes neutralization of free radicals as rep...d by several co-researchers.[38–41]

This investig...ive study demonstrated that ethanol induces severe disrup...ion of stomach mucosa, which results in the reduction of the release of bicarbonates and mucus, and increases the acidity of gastric content. Similar results have been reported by several coresearchers.[15,42,43] The judgment of this study additionally illustrated that *C. barometz* extract significantly decreases the secretion of gastric acid. The results here are analogous to the previous reports.[25,44,45] It is well-established that gastric acid secretion plays a role in gastric ulcer.[34] It is promising that amplification of gastric mucus may contribute to the gastroprotective consequence of *C. barometz* extract. The gastric mucus layer is believed to play a vital role in mucosal protection against endogenous aggressors such as acid and pepsin and also acts as a mediator of restoration of the mucosa.[46,47] The results of the current experiment demonstrated that oral administration of ethanol induces severe destruction of the gastric mucosa, resulting in disruption of vascular endothelium and increased vascular permeability, edema, and leucocyte infiltration of the submucosal layer. Rats fed with *C. barometz* extract showed remarkably protected gastric epithelium. Several previous studies have published results that are in agreement with the observations.[2–4,37,48]

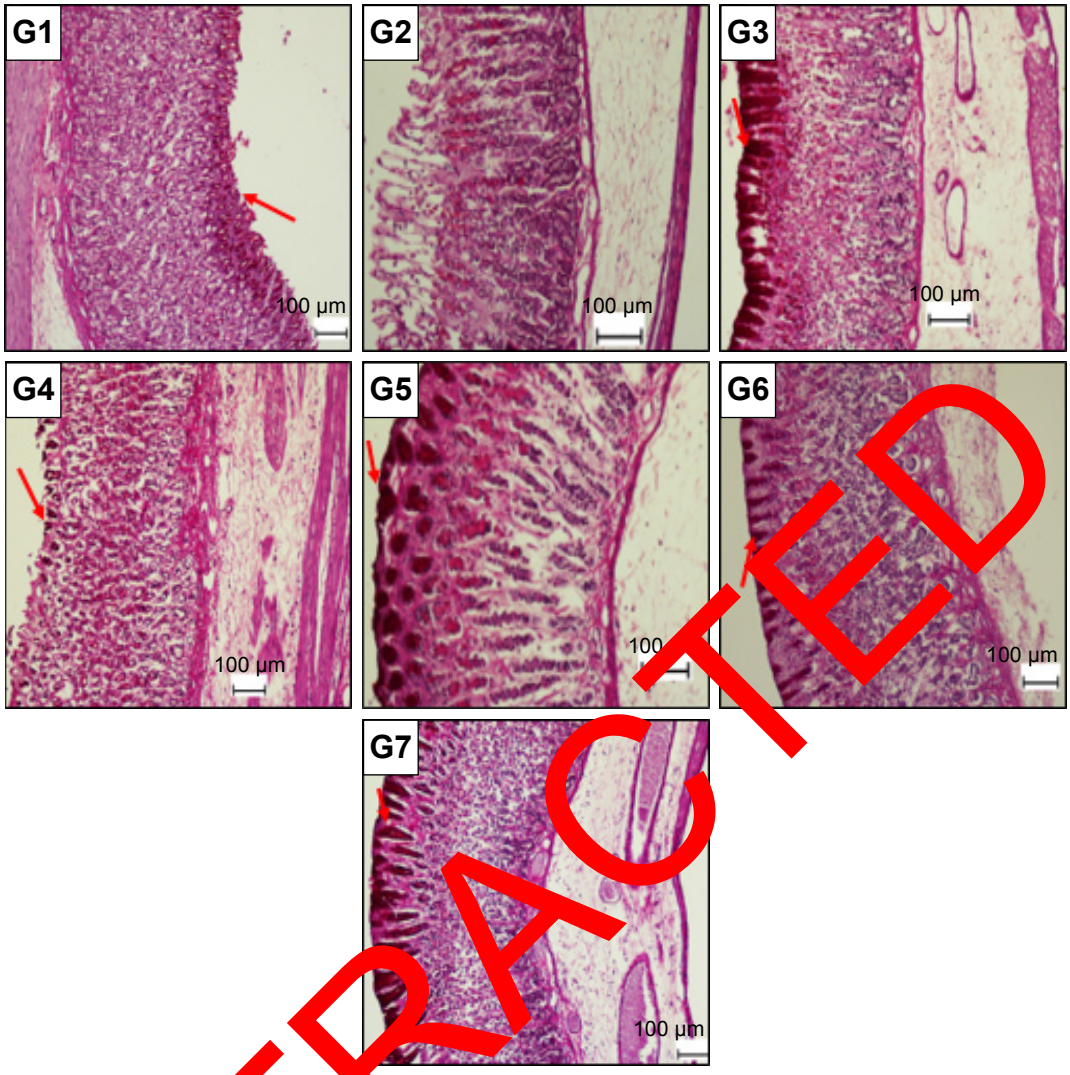

Figure 7 The effect of *Cibotium barometz* ... gastric tissue glycoprotein PAS staining in ethanol-induced gastric ulcers in male Sprague Dawley rats.
Notes: G1 (normal control group) had ... accumulation of the magenta color in the mucosal cell layer; G2 (ulcer control group) had decreased magenta color; G3 (omeprazole), G4 (62.5 mg/kg), G5 (125 mg/kg), G6 (250 mg/kg), and G7 (500 mg/kg) showed an increase in PAS staining intensity through the accumulation of magenta color in the mucosal cell layer compared to the ulcer ... group in a dose-dependent manner. Red arrow indicates PAS staining of the glycoprotein.
Abbreviation: PAS, periodic acid–Schiff.

This investigation demonstrates that administration of ethanol caused a disruption of the gastric mucosa layer that protects the gastric mucosa and a decrease in the activity of protective factors such as SOD, GPx, and CAT enzymes. Moreover, there was an increase in the microvascular permeability and lipid peroxidation of the cell membrane in the stomach epithelium. Therefore, it might be proposed that *C. barometz* extract mediates the protection of the gastric mucosa as a result of its scavenging mechanism.[49] Furthermore, *C. barometz* extract demonstrated protection of the cell membrane via the activities of SOD and CAT elevations from reactive oxygen species (ROS) attack. ROS is one of the main destructive mechanisms of ethanol in gastric cells.[44,50] On the other hand, a significant decrease in the MDA levels of animals pre-treated with *C. barometz* extract was observed, which might be due to the reduction in oxidative gastric injury.[39,51] These findings were matched with the previous study.[52,53] Further, it suggests that GPx plays a remarkable intracellular antioxidant role in the defence of the gastric mucosa from impairment.[10,54–57] In the present study, the lack of a submucosal area in the gastric layer mucosa and reduction of leukocyte infiltration in gastric wall sections is resulted of pre-fed rats with *C. barometz*. Edema and hemorrhagic abrasions in the mucosal layer in the ulcer control group is known as an indicator of ethanol damage. These effects agree with other results reported by several researchers.[32,56,58–62] In the PAS staining assessment, this study demonstrated that *C. barometz* extract heightened the

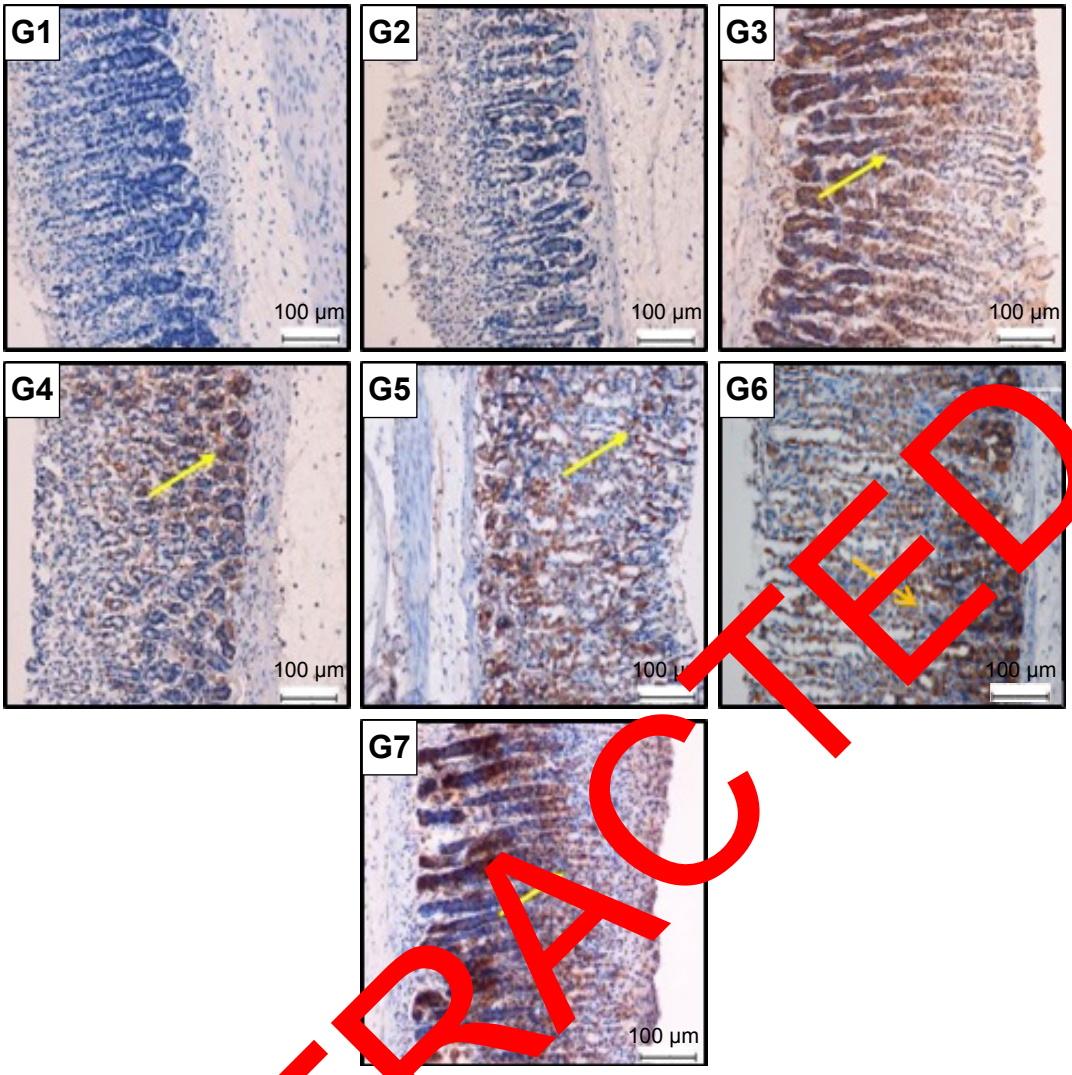

Figure 8 Immunohistochemical analysis of HSP7 ession in the ga ic mucosa of male Sprague Dawley rats.
Notes: G1 (normal control group), G2 (ulc ted co ol group), G3 (eprazole), G4 (62.5 mg/kg), G5 (125 mg/kg), G6 (250 mg/kg), and G7 (500 mg/kg) of *Cibotium barometz* extract. HSP70 protein expressio was upregul d in rats pretreated with *C. barometz* in a dose-dependent manner and also with omeprazole. Downregulation appeared in the ulcerated control grou ellow arrow indic the brown stain of HSP70.
Abbreviation: HSP70, heat shock otein 70.

content of glycop in in gastric ucosa (the magenta color). The i rease the p d tion of mucus was an indicator o cal ga mucosal defence, which is consistent with the re s published by many investigators.[29,63–65] Immunohistochem try results indicated that pretreatment of rats with *C. barometz* extract produced upregulation of HSP70 protein, which protected the cells from oxidative stress or heat shock. Moreover, the downregulation of HSP70 protein expression is one of the types of gastric damage that was characteristic of the ulcer group, which is similar to studies reported by a huge number of coworkers.[21,27,66,67] The generation of ROS by ethanol led to downregulation of HSP70 expression and upregulation of Bax proteins. Otherwise, Bax protein expression was downregulated and HSP70 protein expression was upregulated in the group pretreated with *C. barometz* leaf extract compared to the ulcerated group, which agrees with the results of many researchers reported.[10,12,35,36,68]

Conclusion

To sum up, *C. barometz* leaves presented antiulcer effects against ethanol-induced gastric lesions in the animal model significantly and dose-dependently. The gastroprotective consequence of *C. barometz* could be associated with the effective direct radical scavenging activity and increasing of the cellular antioxidant activities of SOD, CAT, and GPx levels along with decreasing of lipid peroxidation with upregulation of HSP70 protein and downregulation of Bax protein.

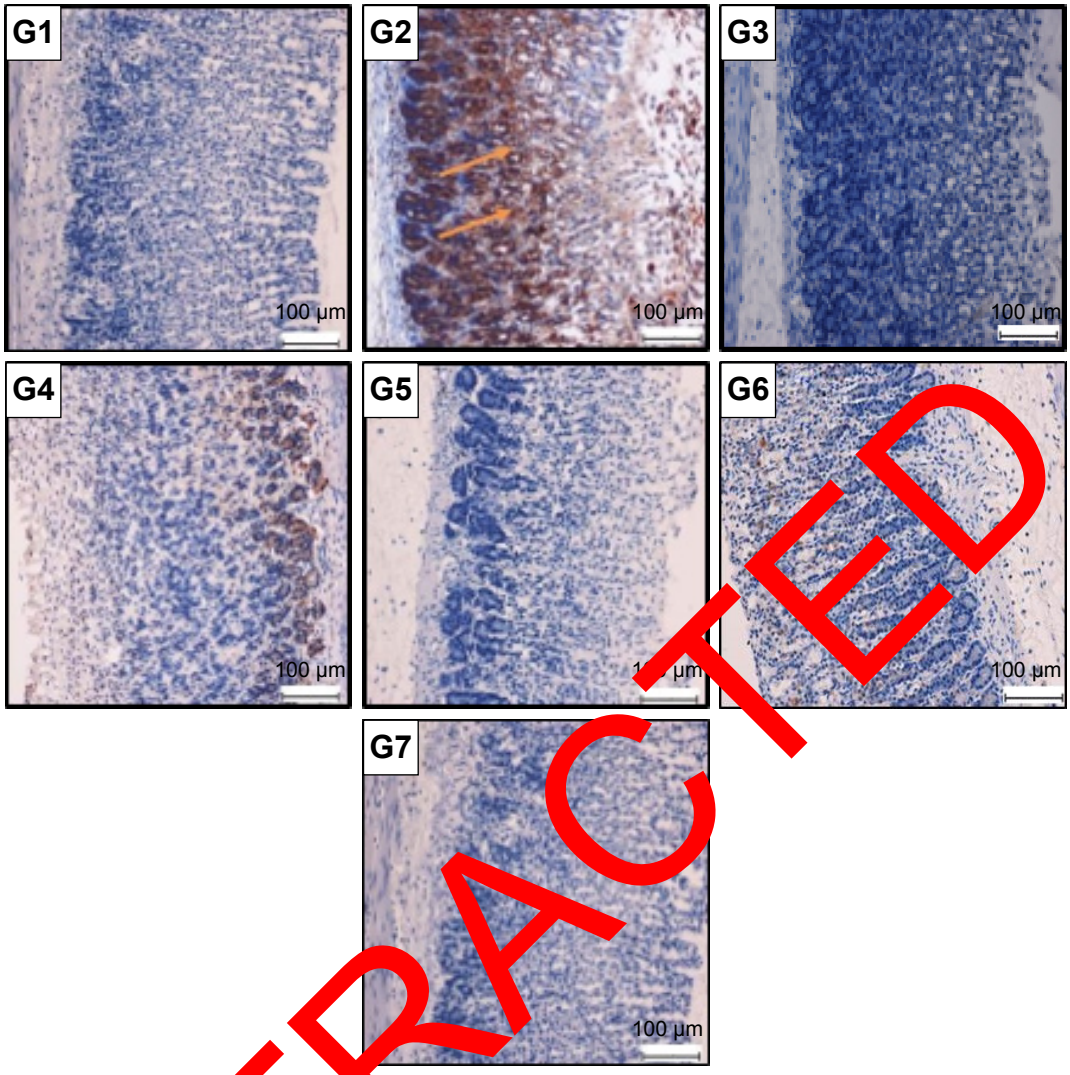

Figure 9 Immunohistochemical analysis of expression of Bax protein in the gastric mucosa of male Sprague Dawley rats.
Notes: G1 (normal control group), G2 (ulcerated control group), G3 (omeprazole), G4 (62.5 mg/kg), G5 (125 mg/kg), G6 (250 mg/kg), and G7 (500 mg/kg) of *Cibotium barometz* extract. Bax protein expression was downregulated in rats pre-treated with *C. barometz* in a dose-dependent manner and also with omeprazole, although upregulated in the ulcerated control group. Orange arrow indicates the stain of Bax protein.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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