

Research Article

Mechanistic molecular approaches to anti-gastric ulcer effects of Maesil Fruit (*Prunus mume*) *in vivo*

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ABSTRACT

Gastric ulcer is a global health burden that demands better intervention because of the associated complications and side effects of conventional chemical synthetics. Hence, the present work was geared to explore the gastroprotective, mucoprotective, and acute toxicity effects of *P. mume* fruit extract (PMFE) in ethanol-mediated gastropathy in rats, focusing on the apoptotic proteins, antioxidants [superoxide dismutase (SOD) and glutathione peroxidase (GPx)], and the aggravating factors (malondialdehyde), as well as histopathological changes. Rats were randomly divided into five cages and were pre-treated either with distilled water, omeprazole (20 mg/kg), or 250, 500 mg/kg of PMFE. After one hour, rats received ethanol-mediated gastropathy. Based on biochemical and histological indications, PMFE supplementation up to 5 g/kg was found to be safe. PMFE (250 and 500 mg/kg) pre-treatment showed significant gastroprotective potential, indicated by increased ulcer inhibition percentages (66.75 and 74.63%, respectively), lower hemorrhagic, mucosal lesions, submucosal penetrations, and lower gastric pH/total stomach acidity. Pre-treated PMFE (250 and 500 mg/kg)-rats exhibited improved gastric defense factors (increasing mucin secretion (1.12 and 1.38 g), higher endogenous antioxidants including GPx, 27.58, 42.10 pg/ml; catalase, 34.57, 44.02 nmol/min/mg, and superoxide dismutase, 11.82 and 14.08 U/mg, respectively). The anti-apoptotic effects of PMFE were confirmed by increased Bcl-2 (B-cell lymphoma 2) and reduced Bax proteins in gastric tissues. Moreover, inflammatory-mediated ethanol gastropathy was down-regulated in PMFE-pre-treated rats, indicated by lower serum (Tumor necrosis factor- α) and interleukin-6 and higher IL-10 contents. This study concludes that PMFE is well tolerated by rats, ameliorates gastric ulcers, and could be considered for integration into the biopharmaceutical/nutraceutical formulation for managing stomach disorders.

1. Introduction

“Once an ulcer, always an ulcer” is a common motto attributed to the frequent recurrence and difficult-to-heal characteristic of gastric ulcers in humans. As a common gastric ulcer disease, peptic ulcer disease (PUD) is recognized by a mucosal penetration of the digestive tract reaching 3–5 mm in size up to the submucosal layer. The global PUD prevalence has reached a peak of 8.1 million in 2019, presenting an increase of 25.82% since 1990 with an annual incidence rate of 0.1% to 0.3% in Western countries (Xie *et al.*, 2022). PUD-associated vague symptoms make the disease harder to manage, and careful clinical attention is crucial before

serious complications are initiated, such as bleeding, gastrointestinal obstruction, perforation, and gastrointestinal obstruction, or even penetrating nearby organs, demanding urgent endoscopic or surgical intervention. *Helicobacter pylori* and non-steroidal drugs are the two most predominant initiators of PUD worldwide. The available applied therapies for PUD are proton pump inhibitors, *Helicobacter pylori* eradication, and H₂ receptor antagonists, all of which come with adverse drug reactions (high recurrence and resistance) despite their therapeutic efficiency (Mohammed *et al.*, 2025). Therefore, seeking alternative anti-gastric ulcer agents is a continuous mission led by scientists to provide more effective and safer therapeutics, such as natural products.

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The mechanism of ethanol-mediated gastropathy is yet to be fully elucidated; however, certain factors can have close correlations, such as a hemostatic disturbance between stressful factors (reactive oxygen species (ROS), gastric acid, and pepsin) and defensive factors (mucus-bicarbonate layer, prostaglandin, and epithelial growth factor). Moreover, ethanol-induced gastric ulcers can occur as a result of provoked mucosal epithelial apoptotic actions, oxidative stress, and inflammatory actions. Pro-inflammatory mediators such as TNF- α and interleukin (IL)-6 (interleukine-6) are important mediators of inflammatory processes in ethanol-mediated gastric mucosal injuries (He et al., 2022). As an aggressive factor, elevated ROS formation generates further lipid peroxides [malondialdehyde (MDA)] and cell death, consequently worsening gastric mucosal injury. In contrast, defensive factors such as antioxidant enzymes (SOD (superoxide dismutase), CAT (catalase), and GPx (glutathione peroxidase)) can have a protective action against ethanol-mediated gastropathy by maintaining a balance between ROS formation/elimination and preserving mucosal layer integrity (Mansour et al., 2022). Cellular imbalance between apoptotic and proliferative action directly affects gastric ulcer initiation and healing, which is modulated by several apoptotic pro-proteins, including p53 and Bax-linked family genes (Bcl-2). Proapoptotic proteins, the Bax gene encodes Bax proteins, which act as apoptotic enhancers and cellular death stimulators. In contrast, Bcl-2 proteins encoded by Bcl-2 genes reverse the process provoked by Bax proteins. Scientists declared that altered apoptotic proteins can provoke cell apoptosis/proliferation, resulting in further ethanol-mediated gastric cell injury (Ahmed et al., 2024). Therefore, the search continues for a new source with effective modulation of apoptotic mediators as one of the pathways involved in peptic ulcer disease (PUD).

Medicinal plants can be a major source of natural products, which have been confidently consumed as a prophylaxis or therapy by nearly 75% of the world's population, according to World Health Organization data (Thomford et al., 2018). Moreover, ethnobotanists revealed that herbal medicine has been predominantly used to avert or cure digestive disorders by 10% of individuals across the globe (Prabhu and Shivani, 2014). *P. mume* fruit is a well-known rich source of phytoconstituents (phenolic, flavonoids, lignans, cyanogenic, furfurals, and organic acids), which have been correlated with its numerous bioactivities, such as antioxidant (Hao et al., 2024), anti-inflammatory (Gao et al., 2024), anticancer (Won et al., 2020), and improvement of blood circulation (Bang and Jeon, 2020). Moreover, the processed fruit of *P. mume*, Fructus mume, has been suggested as a memory enhancer according to its negative modulatory action on cognitive impairment in familial Alzheimer's disease, chronic cerebral hypoperfusion-mediated cognitive impairment (Jeon et al., 2012), and scopolamine-induced memory impairment in animal models (Kim et al., 2015). Moreover, unripe *P. mume* extracts exhibited increased inhibitory action on *Helicobacter pylori* motility, correlated with its chemical contents, mainly Syringaresinol (Miyazawa et al., 2006). *P. mume* extracts ameliorated chronic atrophic gastritis by limiting *H. pylori* infection and inflammation-related mucosal injury (Murali et al., 2014). Despite numerous records regarding its biological potential, concise data on its gastroprotective actions have yet to be reported; herewith, we present the acute toxicity and anti-ulcer effects of PMFE and its possible underlying molecular pathways in ethanol-mediated gastropathy in an animal model.

2. Materials and Methods

2.1 Plant collection and plant extraction

Prunus mume was purchased from China, authenticated by taxonomists in Salahaddin University, air dried under shade (25°C), and sieved to obtain a fine powder and kept in dark vials for later use. An amount of dried powder (1.5 kg) was soaked in methanol 10:1 (v/w) for two days with regular stirring using a magnetic stirrer. After complete filtration using a Whatman paper (gravity filtration), the extract was dried using a rotary evaporator (at 45°C temperature) for nearly an

hour. The concentrated fruit extracts of *P. mume* (yield 22% $\pm$ 1.6%) were stored in tight dark vials (4–5°C) for later experiments.

2.2 Chemicals, animals, and ethics

The necessary chemicals were purchased from Sigma Aldrich and Solar Bio Co. (Wuhan, China). Sprague Dawley adult male/female rats (7–8 weeks, 190–200 g) were taken from Erbil Polytechnic University and placed in steel cages in suitable environmental conditions (12-h light/dark cycles). The animal care/use was according to ARRIVE standards (Percie du Sert et al., 2020). The Erbil Polytechnic ethical committee of Erbil confirmed the study (BIO, 55; 24/11/2024).

2.3 Acute toxicity test

The possible toxic incidence of any therapeutic plant is a major drawback related to their consumption as a remedy for human disorders. As per OECD guidelines, the current study selected a single dose of 2 and 5 g/kg of PMFE as low and high doses of PMFE to ensure its safety in a two-week toxicity trial before its use in a multiple-dose trial (Jonsson et al., 2013). Thirty-six rats (males and females) underwent a one-week adaptation procedure. Rats were randomly aligned into three groups (n=12), fasted overnight, and received either normal saline (A), 2 g/kg PMFE (B), or 5 g/kg PMFE. After that, rats were fasted for another three h, and the routine observations were conducted every 6 hours for 14 days for any toxicity indications (tremors, lethargy, salivation, diarrhea, or skin biting) or mortality incidence. Finally, an injection of anesthesia (3 mg/kg xylazine and 300 mg/kg ketamine) was intraperitoneally injected into all rats, and they were sacrificed using a blade/decapitation procedure. The dissected organs were screened by different histopathology assays, and the separated serum was analyzed for the biochemical contents (Al-Qaisi et al., 2025b).

2.4 Gastroprotective experiment

2.4.1 Experimental groups

Thirty male rats were randomly allocated into 5 steel cages (n=6) with the availability of food and water during a one-week adaptation procedure. After overnight fasting, rats received either 10% Tween 20 (groups A and B), 20 mg/kg omeprazole (C), 250 mg/kg (D), or 500 mg/kg (E). After 1 h, groups B–E were administered 1 ml of absolute ethanol-mediated gastropathy. After one hour, intraperitoneal anesthesia injection was delivered to all rats, and they were sacrificed using a blade/decapitation procedure. The dissected stomach was examined for the macroscopic and microscopic features. The serum was analyzed for biochemical contents (Fig. 1).

2.4.2 Gross study

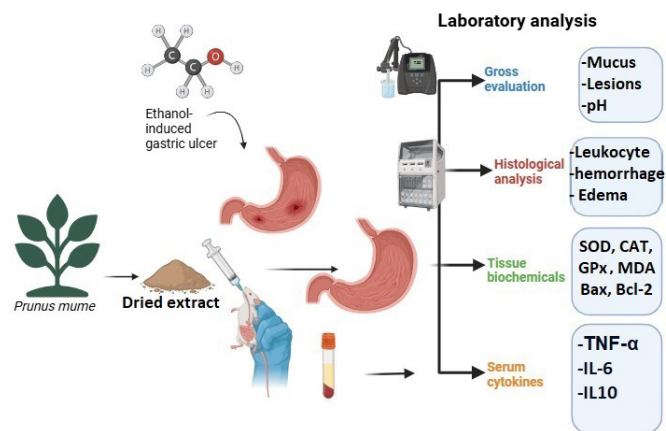


Fig. 1. Experimental design for evaluating gastroprotective effects of PMFE in rats (n=6).

The dissected stomach's organs were water-washed, dried, and opened at the greater curvature. The mucus was collected, and the mucosal lesions were screened macroscopically to estimate the ulcer area. The stomach lesions were recognized as elongated red/dark erosion areas via the ImageJ software.

The surface stomach ulcers were measured by applying the Kauffman and Grossman method with slight changes (Kauffman Jr and Grossman, 1978). The ulcer length/width was measured using a planimeter ($10 \times 10 \text{ mm}^2$) and a dissecting microscope by a histopathologist blinded to rat grouping. The surface of each ulcerated area was estimated by enumerating small squares ($2 \times 2 \text{ mm}$) on top of each ulcer band. The sum of all surface lesions for each stomach was used to determine the ulcer area (UA mm^2), where the sum of small squares $\times 4 \times$ magnification $1.8x$. The inhibition % of the ulcer was calculated as detailed previously (De Araújo et al., 2021) using Eq. 1.

$$\text{Inhibition \% (I\%)} = \frac{\text{UA control} - \text{UA treated}}{\text{UA control}} \times 100 \quad (1)$$

2.4.3 Assessment of gastric indices

The stomachs were washed, and a clean area at the greater curvature was created to collect the mucus and weigh it using an electronic balance (Zhang et al., 2023). The gastric juice was centrifuged (4000 rpm for 10 min), and the gastric pH and mucus volume were estimated for every group replicate. TSA (total stomach acidity) for stomach juice was determined by mixing with distilled water (1 ml) and 2-3 drops of phenolphthalein. The procedure employed NaOH (0.01 N) for titration until a pink color appeared. The TSA was calculated using Eq. 2:

$$\text{TSA} = \frac{V(\text{NaOH}) \times N \times 100 \text{ mEq} / \text{L}}{0.1} \quad (2)$$

Moreover, the Alcian blue assay was employed to evaluate the gastric wall mucus content/mucopolysaccharides, as detailed by previous researchers (Ahmed et al., 2025a).

2.4.4 Histological analysis

The dissected stomach organs were washed (buffer), sectioned (1-2 cm), and placed in 10% (v/v) formalin for fixation. The gastric slices were processed automatically, followed by paraffinization, and a thin-sectioned tissue ($5 \mu\text{m}$) was stained with H&E and PAS on glass slides, and left in the oven overnight to fully dry. The slides were examined using a light microscope (Zeedo, Wuhan, China) for tissue pathological alterations (gastric hemorrhage, epithelial cell disruption, inflammatory cell infiltration, mucosal lesions, submucosal penetration, and submucosal edema (Ahmed et al., 2025b).

2.4.5 Examination of gastric tissue homogenates

The gastric tissues were sliced, buffer-washed, and then homogenized for 10 min with a cocktail of mammalian protease inhibitors. The tissue mixture was centrifuged at $25,000 \times g$ for 15 min at 4°C . The separated supernatant was evaluated for the amount of SOD (Cat. K001650P), CAT (Cat. K002366P), and MDA levels, as detailed previously using ELISA kits (Mao et al., 2016). The concentrations of apoptotic proteins (Bcl-2, K001594P; Bax, K008076P) were also determined using protocols provided with commercial ELISA kits from Solar Bio. (Wuhan, China).

2.4.6 Inflammatory cytokine evaluation

The blood samples from cardiac puncture were obtained from all rats before euthanasia, which were centrifuged at 5000 rpm for 10 min to separate serum for inflammatory cytokines determination using a fully automated Mindray machine (BS 240, Shenzhen, China), following the manufacturer's protocols.

2.5 Statistics

The laboratory data were handled using GraphPad Prism (9.0, California, USA) for normality testing and One-way analysis of variance

(ANOVA). Tukey's post-hoc test was used for group comparison ($n=12$ in toxicity trial; $n=6$ in gastroprotection trial), considering values less than $p<0.05$ as significant. The Results were presented as Mean $\pm$ SEM.

3. Results

3.1 Acute toxicity

Potential toxic effects of any therapeutic can cause liver/kidney impairment (Al-Qaisi et al., 2024). Therefore, to avoid unwanted circumstances, toxicity evaluation was conducted for PMFE in a two-week trial using 2 and 5 g/kg as standard dosages in rats.

The regular checkup did not find any morbidity or mortality throughout the trial. The single intragastric dose delivery of 2 or 5 g/kg to rats did not result in any toxic outcomes such as salivation, tremors, diarrhea, or changes in skin/eye color. Moreover, supplemented rats did not exhibit any abnormal behavior (such as toe/skin biting or salivation) and had comparable food/water intake compared to the normal control group. The histopathological analysis revealed normal tissue arrangement (without indications of hemorrhage, tubular necrosis, or necrosis) of liver and kidney tissues similar to those found in normal control rats. Additionally, serum biochemicals, including ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), creatinine, and urea, were comparable between PMFE-treated rats and normal control rats (available on request). Overall, the data indicate the NOAEL (No observed adverse effect level) level of PMFE is 5 g/kg, and the LD50 dose would be higher than the tested 5 g/kg (Fig. 2).

3.2 Gastroprotection effects

3.2.1 Macroscopic indication

The gross views of dissected stomachs are presented in Fig. 3. The macroscopic views indicated normal, healthy pink stomach tissues in the negative controls. The ulcer controls exhibited clear mucosal lesions (severe hemorrhagic with dark red line), with extended hemorrhage seen as injury bands along the gastric axis. Pre-treatment with omeprazole or PMFE resisted ethanol-mediated gastropathy, indicated by less mucosal damage, more flattened folds, and less swollen/hemorrhagic and congested areas compared to ulcer controls. In a dose-related manner (high dose being more efficient), PMFE showed gastroprotective actions in ethanol-induced gastropathy-based early gross indications.

3.2.2 Histopathological results

Microscopic findings revealed varying rates of gastric tissue changes across treatment strategies. Group A had normal gastric tissue, as evidenced by regular mucosal morphology without signs of tissue deterioration. As expected, ulcer control rats displayed severe gastric tissue injury, denoted by increased mucosal damage, cellular necrosis, vasodilation, elevated inflammatory cells migrating into submucosal areas, oedema, and mucosal erosion. Rats that received omeprazole had the best gastric tissue conditions, comparable to those of normal controls, with slight mucosal injury and epithelial cell disruptions. Interestingly, PMFE pre-treatment alleviated ethanol-mediated gastropathy, denoted by lower mucosal damage, less inflammatory cell infiltration, and smaller edema area compared to ulcer control's stomach tissues (Fig. 4).

3.2.3 Effect of PMFE on physical barriers

The ethanol-mediated gastropathy caused significant modulation of mucus content, gastric pH, total stomach acidity, and ulcer estimations due to different pretreatments. Normal control rats had a reasonable amount of gastric mucus, within range gastric pH, and the highest Alcian blue binding capacities compared to treated rats. Untreated rats displayed the lowest gastric defense factors compared to omeprazole or PMFE-pre-treated rat groups. Ulcer control rats displayed reduced mucus secretion (0.81 g), lowest gastric pH (2.68), and lowered Alcian

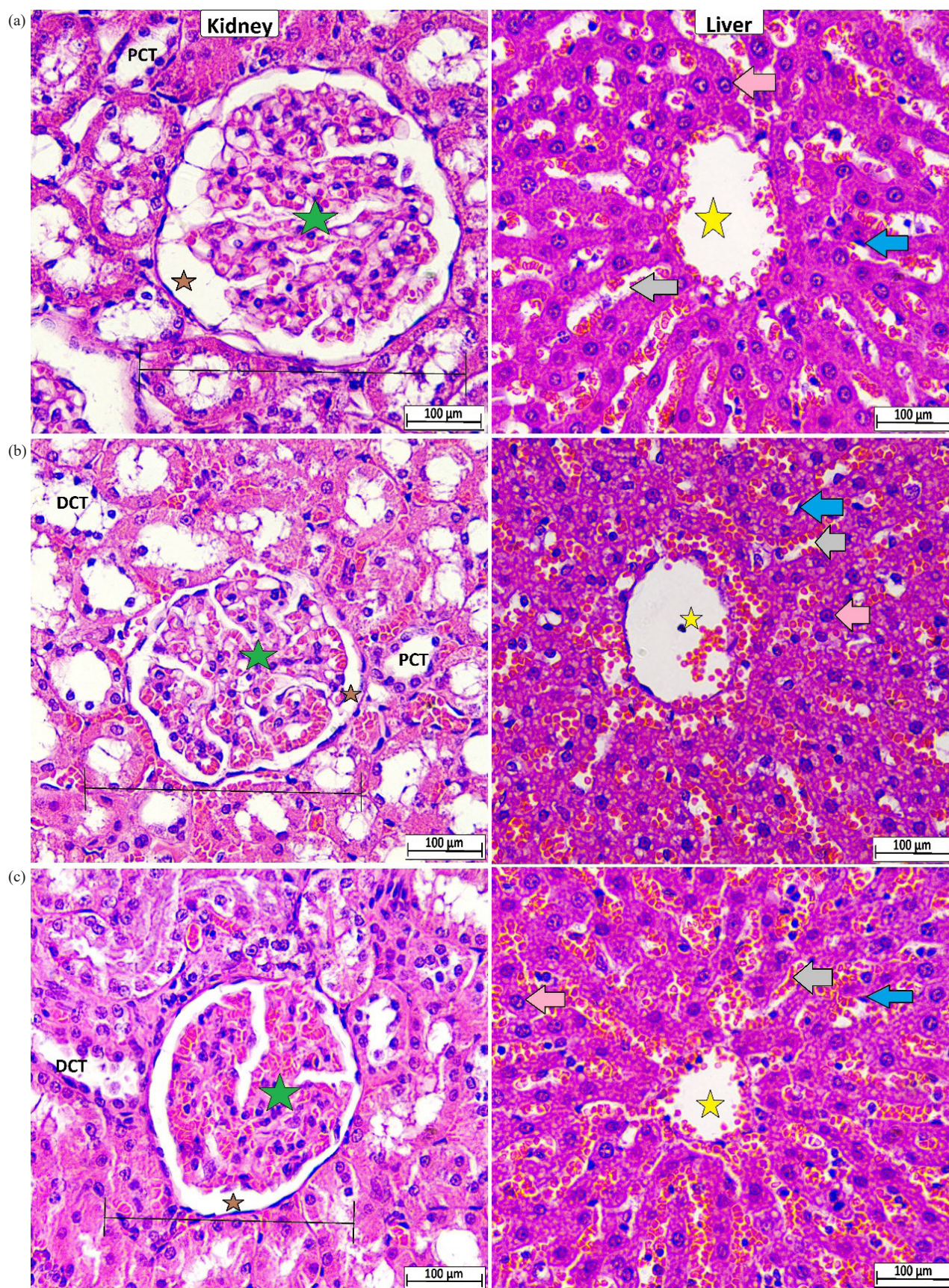


Fig. 2. (a) Microscopic appearance (40X) of liver and kidneys in rats (n = 12) received either normal saline, (b) 2 g/kg PMFE, or (c) 5 g/kg of PMFE. yellow star, central vein; blue arrow, Kupfer cells; pink arrow, sheets of hepatocyte; gray arrow, sinusoids; green star, glomerulus; black line, bowmen's capsule with glomerulus; brown star, bowmen's space (H & E staining, 40X). DCT and PCT, distal and proximal convoluted tubules, respectively.

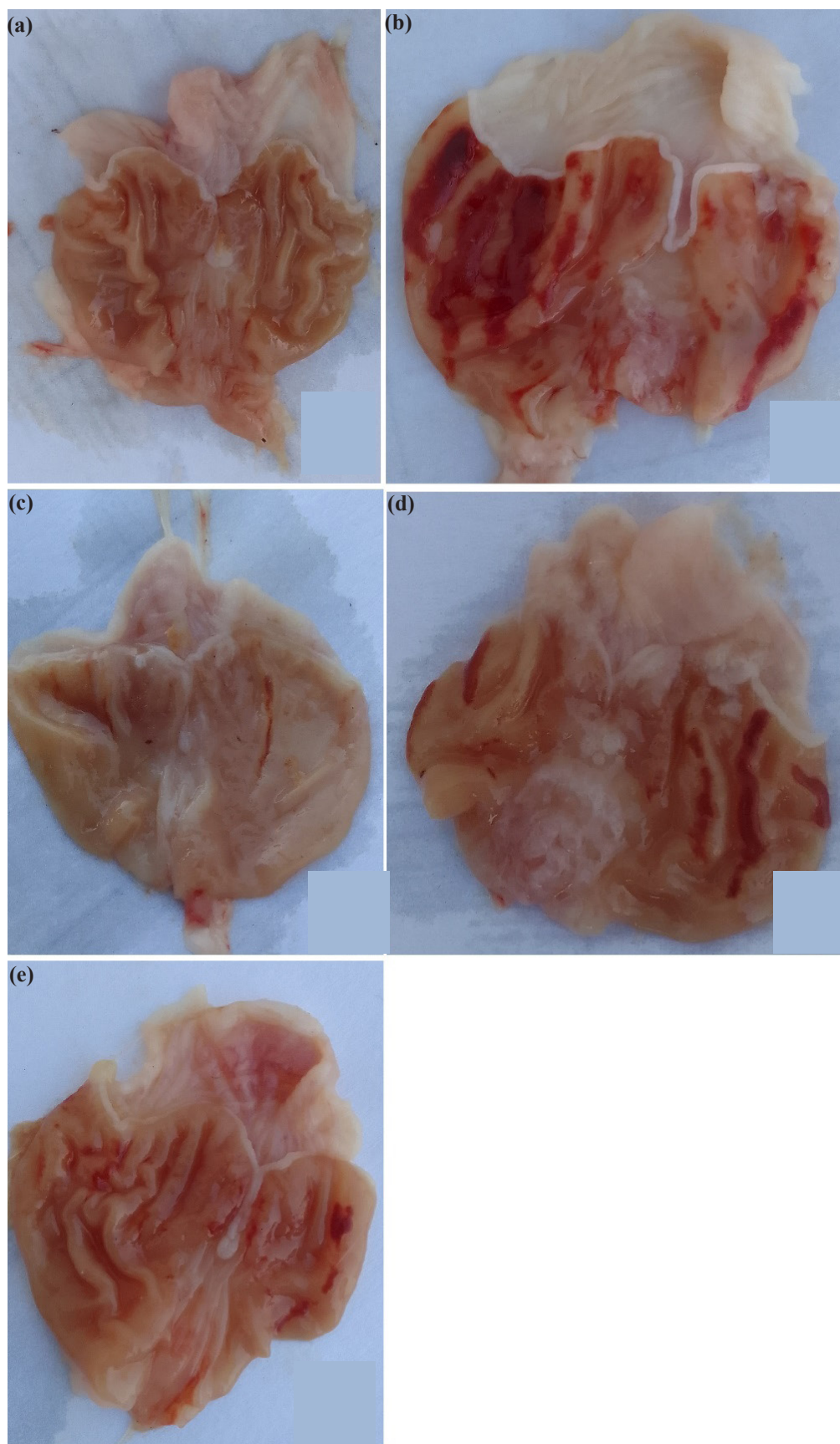


Fig. 3. Effect of PMFE pre-treatment on the macroscopic views of rats' stomachs treated differently + absolute ethanol. (a) Rats received either 10% tween 20+normal saline; (b) 10% tween 20+ethanol; (c) 20 mg/kg omeprazole +ethanol; (d) 250 mg/kg PMFE +ethanol; (e) 500 mg/kg PMFE +ethanol. Pre-treatment with omeprazole or PMFE reduced ethanol-mediated gastropathy, shown by fewer mucosal injuries and fewer areas of black/red hemorrhage.

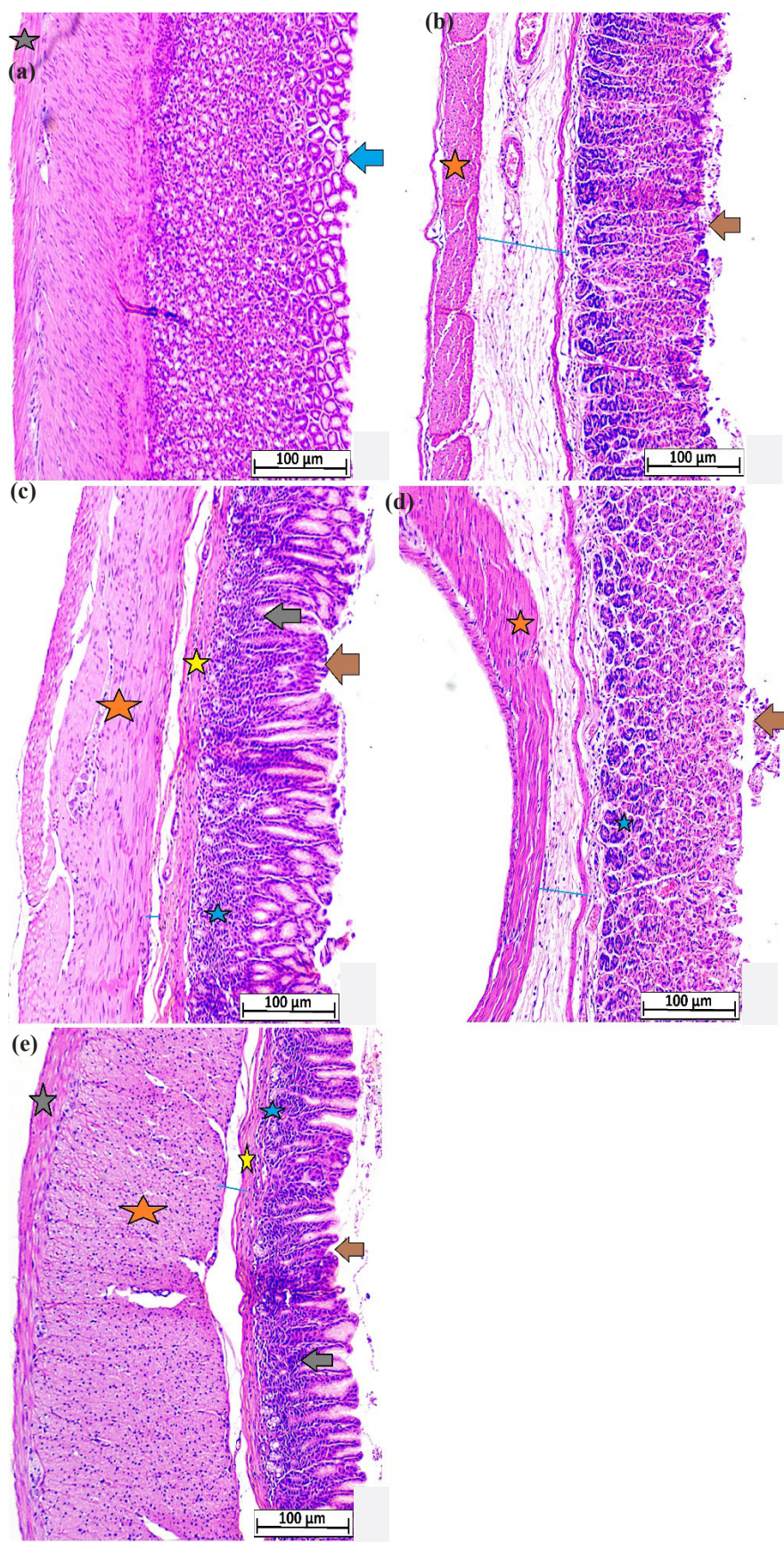


Fig. 4. Microscopic views (10X) of rats' stomachs exposed to pretreatments + absolute ethanol. (a) Rats received either 10% tween 20+normal saline; (b) 10% tween 20+ethanol; (c) 20 mg/kg omeprazole + ethanol; (d) 250 mg/kg PMFE+ ethanol; (e) 500 mg/kg PMFE+ ethanol. The ulcer control exhibited increased epithelial disruption (brown arrow), submucosal edema (blue line), and inflammatory infiltration. Those gastric tissue alterations were ameliorated in omeprazole or PMFE-treated rats. Blue arrow, intact epithelium of normal control; yellow star, muscularis mucosa; orange star, muscularis propria; gray star, serosa; Gray arrow, gastric pits; blue star, gastric glands.

Table 1.
Effect of PMFE on some gastric estimations obtained from experimental rats.

Groups	Mucus (g)	pH	Ulcer area (mm) ²	Inhibition (%)	TSA (mEq/L/100 g)	mg Alcian blue/g tissues
Normal control	2.23±0.40 ^a	6.49±0.34 ^a	-	-	20.41±0.7	590.43±9.3
Ulcer control	0.81±0.32 ^d	2.68±0.54 ^b	569.7±5.20 ^a	-	65.3±2.4	149.8±6.2
20 mg/kg Omeprazole +ethanol	1.54±0.29 ^b	6.60±0.49 ^a	134.2±4.22 ^b	76.44 ^a	33.26±3.9	529.2±6.3
250 mg/kg PMFE+ethanol	1.12±0.43 ^c	5.21±0.65 ^d	162.8±5.51 ^c	66.75 ^b	42.4±3.5	339.1±7.7
500 mg/kg PMFE+ethanol	1.38±0.37 ^b	6.43±0.56 ^a	144.5±3.27 ^d	74.63 ^c	37.31±2.9	440.6±8.2

Values indicated with different superscripts inside the exact column indicate significance at 5%.

binding capacities (149.8 mg/g), increased TSA (65.3 mEq/L/100 g), and elevated ulcer areas (569.7 mm). Omeprazole or PMFE pre-treatments strengthened gastric defense factors, indicated by higher mucus content (1.54, 1.12, 1.38 g), elevated stomach pH (6.6, 5.21, and 6.43), lower ulcer area (134.2, 162.8, and 144.5), decreased TSA (33.26, 42.4, 37.31 mEq/L/100 g), and increased Alcian blue bindings (529.2, 339.1, and 440.6 mg/g). Additionally, pre-treated groups receiving omeprazole or PMFE (250 and 500 mg/kg) exhibited increased ulcer inhibitory percentages (76.44, 66.75, and 74.63%, respectively) compared to the non-inhibitory value of group B (Table 1). The above data could support PMFE's potential as an effective agent for strengthening stomach defense factors that could mediate its ameliorated action on ethanol-mediated gastropathy in rats.

The present estimations indicated an extended range of mucopolysaccharides and gastric mucin contents of pre-treated rats that received ethanol. Rats that received 10% tween 20+ absolute ethanol had the lowest mucin/glycoprotein content, further enhancing gastric mucosal injuries. Rats pre-treated with omeprazole or PMFE, recovered mucosal gland potentials in mucin secretion, improving a defensive gastric barrier that limited ethanol (corrosive)-mediated gastropathy (Fig. 5).

3.2.4 Effect of PMFE on apoptotic indicators

The immunocontent evaluations included a pro-apoptotic Bax protein, usually increased during gastric cell exposure to aggressive factors (ethanol). As seen in Fig. 6, normal control rats had reduced Bax proteins (1.30 ng/ml) in their stomach tissues, denoting reduced apoptosis/stress conditions. In contrast, ulcer control rats showed elevated Bax proteins (6.76 ng/ml) in their tissues, which was expected since this group had only ethanol and thus experienced increased ethanol-stressful (apoptotic) conditions that further enhanced gastric injuries. Pretreatment with omeprazole or PMFE (250 or 500 mg/kg) resisted ethanol-induced apoptosis, indicated by significantly less Bax proteins (1.62, 3.54, and 2.52 ng/ml) in their stomach homogenates, limiting apoptotic action and reducing cellular disruptions because Bax is well-known for their modulatory potentials on the mitochondrial permeability and leakage enhancement of mitochondria-apoptotic molecules such as cytochrome C and AIF.

The gastroprotective study included evaluation of apoptotic protein content in the gastric tissues, which significantly varied because of pre-treatments and ethanol delivery. Normal control rats displayed the usual low amount of Bcl-2 concentration (1.067 ng/ml), denoting the absence of any apoptotic or cellular stress reactions. The ulcer control rats exhibited reduced Bcl-2 contents (2.24 ng/ml) in gastric tissues, promoting apoptosis and gastric tissue injury. Pre-treatment with omeprazole or PMFE displayed an up-regulation of Bcl-2 in gastric tissues (6.16, 3.83, and 5.41 ng/ml), limiting cellular alterations/apoptosis and improving gastric barriers, facilitating the refolding/recovery of semi-altered proteins or up-regulating protein availability for endoplasmic reticulum and mitochondria (Fig. 6).

3.2.5 Effect of PMFE on oxidative stress

Absolute ethanol ingestion can cause significant oxidative stress-mediated tissue injury because of ROS generation/antioxidant

alteration. In the present gastropathy trial, rats that ingested absolute ethanol without any pretreatment exhibited elevated oxidative stress conditions in their gastric tissues compared to other pre-treatment groups. As shown in Fig. 7, normal control rats displayed increased antioxidants (SOD, 23.15 U/mg; CAT, 63.5 nmol/min/mg; GPx, 7.66 pg/ml) and decreased MDA (13.10 nmol/mg) in their stomach tissues. In contrast, ulcer controls showed significantly lower antioxidants (SOD, 9.51 U/mg; CAT, 15 nmol/min/mg; GPx, 5.98 pg/ml) and higher lipid peroxidation indicator (MDA, 102.7 nmol/mg) in their gastric tissues, denoting a severe oxidative stress status that enhanced further gastric tissue injury. Interestingly, PMFE pretreatment resisted ethanol-mediated oxidative damage, increasing SOD by 24.22%, 48.05%; CAT by 130.46%, 193.46%; GPx by 260%, 440%, respectively, using ulcer control value as positive standard. Moreover, as a major end product of lipid peroxidation, MDA levels in gastric tissues were significantly decreased by 80.42%, 75.12%, and 78.41% in omeprazole or PMFE-treated (250 and 500 mg/kg), respectively, using ulcer control value as a positive standard (Fig. 7).

3.2.6 Effect of PMFE on inflammation

Inflammatory mediators are key players in the healing processes; however, elevated pro-inflammatory cytokines can delay healing actions, a condition that occurs during ethanol-mediated gastropathy. In the present trial, normal control rats had reduced pro-inflammatory cytokines (TNF- α , 101.2; IL-6, 21.6 pg/ml) and increased anti-inflammatory (51.8 pg/ml) indicators in their serum. Ulcer control rats experienced a delayed inflammatory condition, indicated by elevated TNF- α (283.1 pg/ml) and IL-6 (64.7 pg/ml) and reduced IL-10 (19.4 pg/ml). While, omeprazole or PMFE (250 and 500 mg/kg) pretreatment resisted ethanol-mediated inflammation, denoted by significantly lower TNF- α (115.3, 172.4, 132.1 pg/ml, respectively) and IL-6 (32.2, 48.3, 41.5 pg/ml, respectively) and higher IL-10 (43.2, 28.9, 36.8 pg/ml, respectively) compared to ulcer controls, altogether led to less inflammatory cell recruitment, microcirculatory alterations, and fewer gastric hemorrhagic. In other terms, ulcer controls exhibited an increase by 279.7% and 299.5%, for TNF- α and IL-6, respectively, compared to normal control, but PMFE pretreatment (250 and 500 mg/kg PMFE) downregulated TNF- α by 39.10%, and 53.34%; IL-6, 25.56% and 34.86%, respectively, compared to ulcer control value. Moreover, IL-10 levels were up-regulated by 49.5 and 89.7% in supplemented PMFE rats (250 and 500 mg/kg, respectively) using ulcer control value as positive standard (Fig. 8).

4. Discussion

Plant-based natural products can have serious toxic effects, which are considered major obstacles associated with their use in daily medicinal practice. Therefore, the present study examines the safety supplementation of PMFE (2 or 5 g/kg) before considering it as a therapeutic for wound healing, considering OECD standards for animal care and concern (Jonsson et al., 2013). The results indicated that rats ingested with a single dose of 2 or 5 g/kg did not experience any toxicity, yet rats seemed completely healthy throughout the trial, with zero morbidity or mortality cases. Moreover, histological and biochemical estimations confirmed the safety of PMFE, denoted by comparable

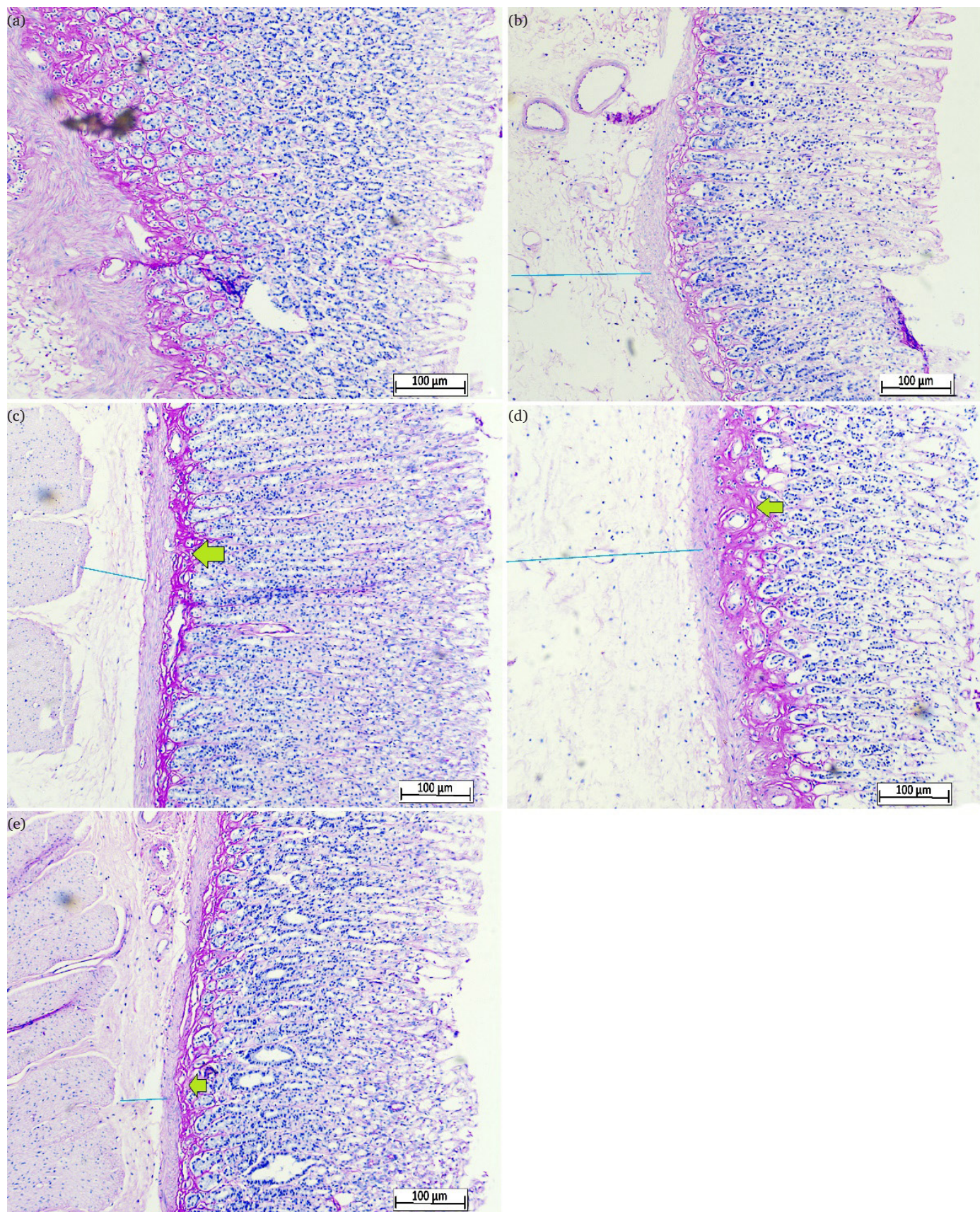


Fig. 5. The gastric tissues were stained with PAS. (a) Rats received either 10% tween 20+normal saline; (b) 10% tween 20+ethanol; (c) 20 mg/kg omeprazole +ethanol; (d) 250 mg/kg PMFE+ ethanol; (e) 500 mg/kg PMFE+ ethanol. Blue line, submucosal edema; green arrow, elevated mucin/glycoprotein production (magnification, 10X).

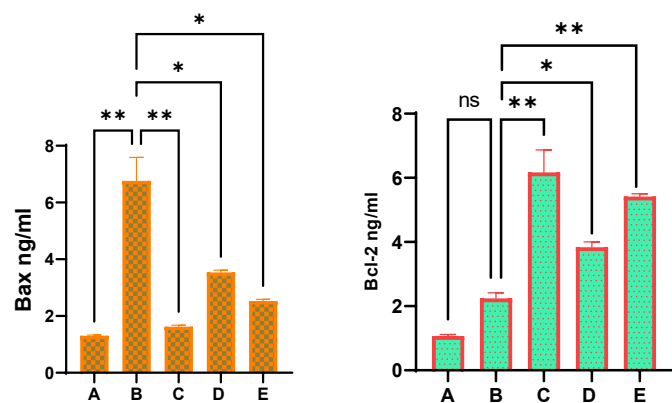


Fig. 6. Effect of PMFE on Bax and Bcl-2 intensity in gastric tissues of rats. (A) Rats received either 10% tween 20+normal saline; (B) 10% tween 20+ethanol; (C) 20 mg/kg omeprazole + ethanol; (D) 250 mg/kg PMFE+ ethanol; (E) 500 mg/kg PMFE+ ethanol. Ethanol-induced apoptotic actions were attenuated in rats pretreated with omeprazole or PMFE. *, $P > 0.05$; **, $P > 0.01$; ns, non-significant.

results compared to normal control rats. Similarly, researchers indicated that the expected lethal dose of *Prunus mume* isolates (mumefural) in an animal model was >5000 mg/kg, declaring non-toxicity incidence at 2500 and 5000 mg/kg (Kim et al., 2020). Accordingly, *P. mume* extract supplementation (0.84–3.33 g/kg) for 30 d did not result in any toxicity or mutagenicity based on indications of hematological, biochemical, and histopathological examination (Lu et al., 2009).

Corrosive agents such as absolute alcohol are considered necrotizing agents that can provoke numerous adverse reactions in

the gastric mucosal epithelium, including damage related to reduced protein attentiveness. The gastric mucosa prevents the penetration of collapsing digestive enzymes, pepsin verbose stomach's partition (Al-Qaisi et al., 2025a). Moreover, limited penetrability was allowed by the gastric mucosa for large molecules as pepsin. Absolute ethanol can alter the permeability of the gastric mucosal layer, releasing vasoactive products and leading to vascular injury. In addition, the up-regulation of mucus production (by gastric mucosa) is considered one of the defense mechanisms to minimize aggressive factors (ethanol)-mediated gastric mucosal injuries (Ahmed et al., 2025a). In this study, ulcer controls exhibited severe mucosal injury, submucosal edema, fibroblast infiltration, epithelial damage, vascular edema, and gastric erosion based on the histopathological examination using H&E. In contrast, PMFE (250 and 500 mg/kg) pretreatment alleviated ethanol-mediated gastropathy, indicated by up-regulating gastric defense factors (gastric pH, mucus content, and glycoproteins) and lower infiltration of inflammatory cells/fibroblast cells. Such gastroprotective potentials of PMFE could be related to its previously reported phytochemicals (phenolics, flavonoids, organic acid, benzyl glycosides, lignans, furfurals, cyanogenic glycosides, steroids, terpenes, and alkaloids (Gong et al., 2021), which were repeatedly declared as alleviators of gastric dysmotility and dyspepsia, gastroesophageal reflux, and constipation in different *in vivo* and clinical trials in animal models. Moreover, researchers revealed increased modulatory potentials of PMFE on interstitial cells of Cajal, which were presented as one of the pathways under its gastroprokinetic-improving effects on gastrointestinal actions (Na et al., 2013; Shin et al., 2021). In addition, researchers reported that methanolic extracts of the unripe *P. mume* can inhibit ($>90\%$, IC_{50} value was $50 \mu\text{g/ml}$) the *Helicobacter pylori* motility (an enhancer of an open sore (ulcer) initiation), which was mainly linked with its isolated chemical content (+)-Syringaresinol (Miyazawa et al., 2006).

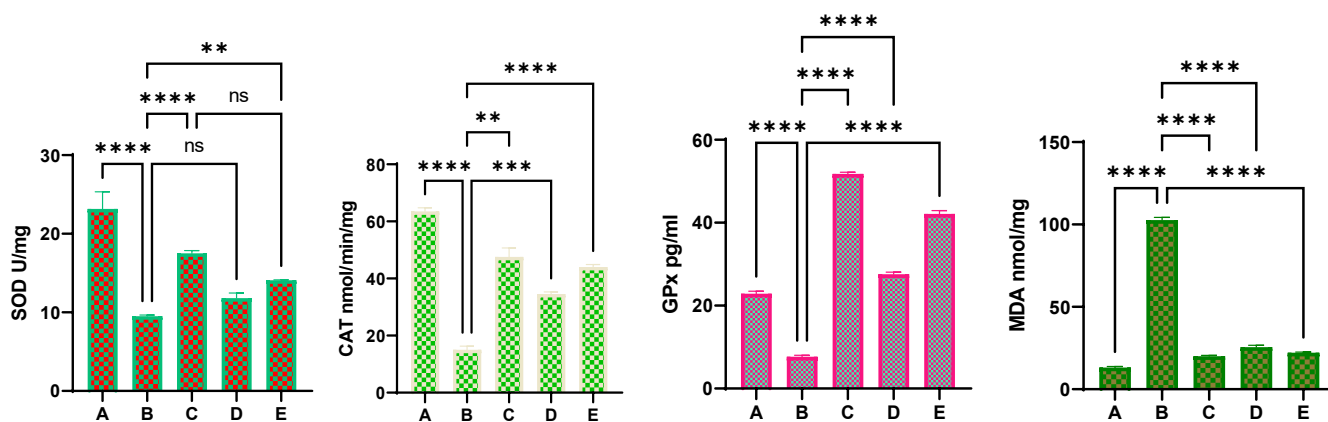


Fig. 7. Effect of PMFE on antioxidants in ethanol-mediated ulcers in rats. (A) Rats received either 10% tween 20+normal saline; (B) 10% tween 20+ethanol; (C) 20 mg/kg omeprazole + ethanol; (D) 250 mg/kg PMFE+ ethanol; (E) 500 mg/kg PMFE+ ethanol. Interestingly, PMFE supplementation attenuated ethanol-mediated ROS/antioxidant imbalance, which facilitates faster ulcer healing. **, $p > 0.01$; ***, $p > 0.001$; ****, $p > 0.0001$; ns, non-significant.

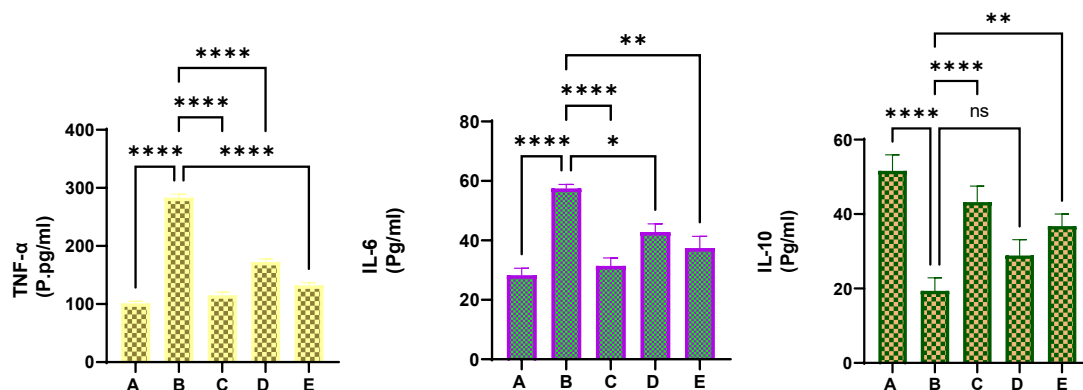


Fig. 8. Effect of PMFE on cytokines in rats with stomach ulceration. (A) Rats received either 10% tween 20+normal saline; (B) 10% tween 20+ethanol; (C) 20 mg/kg omeprazole + ethanol; (D) 250 mg/kg PMFE + ethanol; (E) 500 mg/kg PMFE+ ethanol. PMFE pretreatment ameliorated ethanol-induced gastropathy, resisting gastric tissue disruption, inflammatory cell infiltrations, and mucosal lesions. *, $p > 0.05$; **, $p > 0.01$; ****, $p > 0.0001$; ns, non-significant.

Apoptotic proteins are considered crucial factors that alter the hemostasis between aggressive and defensive factors that will either positively provoke cell survival or negatively stimulate cell death, maintaining or disrupting the gastric mucosal integrity, respectively (Al-Qaisi et al., 2025b). A proapoptotic Bcl-2 protein, Bax, is one of the transcriptional targets for the tumor suppressor gene P53, altering mitochondrial permeability, enhancing DNA damage, and apoptotic initiation (cell death). In contrast, Bcl-2 is a well-known anti-apoptotic/ thus classified as a cytoprotective protein to surpass stressful cellular conditions, facilitating gastric ulcer healing due to its pro-proliferative actions on cell proliferation at the margin of gastric ulcer and promotes angiogenesis inside granulated tissue. (El-Shitany et al., 2024). The present immunocontent evaluation showed that absolute ethanol initiated oxidative stress and an inflammatory process that initiated the intrinsic apoptosis pathway, altogether increasing P53/Bax and decreasing Bcl-2 expression, thereby causing increased cell irregularities and protein denaturation, and gastric cellular death. In contrast, Pretreatment with PMFE (250 and 500) resisted ethanol-mediated apoptosis in stomach tissues, denoted by significantly higher Bcl-2 (mild to moderate) and lower Bax proteins in tissue homogenates, therefore resulting in less P53 activity, fewer cell deaths, and consequently, less gastric mucosal injury compared to positive ulcerated rats. The modulatory potentials of PMFE on apoptotic proteins could be linked with its phytoconstituents (polyphenolics, flavonoids, organic acids, furfural, and terpenoids, including oleanolic acid and ursolic acid) that were already confirmed as regulators of apoptotic proteins in numerous *in vivo* trials (Cheng et al., 2019). PMFE extracts exhibited significant anticancer effects against gastric malignancy and the HL-60 leukemia cells, which were linked with its phytochemicals in modulating several apoptotic proteins/pathways (Adachi et al., 2007). Similarly, Park et al. have correlated the cytotoxicity of *Prunus mume* extracts with their regulatory potentials on the inhibitors of the apoptotic genes, including X-linked inhibitors (Bax), anti-apoptotic Bcl-2, survivin, cleavage of Bic, and up-regulation of FasL. Moreover, PMFE showed provoking actions on caspase-3, -8, and -9, and deterioration of caspase-3 substrate (β -catenin) protein (Park et al., 2011).

Activation of ROS production and oxidative stress can directly or indirectly provoke gastric mucosal injury. Experimental studies confirmed the effectiveness of absolute ethanol or NSAIDs in gastric ulcer initiation and progression through up-regulation of ROS formation. The lipid peroxidation of the gastric cell membrane is another outcome of increased ROS formation, followed by elevated MDA levels, reduced stomach mucus contents, and DNA structural changes (Suchiva et al., 2021). As a biomarker of lipid peroxidation, MDA levels are heightened in gastric tissues exposed to aggressive factor-mediated mucosal injuries. Almost 50% of all ethanol-mediated ROS initiation, neutrophil infiltration, and leukocyte recruitment. Increased ROS formation also downregulates ATP generation, enhancing the release of mitochondrial Ca^{2+} and altering the osmosis/lipid peroxidation balance, consequently leading to mucosal penetration and exacerbation of gastric mucosal injury (Al-Medhtiy et al., 2024). In contrast, antioxidants (SOD and GPx) exhibit a pivotal role in primary cellular antioxidant pathways, maintaining the balance of the cell's redox condition. Endogenous antioxidants, Catalase (H_2O_2 into H_2O and O_2) and superoxide dismutase (catalyzing dismutation of superoxide to H_2O_2) are considered as gastric defense factors that maintain cell redox state (Seetha et al., 2021). The present study evidenced elevated oxidative stress indications in ulcer control rats, denoted by reduced SOD, CAT, and GPx enzymes and increased MDA in gastric tissues that could be attributed to increased free radicals depleting antioxidants during scavenging actions; Nevertheless, PMFE pretreatment alleviated ethanol-mediated oxidative injury, presented by higher SOD, CAT, and GPx and a subsequent reduce in MDA levels compared to ulcer controls. Such antioxidant potential of *p. mume* fruits could be linked with its phytoconstituent potential on modulating numerous cellular and molecular pathways associated with oxidative gastric injury (NF-kappa B and JAK/STAT) (Yan et al., 2014). Similarly, researchers have linked the ameliorating effects of *p. mume* extracts on alcohol-mediated hepatic damage with its phytochemical (mumefural) potentials and its antioxidant properties, inhibiting liver lipogenesis via ROS-mitogen-stimulating protein kinase mechanism axis and decreasing hepatic apoptosis and inflammatory process via

ROS-induced p53 mechanisms (Pan et al., 2016). Similarly, *P. mume* extracts provoked antioxidant productions (SOD, CAT, glutathione) in the hyperuricemia-induced kidney injury model, parallel with its phytochemical potentials on regulating pathways (PI3K/AKT pathway) associated with oxidative stress and inflammation (Zheng et al., 2025).

The ethanol-induced gastric ulcerations are recognized with increased mucosal permeability, vasoactive molecules are released from inflammatory cells (macrophages, mast cells, and leukocytes), and increased ROS molecules activate the NF- κ B pathway through degradation of the I κ B- α protein, provoked by cellular signals (Nam and Choo, 2021). NF- κ B is a well-known transcription factor that provokes various immune and inflammatory mechanisms, producing an intricate orchestration of gastric ulcer initiation. Once activated and migrated from the cytoplasm to the nucleus, the NF- κ B complex will be able to generate transcriptional factors (such as TNF- α and IL-6). TNF- α is a pro-inflammatory chemical that can have pleiotropic effects and can play a pivotal role in ethanol-mediated gastropathy through stimulating an acute inflammatory response in conjunction with inflammatory cell/neutrophil infiltration into stomach mucosal layer (Aziz et al., 2019). Moreover, TNF- α downregulates gastric microcirculation, neutrophil recruitment (required for activating adhesive molecules), blood flow, cell proliferation, and angiogenesis near the injury site, altogether slowing down ulcer healing. (Eraslan et al., 2020). IL-6 is a common multifunctional cytokine and a modulator for acute/chronic inflammation that can stimulate inflammatory cells, releasing several noxious products, lysosomal enzymes, and ROS molecules that exacerbate stomach tissue injury (Sung et al., 2009). IL-10 is a well-recognized anti-inflammatory chemical that lowers the host's immune response to inflammatory chemokines and accelerates tissue restoration. In the present trial, ethanol-mediated gastropathy could be mediated by activation of the I κ B/NF- κ B mechanism, concomitant with up-regulation of TNF- α and IL-6 and reduced IL-10 in ulcer control rats. Fascinatingly, PMFE pretreatment attenuated ethanol-mediated cytokine alterations, evidenced by inflammatory cell infiltration, less TNF- α release, and a subsequent increase in anti-inflammatory cytokines (Yanaka, 2018). Accordingly, *P. mume* extract down-regulated (tumor necrosis factor- α) TNF- α /iNOS, (Cyclooxygenase-2) COX-2, IL-4, STAT6 (Signal Transducer and Activator of Transcription 6), (Interferon-gamma- γ) INF- γ , and STAT1, which were mainly linked with alleviating dextran sulfate sodium-mediated colitis (Jin et al., 2011). The ameliorating effects of fruit extracts of *P. mume* on alcoholic-mediated liver injury in animal models have been linked with its phytochemical potentials in mimicking inflammation/oxidative stress-related pathways (ROS-mitogen-provoked protein kinase communicating axis) (Pan et al., 2016). Similarly, extracts of *P. mume* showed remarkable reducing effects on free radicals and inflammatory markers (TNF- α and IL-6 levels) in the hyperuricemic-mediated renal injury model, which were explained by its regulatory action on the PI3K (Phosphoinositide 3-kinase)/AKT pathway (Zheng et al., 2025).

5. Conclusions

The study subjected *P. mume* extracts to acute toxicity and ethanol-mediated gastropathy evaluation in an animal model. Supplementation with *P. mume* did not cause toxicity, morbidity/mortality throughout the study. PMFE pretreatment ameliorated ethanol-mediated stomach injury, comparable to the omeprazole reference drug. Additionally, it showcased the improved gastric defense factors, antioxidants (SOD, CAT, and GPx), and robust reduction of apoptotic action (decreasing Bax and increasing BCL-2 levels), altogether attenuating infiltration of leukocytes and mucosal necrosis/lesions. PMFE pre-treatment alleviated ethanol-mediated inflammation based on serum indications, possibly because of its down-regulation of pro-inflammatory mediators (IL-6 and TNF- α) and increased IL-10 generation. This suppression lowered stomach acidity, thereby overcoming the effects of consuming conventional chemical anti-secretory medications prescribed in clinics. Given some inherent limitations of the present study (laboratory shortage, lack of specialized diagnostic machines, and small budget), future studies are suggested to unveil the complete molecular pathways that underlie its bioactivities and to isolate the main active ingredients as viable sources for nutraceutical and pharmaceutical formulation.

CRedit authorship contribution statement

Ahmed A.J. Jabbar, Khalid M. Alqais: Conceptualization; Ahmed A.J. Jabbar, Khaled Abdul-Aziz Ahmed: Investigation; Hanan Ibrahim Althagbi, Abdulmohsen I. Algefare,, Noralhuda Ayad Ibrahim, Sheylan Salah Abdullah, Talal Salem Al-Qaisi: Formal analysis and registration; Goran Noori Saleh, Manal A. Alfwuaires: Resources and validation; Mustafa Abdul-Monam, Muzhda Haydar Saber, Ahmed A.J. Jabbar: Software and design; Ahmed A.J. Jabbar: Writing manuscript; All authors agreed on final version of the manuscript.

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.

Data availability

Further data will be made available on reasonable request from the corresponding author (Ahmed A.J. Jabbar, Email: ahmed.abuljabbar@epu.edu.iq).

Declaration of generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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