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Original article

Gastroprotective effects of *Polygonatum odoratum* in rodents by regulation of apoptotic proteins and inflammatory cytokines

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ABSTRACT

In an increasing interest in natural antiulcer compounds that may have gastric healing effects and possibly prevent ulcer recurrence, *Polygonatum odoratum* appears as a strong candidate. The gastroprotective potentials of *P. odoratum* rhizome extract (PORE) were explored on ethanol-induced gastric ulceration in rats. *Sprague Dawley* rats were caged in 5 groups, normal and ulcer control rats received CMC (1% carboxymethyl cellulose). Omeprazole (20 mg/kg) was given to reference Rats. Experimental rats were treated with 250 mg/kg and 500 mg/kg PORE, respectively. After an hour, the normal control rats received 1% CMC, whereas rat groups 2–5 were given absolute ethanol by oral gavage. After 60 min, rats received anesthesia and were sacrificed. Dissected gastric tissue was analyzed by histopathological and immunohistochemical techniques. PORE treatment significantly lowered the ethanol-induced gastric injury, as shown by up-surging gastric pH and mucus content, reduced leukocyte infiltration, lower ulcerative areas in mucosal layers, and increased antioxidants (SOD and CAT) and (MDA) levels. Furthermore, PORE pretreated rats showed significantly increased expression of the Periodic acid-Schiff (PAS), HSP-70 protein, and decreased Bax protein in their gastric epithelial layers. PORE treatment showed an important regulation of inflammatory cytokines shown by decreasing the TNF-a, and IL-6 and increasing the IL-10 values. The detected biological activity of PORE is encouraging and presents the scientific evidence for its traditional use as a gastroprotection agent however further studies are required to determine the exact phytochemicals and mechanism pathway responsible for this bioactivity.

1. Introduction

Gastric ulcer is still the most known digestive system disorder around the globe. Recent statistics revealed that nearly four million cases were recorded each year with a prevalence of 5–10% during the lifetime of a nation (Abbasi-Kangevari et al., 2022). Worldwide, numerous diversity exists in following the healthcare protocols of gastric ulcers in terms of prevention, identification, management, and curing (Kruk et al., 2018). Scientists have proven that imbalance between ulcerogenic and defensive factors will lead to the production of gastric mucosal damage and gastric ulcers. Aggressive factors such as an unhealthy diet, alcohol overconsuming, increased gastric acid secretion, bacterial infection, genetic disorder, and chemical synthetics. While, protective factors of the stomach include any molecules that induce gastric mucus secretion and preserve intact mucosa in case of changes in the pH, temperature, and microbial infection that can stimulate the inflammatory process (Bitar and Moussa, 2022). Synthetic chemicals (Omeprazole) is a well-known proton pump inhibitor (PPI) used widely for gastric disorders, but recent studies have reported several adverse

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effects of this drug including osteoporotic fracture (Lespessailles and Toumi, 2022), heart attack (Oo and Lim, 2022), and galactorrhea in kidney transplant cases (Dorji et al., 2022). Recently, a population based-study revealed that significant correlation between intake duration intake and dose-dependence of administering PPI with the increasing incidence of stomach cancer (Cheung et al., 2018). The mentioned data reinforce the search for alternatives, such as natural products, which were continuously explored for gastric healing and gastroprotection effects (Shady et al., 2022; Shareef et al., 2022).

Polygonatum species (family Asparagaceae) is well-known plant species that grows in the Northern Hemisphere that includes 71 species with only 37 species named as folkloric herbal medicine based on records in Traditional Chinese Medicine (TCM), Ayurveda and Iran traditional medicine (Zhao et al., 2018). In TCM, PORE is identified as "Yuzhu", and has been used for centuries for various health problems, Yin-nourishing (action-oriented energy) and lung diseases, muscular disorders, and osteoporosis (Zhao et al., 2019). Most of these pharmacological activities of *P. odoratum* were correlated with its phytochemical contents including increased polysaccharide levels in its rhizomes (Cui et al., 2018; Zhao et al., 2018). In the past decade, scientists have explored *Polygonatum* polysaccharides for different biological activities. The chemically prepared and natural polysaccharides from *P.* cyrtonema Hua. showed significant antiherpetic activity against herpes simplex virus (HSV) (Liu et al., 2011). Furthermore, researchers have reported immunoregulatory roles of isolated polysaccharides from *Polygonatum sibiricum* shown by down-regulation of IL −2, TNF −α, and up-regulation of IL −4 and IL −10 cytokines (Wang et al., 2020).

Polysaccharides are commonly known as polymers bonded by glycosidic bonds, as biomolecules play various roles in cellular processes. In recent years, searching and isolation of natural polysaccharides have increased due to their various bioactivity (immune stimulation), lesser toxicity, and minimum side effects (Jabbar et al., 2022b; Zhao et al., 2021). Previously, scientists have shown the polysaccharide potentials in the stimulation of phagocytic activity (macrophage activation) by increased formation of ROS (reactive oxygen species), facilitating the release of cytokines and chemokines (Devi et al., 2015). Furthermore, in vivo, studies have revealed the significant potential of natural polysaccharides in boosting the immune system in different animal trials with reduced immunity mitigated by viral disease, environmental pollution, or synthetic chemicals (Li et al., 2022). Polysaccharides have been also linked with the reduction of free radicals and oxidative stress, an important risk factor for a gastrointestinal disorder (gastric ulcer) (Ma et al., 2022). *P. odoratum* has been well-known as a rich source of polysaccharides bearing different biological activities, anti-diabetic (Shi et al., 2023), antioxidant (Zhou et al., 2015), and anti-inflammatory (Wang et al., 2018). The phytochemical profiling of *P. odoratum* revealed different chemical compounds belonging to the class of polysaccharides (mannose, rhamnose, glucose, galactose, arabinose), homoisoflavanones ((E)-5,7-dihydroxy-6,8-dimethyl-3-(4′-hydroxybenzylidene)chroman-4-one and 4′-demethylleucomin 7-O-D-glucopyranoside or (E)-7-O–D-glucopyranoside-5-hydroxy-3-(4′-hydroxybenzylidene)chroman-4-one) serving as hypoglycemic agent (Kwon et al., 2020), and saponine (polygodoside A-F and (25S)-L-O-acetylspirost-5-ene-1β,3β-diol 3-O-β-D-glucopyranosyl-(l->2)-[β-D-xylopyranosyl-(l->3)]-β-D-glucopyranosyl-(l->4)-β-D-galactopyranoside) (Kwon et al., 2020). playing as hypoglycemic, immunoregulator, procoagulant, antifungal, and anticancer agents (Bi et al., 2023).

As declared by now, numerous studies have reported different bioactivities of *P. odoratum*, however, the present investigation is considered the first scientific exploration of the antiulcer potentials of PORE. The investigation of gastroprotection roles of PORE was explored by assessing its alteration of different biological signaling pathways.

2. Materials and methods

2.1. Plant collection

PORE was found on the Zine-Asterokan Mountain (Karokh mountain) Choman – Erbil, Iraq. Authentication of the plant was made by botanist Prof. Dr. Abdul Hussain Al Khayat based on common features of this genus found in Iraqi flora. The voucher number (6732) was obtained from the Herbarium of Biology department, College of Science, Salahaddin University, Iraq.

Plant roots were washed with water and dried in a shaded place at room temperature (20–25 °C). Then, the roots were converted to powder by grinding mill until particle size became 710 um sieves. Obtained Powder was stored in dark bottles for later analysis.

2.2. Acute toxicity test

The test was displayed as an obligatory safety test for PORE biological activity to avoid any undesirable changes. Acute toxicity protocol followed as specified in the OECD guidelines, 2002. Rats were purchased from the Animal House Unit of Cihan University-Erbil. Rats were receiving regular pellet ad libitum and water. Albino Dawley rats (36 in both gender) were divided equally into 3 clusters, vehicle (G1, 1% CMC); low dose (G2, 2500 mg/kg PORE), and high dose (G3, 5000 mg/kg of PORE) groups. Dose selection was based on previously published toxicity studies (Jabbar, 2021; Salama et al., 2012). Food was removed before one day of treatment with free access to water. The observation process continued for 24 h (every 30 min) and 24 h after dosing for possible toxic symptoms or death. On the last day of the experiment, food was removed from rats for 24 h, and then rats were sacrificed applying common anesthesia (Ketamine and Xylazine) at 3 mg/kg, 100 mg/mL dosage (Al-Medhtiy et al., 2022; Mahmood et al., 2010). Intracardiac puncture Blood was withdrawn for the biochemical analysis (liver and kidney parameters). Rats were eventually sacrificed and dissected kidney and liver organs were analyzed histopathologically by H & E stain to determine any tissue damage or structure disturbance (Jabbar et al., 2023).

2.3. Ethical approval for rat usage

Rats weighing 220–260 g were provided by the Animal House Unit, (Ethics form BIO/14/https://doi.org/10/2022/M.A.A.), Cihan University-Erbil. Rats were handled according to the standards bordered "Director Care Usage Research Laboratory Animals" organized set "Nationwide Conservatory Knowledge" available via "Nationwide Institution Healthiness (USA)" (Garber J et. al. 2011).

2.4. Induction of stomach ulcer

Rats were clustered (5 groups with 6 rats each) in regular cages (widespread net bottom) to avoid coprophagia. They were fed on a standard diet and water. For easy adaptation, rats stayed in these cages for 7 days. Then treated as follows:

Groups A & B were treated with 1% CMC by oral gavage.
Group C was treated with Omeprazole (20 mg/kg dissolved in CMC).
Group D & E were treated with PORE 250 mg/kg and 500 mg/kg, respectively.

After 1 h, all rats (except normal control) received alcohol (100% intensity, 5 ml/kg) by oral gavage to induce peptic ulcer. After another hour, rats were given a high dose of ketamine & xylazine, sacrificed, and blood samples were taken by an intracardial perforation for the biochemical investigation (Jabbar et al., 2022).

2.5. Gross study

The obtained stomach opened at larger curvature and the obtained stomachs were cleaned and observed by microscope. Gastric injuries on the epithelial layers were observed as a thick red-line lesion. Several photos of gastric gross views obtained from all rat groups were taken and the area of the stomach lesion was identified by applying Image J software.

Inhibition % (I %) is measured as follows:

I% = UAcontrol– UAtreated / UAcontrol × 100 (Al-Wajeeh et al., 2017).

2.6. Evaluation of gastric pH

Stomach fillings were collected gathered and hydrogen ion intensity was found by using a pH meter (mEq/L) (inoLab pH 7310P BNC, Weilheim, Germany) and 0.1 normality of NaOH as titrant (Saremi et al., 2019).

2.7. Estimation of gastric mucus

The dissected stomachs were cleaned with saline (ice-cold phosphate buffer) and the mucosal layers of the stomach were carefully removed by clean slide. The collected mucus was weighted by electrical balance (Abdulla et al., 2010).

2.8. Histological evaluation

Gastric slices (1–2 cm) were kept in a buffer (10% formalin) for 24 h at room temperature. Gastric tissues were dried by using ethanol, cleaned with xylene, and then, gastric tissues were treated with paraffin in a tissue processing machine. Finally, tissue biopsy was blocked in paraffin and a small sliced piece 5 μm was put on a slide for microscopic observation (Leica Rotation Microtome) (Jabbar et al., 2022a). Scoring for histological Semi-quantitative analysis was explored based on the previous guidelines (Arismendi Sosa et al., 2022) and the scores are given based on the appearance as follows:

Submucosal oedema, 0 None; 1 Mild; 2 Moderate; 3 Severe.
Damage/necrosis, 0 None; 1 Mild; 2 Moderate; 3 Severe.
Inflammatory cell infiltration, 0 None; 1 Mild; 2 Moderate; 3 Severe.
Haemorrhage, 0 None; 1 Mild; 2 Moderate; 3 Severe.
Perforation, 0 None; 1 Mild.

2.9. H and E stain

The slides were histologically examined by using Hematoxylin and Eosin stain as previously explained (Grant et al., 1989).

2.10. Periodic acid–Schiff stain (PAS)

The mucus content in the gastric epithelium was determined by staining a 5 μm sliced gastric wall with PAS stain to estimate the glycoprotein (high or low pH) of the gastric mucus according to the producer's instructions (Sigma (PAS) Kit, Merk, Germany). Photomicrograph was taken by Image J software (Alrdahe et al., 2010).

2.11 Immunohistochemical investigation

The gastric tissues were sliced at 5 μm and were stained with antibodies against immunostains (HSP70, 1:100 and Bax, 1:50) by using a standard Animal research kit (Santa Cruz, USA). Estimation of the stained cells was made by a light microscope. The positive brown-stained areas were calculated (μm2) and evaluated by applying Image J software. Then, the stained percentage of gastric cells in the pretentious areas was determined (Ismail et al., 2018).

2.12. Preparation of tissue homogenates and antioxidant enzyme evaluation

Stomach sections were cleaned gently with PBS. Sliced stomach layers were homogenized (10% w/v) and mixed with PBS forming a cocktail of mammalian protease inhibitors. Pooled gastric tissues from each group were homogenated for 10 min at 4 °C. Obtained supernatants were analyzed for antioxidant enzymes (SOD and CAT) and lipid peroxidation MDA levels. Laboratory protocols follow the manufacturer's guidelines (Elabscience, Wuhan, China) (Jabbar et al., 2022a).

2.13. Inflammatory cytokines

The obtained samples were also analyzed to determine the inflammatory cytokines (TNF-α, IL-6, and IL-10) by using an ELISA kit (Elabscience, Wuhan, China) based on the producer's instructions. Estimation of the cytokine strength was performed by utilizing the recombinant sanitized cytokines (Jabbar et al., 2022).

2.14. Statistical analysis

Statistical study information is accomplished by using Graph Pad Prism software (version 9), ANOVA, *t*-test correlation, and descriptive statistics. Data were presented as Mean ± SEM. The significance level for numbers was set at $p < 0.05$.

3. Results

3.1. PORE toxicity results

PORE treatment at 2500 and 5000 mg/kg dosage in rats showed the absence of undesirable changes throughout the 14 days of the trial. The rats looked very active without any abnormal or toxic signs. The observation process revealed the absence of significant changes in the food intake, body weight, attitude, or daily practice between all three rat groups without any record of death records even after the test finished. The histological analysis of kidney and liver tissues from different rat groups revealed non-significant changes in their tissue structure without any noticeable damage in their gastric layers (Fig. 1). The results of the blood parameters of experimental and normal control rats were found statistically non-significant. These results indicate that the toxic dosage of PORE could be higher than 5000 mg/kg (see Table 1A and Table 1B).

3.2. PORE effects on stomach gross, H and E, and PAS stain

The current results revealed that group A had a normal gross view of their gastric mucosal layers. Rats treated only with ethanol (B) experienced severe gastric lesions with multiple observable areas of gastric lesions based on their gastric gross analysis. Reference rats (C, receiving 20 mg/kg Omeprazole) showed minimum gastric mucosal damage in their mucosal linings compared to ulcer control rats. Rats addressed with 250 mg/kg of PORE (D) revealed

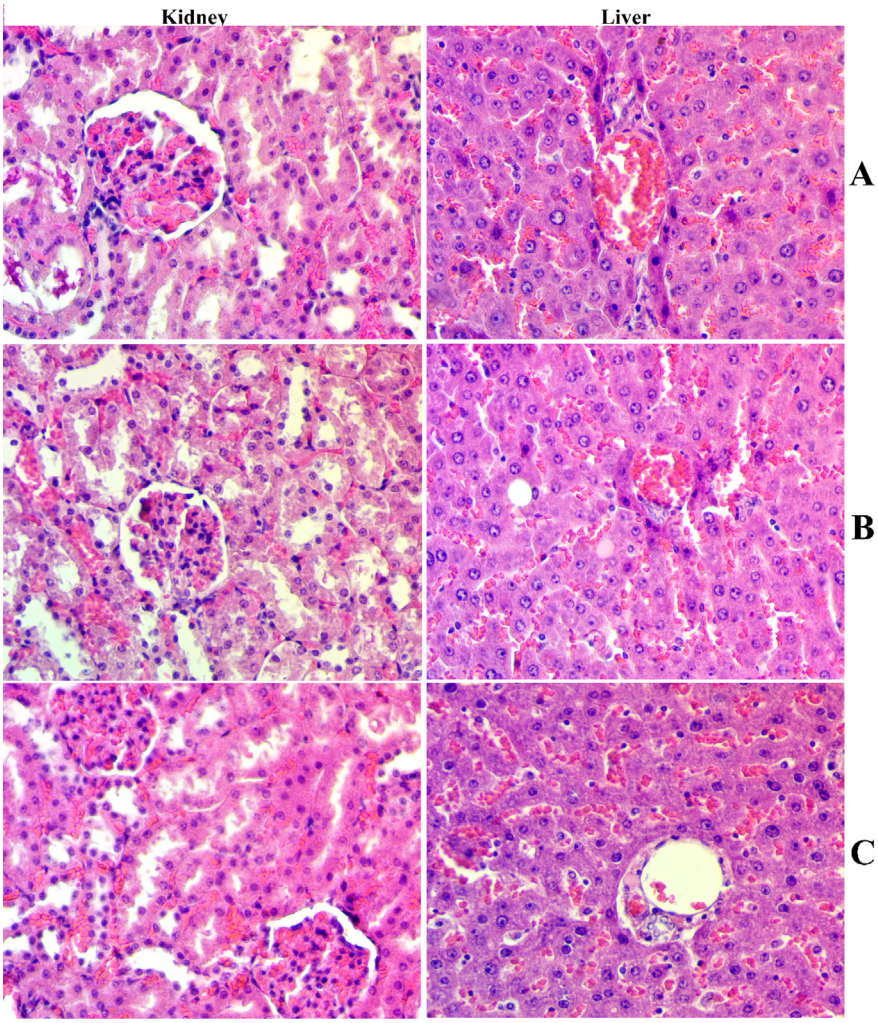

Fig. 1. Microscopic views of rat tissues in acute toxicity experiment. A, rats had only 1% CMC; B, 2500 mg/kg PORE-treated rats; C, 5000 mg/kg PORE-treated rats. The tissue structure of both organs was comparable between PORE-treated and normal control rats (hematoxylin and eosin, 40x).

Table 1A
Influence of PORE on blood (liver) parameters of rats in toxicity trial.

Groups	Albumin g/ L	Total bilirubin mmol/L	Alkaline phosphatase U/ L	Alanine aminotransferase U/ L	G-glutamyl transferase U/ L
G1	34.5 ± 1.8	<2	168.4 ± 3.1	82.11 ± 9.2	0.10 ± 0.4
G2	32.2 ± 2.0	<2	161.42 ± 2.5	79.24 ± 10.2	0.09 ± 0.3
G3	36.4 ± 3.0	<2	164.50 ± 5.2	80.22 ± 11.8	0.11 ± 0.3

Values are shown as Mean ± SEM (n = 6). Values were non-significant at $p < 0.05$. G1, normal rats were receiving 1% CMC; G2, rats ingested 2500 mg/kg of PORE; G3, rats ingested 5000 mg/kg of PORE.

Table 1B
Influence of PORE on blood (kidney) parameters of rats in acute toxicity test.

Groups	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Carbon Dioxide mmol/L	Anion gap mmol/L	Urea mmol/L	Creatinine umol/L
G1	140.2 ± 5.2	4.9 ± 1.6	106.2 ± 4.3	37.9 ± 2.8	15.3 ± 2.2	6.30 ± 1.0	34.40 ± 3.2
G2	150.2 ± 3.2	5.1 ± 1.8	108.30 ± 3.2	38.40 ± 1.3	13.20 ± 1.7	6.33 ± 1.8	30.34 ± 3.0
G3	159.0 ± 4.5	5.3 ± 2.1	104.5 ± 8.8	35.4 ± 5.3	12.2 ± 1.8	7.50 ± 2.0	29.9 ± 3.4

Values are shown as Mean ± SEM (n = 6). Values were non-significant between groups at $p < 0.05$. G1, normal rats were receiving 1% CMC; G2, rats ingested 2500 mg/kg of PORE; G3, rats ingested 5000 mg/kg of PORE.

significantly fewer ulcerated areas in their gastric mucosa than that found in ulcer control rats (Fig. 2). Rats ingested 500 mg/kg of PORE (E) showed a more flattened surface in their gastric epithelium with significantly fewer gastric mucosal injuries than that of the B and D groups based on the gross evaluation (Fig. 2).

The histopathological analysis by using H & E stains revealed that ulcer control rats (B) had several deep injuries in their gastric epithelia; ulcerations mainly due to significant penetration in the stomach mucosal epithelium with infiltration of edema leukocytes. PORE-treated rats showed significantly fewer gastric epithelial

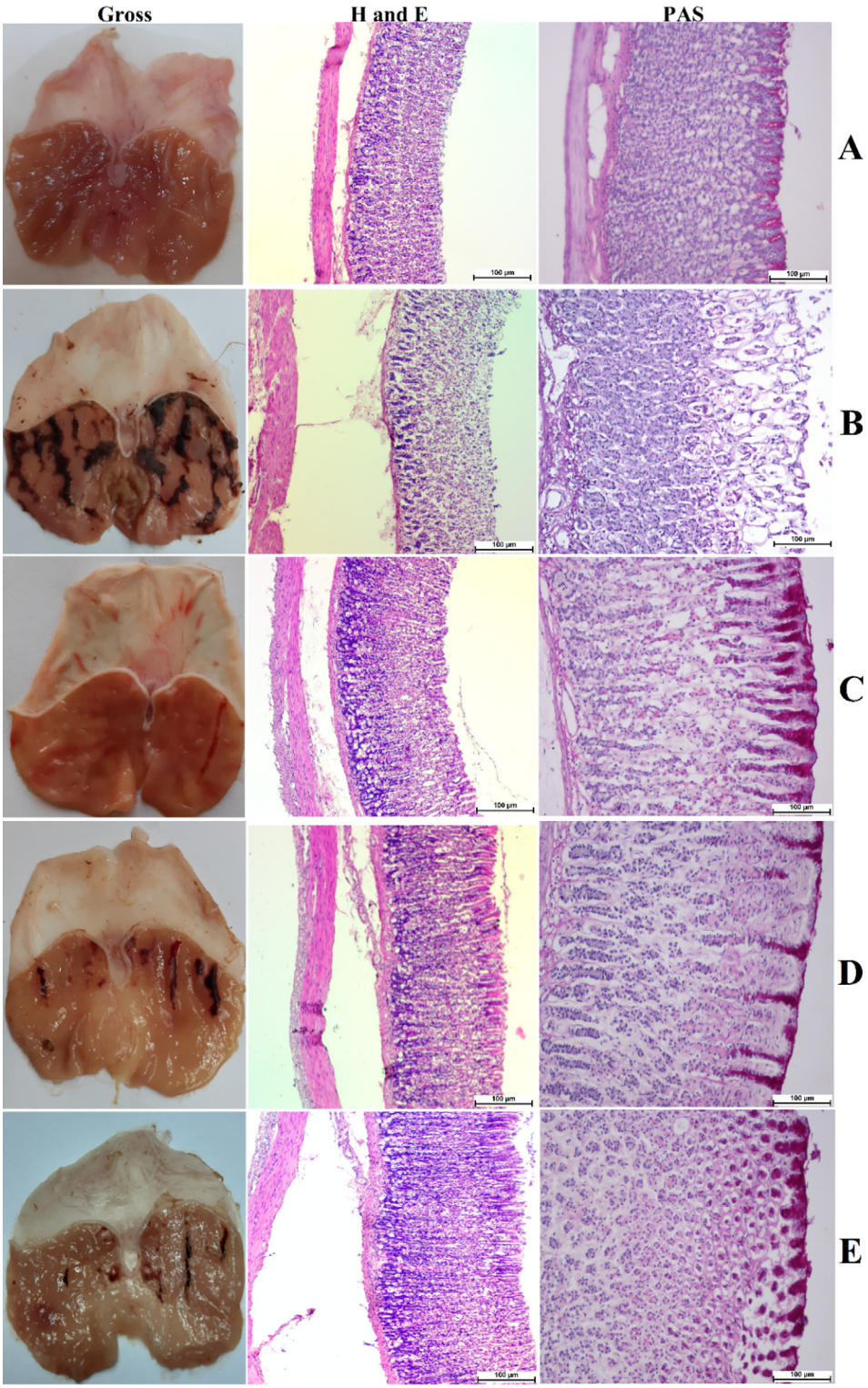

Fig. 2. Protective Effects of PORE on gross view and histological view (stained by H&E and PAS) of the gastric mucosa layers in ethanol-induced stomach ulcer rats. A, normal control rats; B, ulcerated rats; C, standard rats (omeprazole, 20 mg/kg); D, 250 mg/kg PORE-treated rats; E, 500 mg/kg PORE-treated rats.

injuries, less ulcer area (hemorrhoid bands), and edema leukocytes than that ulcer control rats (Fig. 2).

PAS staining technique provides an amount of the mucus polysaccharides, evidenced by the higher color intensity in the experimental (C, D, E) and normal control (A) groups. Ulcer control rats (B) had lower intensity PAS in their gastric mucosa than that of pre-treated rats. The PAS expressions were very comparable between reference (C) and PORE-treated rats (D and E). The outcome indicated that PORE has the potential to up-regulate the secretion of gastric mucus, which acts as a defense barrier against ethanol-inducer gastric injury (Fig. 2).

The microscopic scoring of gastric tissue damages showed higher scores for rats that received only ethanol (B group) than that of rats in other treated (C, D, and E) groups (Fig. 3).

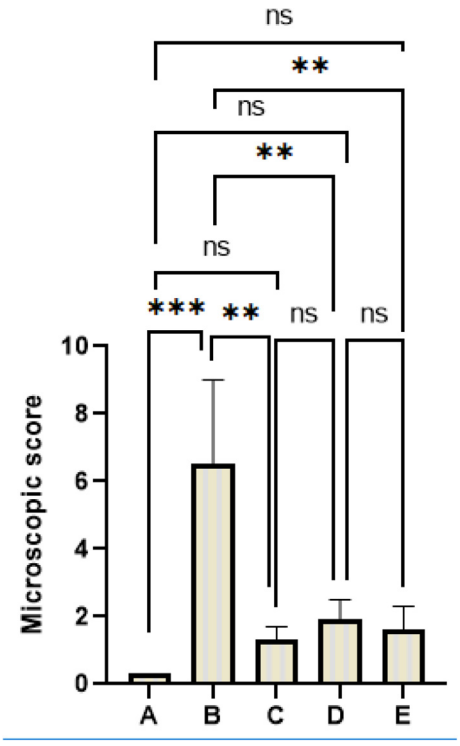

Fig. 3. Microscopic scores of the gastric injury from different groups. A, normal control rats; B, ulcerated rats; C, standard rats (omeprazole, 20 mg/kg); D, 250 mg/kg PORE-treated rats; E, 500 mg/kg PORE-treated rats.

3.3. PORE effects on mucus levels

Rats treated with PORE at low and high doses (250 and 500 mg/kg) revealed significant ($p < 0,001$) inhibition percentage of stomach ulcer formation (74.80% and 77.41%, respectively), compared that of ulcer control rats. Rats pre-ingested Omeprazole (20 mg/kg) also revealed a significant inhibition percentage (78.81%) of ulcer formation. These statistical results indicated that PORE treatment produced comparable inhibition effects in preventing the formation of stomach ulcers to that of the reference drug (Table 2). Furthermore, Ulcer areas were inhibited by the PORE treatment in a dose-based manner.

3.4. PORE effects on the gastric pH

Immunohistochemical results revealed that gastric pH was very comparable between normal control, PORE-treated, and reference drug-treated rats. Rats that received only ethanol (B) showed the minimum stomach pH (3.25 ± 0.04) compared to all rat groups. Rats pre-treated with standard drug (C) had almost similar gastric pH (5.82 ± 0.43) as normal control rats (5.98 ± 0.18). PORE treatment caused a gradual increase in gastric pH in a dose-dependent fashion. Which, rats ingested 250 mg/kg PORE showed a retrieval from reduced stomach pH caused by ethanol, but not as significant as rats ingested 500 mg/kg PORE. This indication shows PORE as a significant preservative of gastric pH in ethanol-induced gastric ulcers in rats (Table 2).

3.5. PORE effects on immunohistochemical stains

The normal control rats showed the lowest Bax reaction in the gastric mucosa of the basal part and apical part of the lining surface epithelium (Fig. 4). Results also showed that Significantly increased Bax protein expression was detected in the mucosal layer of the stomach obtained from group B (rats received only ethanol) compared to that of normal control rats. In the mucosa area, the observable reaction in the disrupted mucus cells was found (Fig. 4). By comparison, reference rats showed similar Bax stain intensity to that of normal control rats. PORE pre-treatment at the dose of 250 mg/kg and 500 mg/kg dose-dependently increased the expression of Bax protein in their gastric epithelial layers in ethanol-induced ulcers in rats.

The current immunohistochemical analysis by HSP-70 revealed that ulcer control rats had a decreased immunolabeled area in their gastric tissues compared to all rat groups. By comparison, PORE-treated rats had significantly higher immunolabeled areas for HSP-70 than that of ulcer controls, and almost similar brown color intensity of HSP-70 expression as reference drug-treated rats (Fig. 5).

3.6. Effect of PORE on gastric antioxidant enzymes

An alteration in the local ROS production and the endogenous antioxidants may cause oxidative stress-related gastric disorders (A. Ketuly et al., 2013). Data results have found that rats normal control rats (A) showed standard levels of antioxidant enzymes and lipid peroxidation (MDA) content. Rats with only ethanol treatment (B) showed significantly decreased antioxidant (SOD and CAT) enzymes activity and higher MDA (oxidative stress) content than that of rats pre-ingested reference drug (C) or PORE-treated rats (D and E). Reference drug treatment in ethanol-induced ulcer rats showed non-significant alteration in the gastric antioxidant enzymes compared to that of normal control rats. PORE treatment lead to a significant up-surging of antioxidant enzymes in a dose-related manner compared to ulcer control rats. Rates administered 250 mg/kg PORE (D) showed higher activity of SOD (399.87 ± 4.59 **U/mg**) and CAT (105.25 ± 3.13 **nmol/min/mg**) than that (165.15 ± 2.38 **U/mg** and 62.83 ± 2.15 **nmol/min/mg**) of ulcer controls; but not as significant as high dose 500 mg/kg PORE treated rats (D). Lipid peroxidation (MDA) statistics were significantly lower in high dose PORE-treated rats (115.17 ± 1.21 **μM/g**) than that (210.07 ± 3.17 **μM/g**) of ulcer control, but not significantly changed compared to A, C, and D groups, respectively (see Table 3).

Table 2
Show PORE effects on various stomach parameters of different experimental rats.

Groups	Treatments	Mucus weight	Gastric pH	Ulcer area	Inhibition %
G1	1% CMC	1.733 ± 0.15^{a}	5.98 ± 0.18^{a}	–	–
G2	100% ethanol	0.708 ± 0.17^{b}	3.25 ± 0.04^{b}	496.25 ± 4.28^{a}	–
G3	20 mg/kg Omeprazole	1.91 ± 0.36^{a}	5.82 ± 0.43^{a}	105.17 ± 3.23^{b}	78.81^{a}
G4	250 mg/kg PORE	1.62 ± 0.27^{a}	$5.65 + 0.27^{a}$	125.06 ± 3.73^{b}	74.80^{a}
G5	500 mg/kg PORE	1.89 ± 0.31^{a}	5.73 ± 0.36^{a}	112.12 ± 4.16^{b}	77.41^{a}

Values are shown as Mean ± SEM (n = 6). Values with distinct superscription within the same column means significant at $p < 0.05$. PORE, *Polygonatum odoratum* rhizome extract.

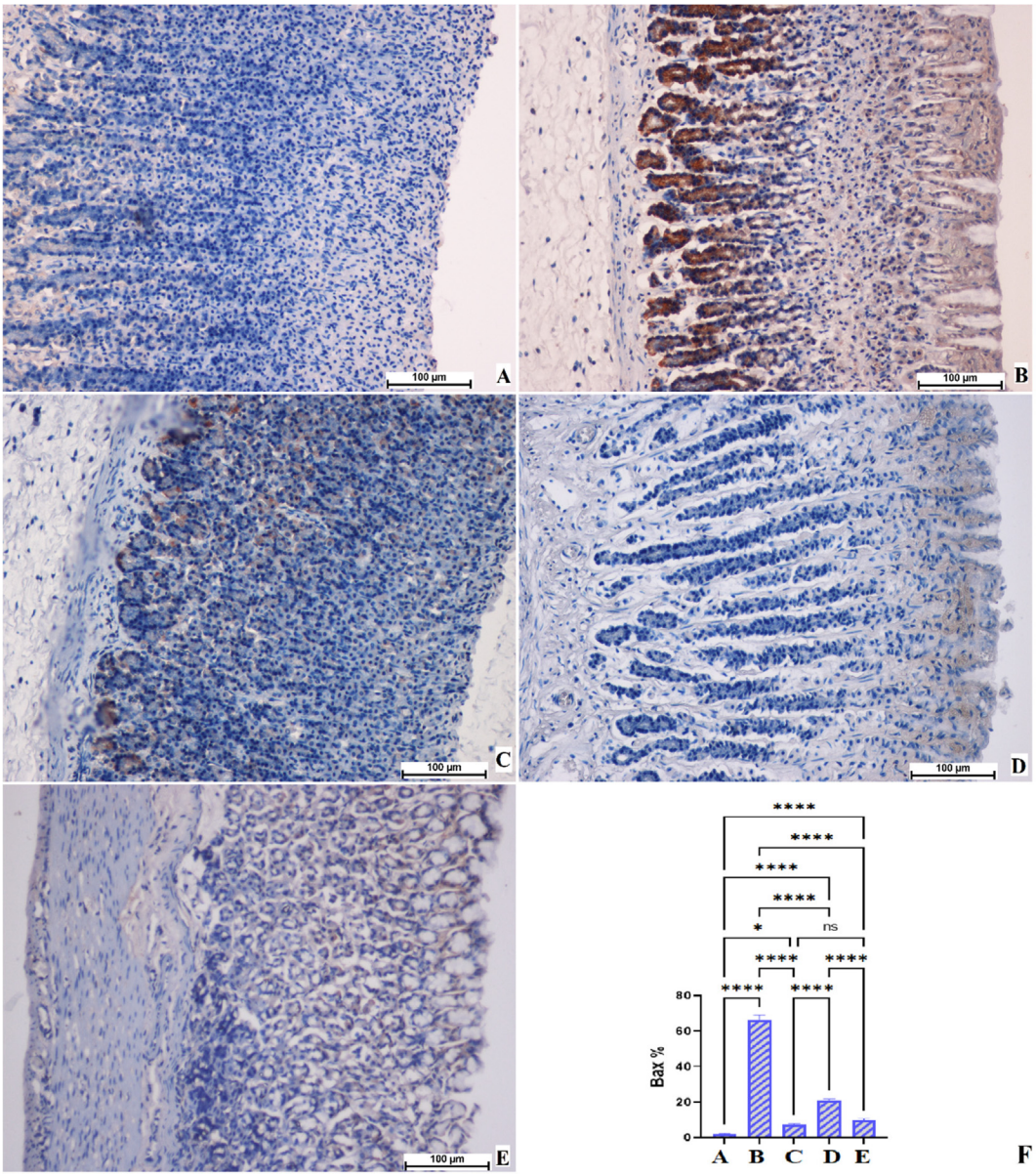

Fig. 4. PORE effects on the microscopic view of Bax protein expression in ethanol-induced gastric ulcer in rats. A, Negative controls were showed normal microscopic architecture of gastric layers; B, ulcer control rats experienced numerous tissue structural damage in the mucosal and submucosal layers, ulceration of the upper part, desquamation of the lining epithelium, dilated blood vessels with many edema and leucocytes in the submucosa; C, the omeprazole group showed a mild injury in their gastric mucosa; D, PORE 250 mg/kg treated rats had showed tissue damage in their gastric mucosa and an improvement in the tissue architecture; E, PORE 500 mg/kg treated rats were experienced mild disruption in the mucosa layer with almost same tissue architecture to that of reference rats and many, chief and parietal cells in the base parts with still some degenerated and rearrangement of gastric tissue (magnification, 20x).

3.7. Evaluation of PORE influence on inflammatory cytokines

Absolute ethanol ingestion stimulates an inflammatory process, causing increased production of TNF-α and IL-6 cytokines and decreased IL-10 cytokine (Zhou et al., 2020). Accordingly, current results have found up-regulation of pro-inflammatory and down-regulation of anti-inflammatory cytokines in ulcer control rats compared to normal control rats (B). PORE treatment established an immunomodulatory effect in the mucosal layer of stomach in ethanol-induced gastric ulcer rats by significantly ($p < 0.05$) decreasing pro-inflammatory TNF-α (143.2 pg/ml for E group) and IL-6 (162.4 pg/ml for E group) than that (612.2 and 389.4, respectively) for ulcer control rats. Furthermore, rats treated with 500 mg/kg showed significantly increased anti-inflammatory IL-10 levels (189.3 pg/ml) than that (110.2) of ulcer control rats (Fig. 6).

4. Discussion

The present study investigates the stomach protective effects of PORE in ethanol-mitigated ulcers in a rat model and its potential pathophysiology mechanism. PORE displayed a dose-related effect and showed substantial significant gastroprotective action against gastric ulcer formation. PORE doses (250 and 500 mg/kg) were used were delivered to rats in the present ulcer experiment. Ethanol was delivered by oral gavage and significantly induced ulcers in different gastric areas by many possible mechanisms, including stimulation of reactive oxygen species (NO and H_2O_2), inducing

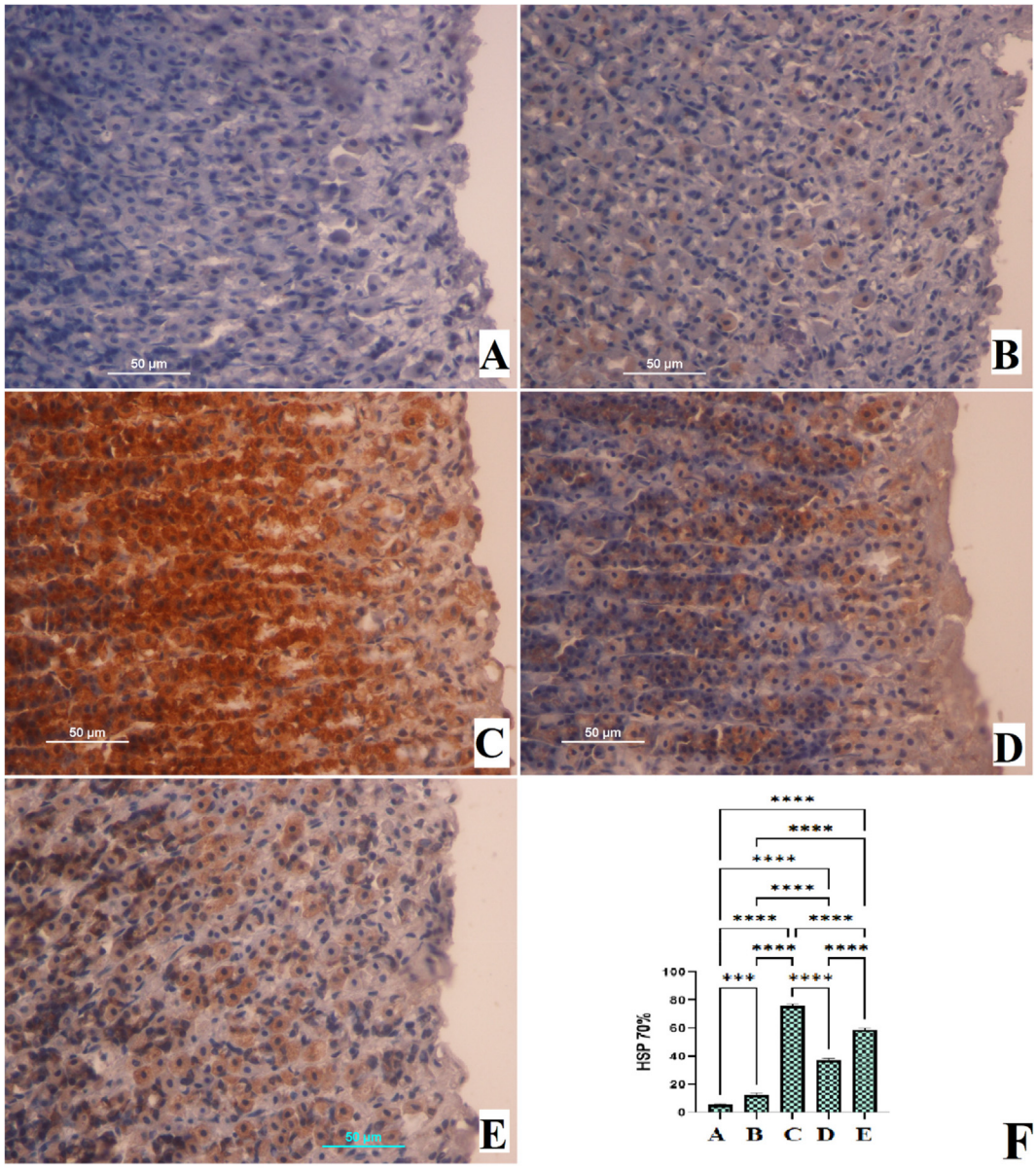

Fig. 5. PORE effects on the HSP-70 protein expression in the gastric mucosa obtained from different treated and normal control rats. **A**, normal control rats showed standard tissue structure of the gastric mucosa; **B**, ulcer control rats revealed numerous gastric injured areas with mild magenta PAS stain; **C**, reference rats, showed almost comparable gastric tissue views as normal control rats with PAS staining intensity; (**D**), rats were treated with 250 mg/kg PORE showed mild to moderate gastric epithelial injury with mild PAS intensity; (**E**), rats received 500 mg/kg PORE revealed moderate gastric epithelial injury with increased representations of HSP-70 protein (HSP-70 stain magnification 20×).

Table 3
Effect of PORE on antioxidant enzyme and MDA levels in gastric tissue homogenates obtained from different treated and normal control rats.

Groups	Treatment	SOD (U/mg protein)	CAT (nmol/min/mg protein)	MDA (µM/g protein)
A	1% CMC	426.14 ± 4.61[a]	118.14 ± 1.74[a]	96.16 ± 2.06[b]
B	100% ethanol	165.15 ± 2.38[b]	62.83 ± 2.15[b]	210.07 ± 3.17[a]
C	5 mg/kg Omeprazole	415.55 ± 5.43[a]	120.12 ± 3.26[a]	104.13 ± 1.03[b]
D	250 mg/kg PORE	399.87 ± 4.59[a]	105.25 ± 3.13[a]	120. 05 ± 1.55[b]
E	500 mg/kg PORE	407.27 ± 3.67[a]	111.33 ± 2,48[a]	115.17 ± 1.21[b]

Data are shown as means ± SEM (n = 6). Values with different superscription within same column considered significant at $p < 0.05$. PORE, *Polygonatum odoratum* rhizome extract. A, normal control; B, ulcer control; C, reference rats (omeprazole, 20 mg/kg); D, rats treated with 250 mg/kg of PORE; E, rats treated with 500 mg/kg of PORE.

pro-inflammatory enzymes (cytokines), and reducing cellular defense mechanism (Al-Wajeeh et al., 2017).

The ethanol-mitigated gastric ulcer is a well know experimental technique widely used for preclinical estimation of interested products with possible anti-ulcer potentials (Saremi et al., 2019).

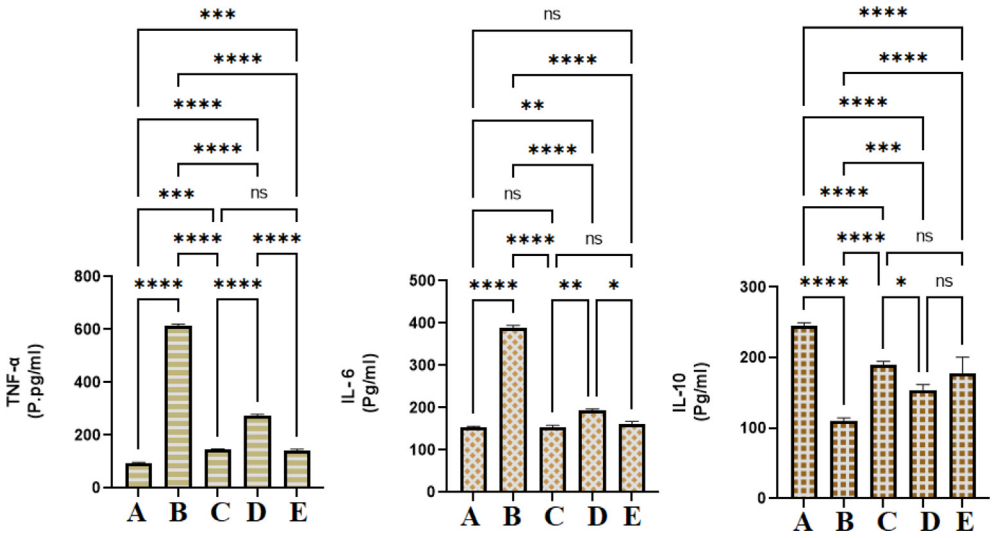

Fig. 6. PORE effects on inflammatory cytokines in the serum of ethanol-induced gastric ulcer rats. A, normal control; B, ulcer control; C, reference rats (omeprazole, 20 mg/kg); D, rats treated with 250 mg/kg of PORE; E, rats treated with 500 mg/kg of PORE.

Gastric barriers and defensive pathways were significantly impaired by alcohol (ethanol), an effective necrotizing chemical that causes forms lesions in the mucous lining of the stomach. This gastric layer injury starts with the interruption of vascular endothelium (microvascular injury) leading to increased vascular permeability, edema formation, and distortion of epithelial walls (Shams and Eissa, 2022). Our results were comparable to those of previous studies (Gupta et al., 2021; He et al., 2019). The same evident features were seen in the ulcer control rats, but they were amended in the gastric tissues obtained from PORE-treated rats.

The gastric mucus layer is considered the first defense barrier against aggressive factors (microbial infection and alcohol). The mucus layer is usually a gel-like substance (water and glycoprotein) that produces a protective barrier over the surface of the gastric mucosa. Thereby preserving the neutral pH of epithelium despite the acidic pH of the gastric lumen. Another mucus participation in ulcer prevention is through avoiding hydrogen ion reflex and participating in the buffering of stomach juice (Li et al., 2021). The PAS staining technique is a common histopathological procedure that reveals the ability of carmine staining of gastric tissues that have mucopolysaccharides such as gastric mucus. The higher the mucus amount in the stomach will result in the higher the expression of PAS stain. The current results have shown ulcer control rats had the lowest PAS stain intensity (Halabi et al., 2014). Accordingly, the current study showed that ethanol reduced the mucus barriers, but the PORE treatment significantly promotes the mucus layer coverage of the gastric mucosa (shown by increased PAS expression), thereby decreasing gastric injury and providing evidence that PORE enhances first line barrier of the gastric immunity.

Ethanol ulcerative mechanism could be also correlated with its ability to promote leukocyte infiltration at the ulcer site (Figueiredo et al., 2022). Neutrophils are specific immune cells that were considered important participants in the cellular defense system by the initiation of phagocytosis and generation of ROS (Othman et al., 2022). The stimulation and infiltration of neutrophils will generate gastric lesions in the mucosal and submucosal layers. Therefore, reducing the infiltration of inflammatory cells is considered an important mechanism by which effective antiulcer products function (Beiranvand, 2022). Microscopic investigation of gastric tissue (stained with H and E) revealed significantly lower leukocyte infiltration in PORE-treated rats than that of ulcer control rats. This bioactivity of PORE could be correlated with its phytochemical contents mainly polysaccharides which have been repeatedly reported as a gastroprotective product against various chemical-induced gastric ulcers (Neto et al., 2022; Tian et al., 2022; Wang et al., 2022).

Natural products and phytochemicals could benefit or harm human health. Similarly, synthetic drugs could be curative at a certain dosage and hazardous at another dosage (Jabbar et al., 2022; Windolf et al., 2022). Therefore, to avoid toxicity and find suitable safe dosages of plant products, some quality tests were performed on herbs before they become available for consumers. Acute toxicity tests are one of the most commonly used tests to find the healthy or toxic dosage of plant products and their phytochemicals (Hajrezaie et al., 2015). The genus Polygonatum has been utilized as a food and curative plant by the Chinese for two millenniums, without any toxic reports. In 2002, the Chinese ministry of health identified Polygonatum as the first group of products that can be depended upon in diet and disease treatment without any restriction in terms of utilization and dosage (Shi et al., 2023). Numerous studies reported the safety of *Polygonatum* Rhizomes in various toxicity trials. Previously, acute toxicity test revealed in safety (absence of toxic effects) of oral administration of 10 g/kg *Polygonati Odorati* in 10 weeks of rat trial (Chen et al., 2001). The chronic toxicity test reported no toxic effects of oral ingestion of 16 g/kg *Polygonati Odorati* in a 6 six-month rat trial (Chen et al., 2001). Antinociceptive and toxicity experiment for 600 mg/kg of *Polygonatum verticillatum* showed the absence of any physiological or behavioral changes of rats (Khan et al., 2011). Accordingly, the current results have shown that rats administered with 2500 and 5000 mg/kg did not experience any behavioral or physiological abnormalities, indicating the toxic dosage of this plant to exceed 5000 mg/kg.

The Bcl-2-associated X)Bax(pro-apoptotic protein is a well-known pro-apoptotic factor that regulates apoptosis by interfering with outer membrane permeability, facilitating the release of cytochrome *c* (Osman Mahmud et al., 2022). Contrarily, Heat-shock protein 70 (Hsp70) is commonly known as an anti-apoptotic protein that conjugates with apoptosis protease activating factor-1 (Apaf-1), which leads to blocking the apoptosome formation and inhibiting the Apaf-1/cytochrome *c*/caspase-9 cascade (Fahmy et al., 2020). In the present studies, PORE treatment significantly down-regulated the Bax and up-regulated the HSP-70 protein

expressions in gastric tissues obtained from ethanol-induced ulcers in rats. This bioactivity could be correlated with its phytochemical contents (polysaccharides, triterpenoid saponins, homoisoflavanones, and lectins) as previously determined (Liu et al., 2018; Zhao et al., 2018). Similarly, researchers have revealed significant potentials of *polygonatum* polysaccharides in lowering Bax (pro-apoptotic) and decreasing Blc-2 (anti-apoptotic) protein expression in hypoxia–induced apoptosis of neurons, indicating a nephroprotective effects (Hu et al., 2014).

Oxidative stress is a well-known causative of inflammation that results from an imbalance in the formation and excretion of ROS (free radicals), thereby decreasing gastric antioxidants (antiradical enzymes) (Jabbar et al., 2022). Antioxidants including phase 2 enzymes and genes responsible for cell protection (NQO1 and HO-1), were stimulated by Nrf2, known as an effective bio-factor in the reduction of pro-inflammatory and free radical compounds (Liu et al., 2022). Nrf2 usually undergoes dimerization with Maf proteins in the cell nuclei and binds with the antiradical response substances to initiate the transcriptional process. This biological pathway possibly will be reduced by various genes, such as NQO1 and HO-1 according to previous outcomes. Furthermore, the NF-Kb signaling mechanism is a well-known reducer of the antioxidant enzymes because of suppressing action on the Nrf2-Keap1 route by different gene activation (Keap1 and the p65) (Motohashi and Yamamoto, 2004). Therefore, searching for new anti-inflammatory substances for Nrf2 modulation is an important enhancer of the cellular defense system because of its biological roles in the up-regulation of cellular defensive genes (HO-1, NQO1, and GCLC) (Hasanvand et al., 2018). Present results showed increased antioxidant activity of PORE shown by increased antioxidants (SOD and CAT) and reduced MDA levels in gastric homogenates. Similarly, researchers have shown that ingestion of 150, 300, and 500 mg/kg of polygonatum polysaccharides significantly increased the SOD, CAT, GSH-Px, and lowered liver malondialdehyde (MDA) contents in CCl_4-induced liver oxidative injury (Jiang et al., 2013). Similarly, researchers have isolated 3 compounds of homoisoflavonoids from isolated PORE and showed significant in vitro antioxidant activity (IC_{50} values at 3.8–4.9 μg/mL) compared to ascorbic acid (IC_{50} value at 5.3 μg/mL) (Zhou et al., 2015). Furthermore, researchers have shown different antioxidant potentials of *P. odoratum* flavones based on the food processing method. Yeast fermentation is the most suitable method for PORE preparation due to its fewer disruption effects on the antioxidant potentials compared to extrusion and high-pressure methods, which both significantly reduced the antioxidant potentials of flavones isolated from PORE (Xia et al., 2021).

Cytokines are playing an important role in the sustained homeostasis of immunity by regulating the activity of immune cells (and the cellular communication between them (Wang et al., 2023). The researcher has indicated that innate immunity is stimulated by increasing Th1 (TNF - α, IL − 2) and decreasing Th2 (IL −4, IL −10) levels. However, hyperactivity of immune system can have extreme destructive effect on various pathways, which is more destructive than that of immunodeficiency patients. Thus, maintaining the Th1/Th2 ratio is crucial for a sustained immune system (Raish et al., 2021). In the present study, ethanol treatment significantly weakened the immune system in ulcer control shown by increased pro-inflammatory (TNF - α, IL − 6) and reduced anti-inflammatory (IL −10) cytokines. Contrarily, rats pre-treated with PORE had down-regulation of TNF - α, IL – 6 cytokines and up-regulation of IL –10 cytokines. Similarly, researchers have shown the immune boosting effects of *P. odoratum* and correlated this bioactivity with its polysaccharide contents that were mainly synthesized by 18 enzymes found in rhizomes more than in leaf or stem (Zhang et al., 2020). Accordingly, immunomodulatory effects of polysaccharides from *Polygonatum sibiricum* were reported as shown by significant activation of anti-inflammatory cytokines in cyclophosphamide-induced immunodeficient mice (Wang et al., 2020).

5. Conclusion and future perspectives

The present study, based on the systematic search, could be the first report on the gastroprotectiveeffects of PORE in ethanol-induced gastric ulcers in rats shown by photomicrographs, immunohistochemistry, histopathology, gastric antioxidant and lipid peroxidation evaluations. PORE treatment significantly increased the CAT & SOD levels, whereas it decreased the MDA level. PORE-treated rats had a comparable inhibition percentage of foci formation and increased mucus content (PAS expression) to that of reference-treated rats. PORE treatment caused significant up-regulation of anti-apoptotic (HSP-70) and down-regulated the pro-apoptotic (Bax) protein expressions. Pro-inflammatory cytokine (TNF-α and IL-6) were lower and anti-inflammatory cytokine (IL-10) was higher in rats treated with PORE. This bioactivity of PORE could be correlated with its potential to suppress oxidative stress and stimulate the gastric antioxidant enzymes possibly through its phytochemicals (polysaccharides, homoisoflavonoids, and saponins). The provided information could be scientific evidence for the traditional use of this plant, however, future studies are suggested to determine the phytochemicals and the exact biological pathway bearing this bioactivity.

CRediT authorship contribution statement

Abdalbasit A. Mariod : Software. **Ahmed A.J. Jabbar:** Writing – review & editing, Writing – original draft, Data curation, Formal analysis, Validation, Conceptualization, Methodology. **Zaenah Zuhair Alamri:** Validation. **Ahmed Salim Al Rashdi:** Validation. **Mahmood Ameen Abdulla:** Writing – review & editing, Conceptualization, Methodology, Investigation.

Data Availability Statement

Further details are available on the request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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