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Gastroprotective, Biochemical and Acute Toxicity Effects of *Papaver decaisnei* against Ethanol-Induced Gastric Ulcers in Rats

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Abstract: *Papaver decaisnei* (*P. decaisnei*) has been used as folkloric medicine for many health issues including gastric problems. The current study investigates the gastroprotective roles of *P. decaisnei* against ethanol-induced ulcers in rodents. Sprague Dawley rats (30) were separated into five groups: the normal group (G1) and the ulcer control group (G2) were orally administered 0.5% carboxymethylcellulose (CMC); the reference group (G3) was administered 20 mg/kg of Omeprazole; two experimental groups were fed with 200 mg/kg (G4) and 400 mg/kg (G5) of the *P. decaisnei* extract (PDE), respectively. Next, the rats were given absolute ethanol and sacrificed for the analysis of the gastric mucosal injury through microscopic, enzymatic, histologic, and immunohistochemistry assays. The ulcer controls showed significant superficial hemorrhagic gastric mucosal lesions, with a decreased gastric wall mucus and edema production, whereas fewer were found for the reference and plant-treated rats. Furthermore, the PDE pre-treated rats had a significantly reduced the periodic acid-Schiff (PAS) staining intensity, produced the upregulation of the HSP70 protein, and the downregulation of the Bax protein expressions in the stomach epithelium. *P. decaisnei* displayed a significant role in the upregulation of endogenous antioxidant enzymes (SOD, CAT, and PGE2), significantly reduced malondialdehyde (MDA), TNF-a, IL-6, and upraised the IL-10 levels. Based on the positive impacts, the PDE can be proposed as the protective/treatment agent against gastric ulcers and stomach lesions.

Keywords: *P. decaisnei*; gastric ulcer; histology; immunohistochemistry; antioxidant; cytokine enzymes

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1. Introduction

Peptic ulcers are one of the most common types of diseases in the 21st century, and are identified by the occurrence of mucosal injuries, which are mainly due to Helicobacter pylori infections, platelet agglutination inhibitors, such as non-steroidal anti-inflammatory and acetylsalicylic acid drugs, serotonin reuptake inhibitors, and alcohol and tobacco use [1,2]. The mentioned diseases could cause the submucosal erosion and reduce cyclooxygenase, as well as the distortion of the stomach's mucosal layers [3]. Peptic ulcers are usually found in the stomach and in the proximal duodenum as open sores. Gastric ulcers are the outcome of interferences in the body's defenses (blood flow, mucus content, mucosal membranes,

cell regeneration, and endogenous defense enzymes) and the bioactivities of the digestive system [4].

Alcohol-induced gastric lesions cause the dysfunctionality of gastric defense factors, mucosal circulation, and mucus secretion [5]. Alcohol (Ethanol) consumption could lead to necrotic lesions in the gastric mucosa via various mechanisms, direct necrotic lesion production, which in turn lowers the defense system, secretion of bicarbonate, and mucus formation [6]. The mucus gastric layers are considered a crucial factor in the protection against gastrointestinal injuries. Mucosal secretion also has been known as a key defensive factor in the prevention of gastric lesions. The produced gastric mucus is evaluated as an amount of gastric mucosal secretion [7].

Medicinal plants have been used for numerous biological, clinical, pharmaceutical, and medicinal purposes for many years; however, their use as bio-factories for preparing desired pharmaceutical and biomedical compounds is relatively modern [8]. The presence of phytochemicals; terpenoids, alkaloids, and phenols, which are common plant organ components, supports the use of medicinal plants as alternatives to traditional medicines [8]. Phenolics, flavonoids, and terpenoids are compounds that contribute to human health as antioxidants, and through their free radical scavenging, and gastroprotective actions [9,10].

The *Papaver* plant (field poppy) is a cosmopolitan edible plant with multiple biological and medicinal potentials, thus it has been consumed from early times for many human health problems, including insomnia, oxidative stress, inflammation, and digestive issues [11]. The efficiency of the *Papaver* species, as a traditional and alternative medicine, could be linked to the species' moderate hydrophilic secondary metabolites (flavonoids, phenolic acids, alkaloids, sterols, zeaxanthins, tocopherols, tocotrienols, glycosides, and carotenoids) that were well known antioxidants against oxidative stress-related diseases [11–13]. The present research project explores the mechanism of the anti-ulcerogenic actions of *P. decaisnei* and the role of anti-inflammatory cytokines and endogenous antioxidants in alcohol-induced ulcerogenic rats.

2. Materials and Methods

2.1. Plant Collection

The aerial parts (Figure 1) of *P. decaisnei* were collected near Shaqlawa/Erbil, Iraq during the spring of 2021 (Altitude: 36.609153, Latitude: 44.526220). The plant was identified by Prof. Dr. Abdullah Sh. Sardar and the voucher specimen was deposited at the Salahaddin University Herbarium-Education College (ESUH) (voucher no. 6548).

Figure 1. General appearance of *P. decaisnei.* (taken by A.A.J.). A, Leaves; B, Stems; C, Flowers; D, poppy capsules.

2.2. Plant Extraction

The plant leaves of *P. decaisnei* were washed and air dried, and an amount of 500 g was weighed for the extraction. The leaves were macerated with 500 mL methanol for 24 h. The

filtration was performed using Whatman grade 1 paper. Next, the methanol was separated using a rotary evaporator. The obtained extracts were stored at +4 °C for later analysis and in-vivo experiments.

2.3. Chemicals and Reagents

The study project purchased all of the reagents from Sigma-Aldrich Chemical Co. (Burlington, MA, USA). The reference drug (Omeprazole) was purchased locally from a Kurdistan pharmacy. Omeprazole was blended with 0.5% carboxymethylcellulose (CMC) and orally delivered to the laboratory rodents at a 20 mg/kg (5 mL/kg) dosage, as several researchers have suggested [14].

2.4. Moral Declaration and the Animal Study

The current study was performed at the Animal House Unit, College of Science, Cihan University. The study was approved by the Ethical Committee of Cihan University (Ethical No. 34 BIO/04/06/2021/Y.G.). The experimental rats were kept in accordance with the rules and regulations set by the "Director Care Usage Research Laboratory Animals" of the "Nationwide Conservatory Knowledge" set by the "Nationwide Institution Healthiness (USA)".

2.5. Acute Toxicity and Rat Trials

For the acute toxicity test, thirty-six healthy Sprag Dawley rats (6–7 weeks old, 180–210 g in weight) were purchased from the rodent unit, Cihan University-Erbil. The rats were fed on common tap water ad libitum and rat pellets, in different cages and a flat-mesh wire was laid on the ground to prevent coprophagia. For adaptation purposes, the rodents were left in cages for one week. The rats (36) were divided into three groups: the reference group administered with 5 mL/kg CMC (G1), 200 mg/kg *P. decaisnei* (G2), and 400 mg/kg *P. decaisnei* (G3). Prior to the supplementation, the animals fasted overnight. The supplementation consisted of food, but not water. Following 3–4 h of the supplementation, the food was taken away from all of the animals. The animal groups were observed for 24–48 h following the administration of *P. decaisnei* for any unusual or toxicity clues. The rate of animal death rate was estimated to over two weeks. Following this, rats were injected with anesthetics (xylazine and ketamine) on the 15th day. The blood samples were drawn via intracardial perforation and serum samples were biochemically analyzed [15].

2.6. Gastric Ulcer Experiment

Rats were put into separate steel cages with la arge size net base to prevent coprophagia. The rats had free access to food and tap water. The rats formed into five groups (siz rats in each). For their adaptation to this environment, the rats were housed in cages for one week and fed as follows:

(1) Group 1 and 2 received carboxymethylcellulose (CMC, 0.5%), orally (normal and negative control).
(2) Group 3 was orally administered 20 mg/kg of omeprazole (reference).
(3) Groups 4 and 5 were administered 200 mg and 400 mg/kg of the PDE, respectively.

Following a period of one hour, all of the animal groups except for normal control group (G1), were orally administered 5 ml/kg of absolute ethanol. Then, (60 min later), the scarification of the rats was performed via two common anesthesia (ketamine & xylazine) and this was followed by the intracardial perforation for the blood sampling. The serum was separated and was analyzed to determine the biochemical profile of the rats [15].

2.7. Gastric Gross Study

The rats' stomachs were opened in the greater curvature. Next, all of the stomachs were washed with an ice-cold saline buffer. The gastric lesions on the stomach epithelium appeared as red injury bands. The stomach ulcers usually looked very similar to the gastric

axis. They were photographed and the damaged stomach areas were analyzed via Image J software [16].

Inhibition % (I %) estimate via succeeding formulation

I% = UAcontrol − Uatreated/UAcontrol × 100

Measurement of the stomach pH.

The stomach juice specimens were collected and mixed in order to estimate the hydrogen ion concentration in stomach fluid via a pH meter and titration using a solution of sodium hydroxide 0.1 N (mEq/L) [15].

2.8. Measurement of the Stomach Mucus Content

The gastric tissues were rinsed with an ice-cold phosphate-buffered saline (PBS). The mucosal tissues were softly scrubbed off via a fresh glass slide and the gastric mucus weight was measured via an electrical balance [15].

2.9. Formulating the Stomach Tissue Homogenate

Small sections of the glandular stomach were rinsed with an ice-cold PBS. Portions of the stomach walls were homogenized (10% w/v) with a cold PBS bearing a mammalian protease inhibitor cocktail via a homogenizer. Gastric homogenates (1000 g) were spun for 10 min at 4 °C. The obtained fluid was analyzed for prostaglandin E2 (PGE2), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels. The examination was carried out based on the manufacturer's guidelines (Cayman, Ann Arbor, Michigan 48103, USA) balance [15].

2.10. Evaluation of the Antioxidants

The endogenous antioxidants (SOD, CAT, and PGE2) in the gastric homogenates were measured via commercial kits (Elabscience, Wuhan, China). The manufacturer's guidelines were followed during the procedure balance [15].

2.11. Lipid Peroxidation (MDA) Status of the Tissue Homogenates

The MDA (malondialdehyde) levels in the tissue homogenate were estimated via thiobarbituric acid reactive substance (TBARS) methods via profitable kits (Elabscience, Wuhan, China), according to the manufacturer's guidelines [15].

2.12. Histological Analysis

The gastric glandular epithelium was sectioned in small pieces (1–2 cm) and then fixed instantly in 10% formalin for one day at room temperature. The gastric tissues were dehydrated with ethanol for shadowing, the tissue processing machine was used for the clearance with the penetration of xylene paraffin. The biopsy of the tissues were embedded in paraffin in a 5 µm slice for sliding (Leica Rotation Microtome) [15].

2.13. Hematoxylin and Eosin Stains

The prepared slides were stained with routine hematoxylin and eosin stains and analyzed histopathologically via a compound microscope [15].

Periodic acid–Schiff stain (PAS)

The secreted mucus of the gastric glandular epithelium was measured using the PAS staining of a 5 µm glandular sliced layer of the gastric tissue in order to differentiate between the glycoprotein levels (acidic or basic) in the mucus, following the manufacturer's instruction (Sigma Periodic Acid–Schiff (PAS) Kit, Merk, Germany). The microscopic image of the gastric PAS stained slice was obtained via Image J software [15].

2.14. Immunohistochemical Staining

The gastric sliced tissues (5 µm) were immunostained using the Animal Research Kit in order to find the local HSP70 (1:100) and Bax (1:50) protein expressions. The standard proteins were purchased from "Santa Cruz, USA". The immunohistochemically stained

positive cells were enumerated via microscope observations. The microscope images were evaluated by using Image J software to evaluate the intensity of the stain on positive cells. Then, the percentage amount of the stained cells in the overall pretentious part was estimated [15].

2.15. Evaluation of the Cytokines

The determination of the TNF-α, IL-6, and IL-10 was carried out via ELISA kit, My BioSource, USA, and following the manufacturer's instructions. The evaluation of the cytokine strength was possible via the recombinant cytokines with the sanitized properties [17].

2.16. Statistical Analysis

The statistical process of the acquired data was made by utilizing SPSS (version 24 for Windows). The variance (ANOVA) for the One-way analysis was followed, shadowing Tukey's post-hoc trial. The standard test used the Kolmogorov–Smirnov test and the values were designed as mean ± SEM. The value of $p < 0.05$ for example, measured significantly similar with the ulcer control group.

3. Results

3.1. Acute Toxicity

The in-vivo acute toxicity trial was performed on the 36 rats, they were separated into three groups; the vehicle (G1) left without supplementation (5 mL CMC/kg), the experimental groups (G2 and G3) addressed with one dosage of 200 mg/kg and 400 mg/kg *P. decaisnei,* respectively. The rats were observed for 14 days, every eight hours. Through the observational process of all of the rats, the acute toxicity results showed that all of the experimental rats passed the test and none showed any signs of toxicity. Furthermore, the follow-ups found no abnormalities in the rats' weight, behavior, food, and liquid consumption. Additionally, the histopathological analysis revealed no tissue damage in the liver and kidneys of the experimental rats (Figure 2).

3.2. The Stomach Gross Analysis

The current results revealed that the rats treated with *P. decaisnei* had significantly fewer ulcerated gastric lesions than those of control group (Figure 3). Furthermore, the rats supplemented with *P. decaisnei* had less mucosal damage of the gastric epithelial surface compared to the ulcer control group (Figure 3). The ulcer control group rats (G2) had significantly more gastric lesions in the submucosal linings than those of all of the other experimental groups (G1, G3, G4, and G5).

3.3. Gastric Mucus Level

The data results have shown that the rats treated with *P. decaisnei* had a significantly higher gastric mucus output compared to the ulcerated reference group. The mucus weight was significantly reduced in the ethanol-induced gastric ulcer controls (G2) (Table 1). The mucus weights of the ulcer control rats (G2) was significantly lower (0.67 ± 0.32 g) than that (1.75 ± 0.65, 1.77 ± 0.48, 1.52 ± 0.33, and 1.76 ± 0.58) of G1, G3, G4, and G5, respectively.

The *P. decaisnei* treatment prior to the administration of the absolute ethanol significantly increased the mucus weight compared to the negative (ulcer) control (Table 1).

3.4. P. decaisnei Effects on the Stomach pH

The pretreatment of the experimental rats with *P. decaisnei* before the ethanol gavage had a significantly higher stomach pH compared to the ulcer reference. The ethanol ingestion caused a significant drop in pH in G2 (2.9 ± 0.8), creating a higher gastric ulcer area (655.78 ± 8.55 $(mm)^2$) than that (98.33 ± 5.43, 152.66 ± 7.30, and 108.16 ± 4.89 $(mm)^2$) for G3, G4, and G5, respectively. The recorded pH for G1 and G3 had similar values at 6.10 ± 0.55 and 6.2 ± 0.60, respectively. The rats pretreated with 400 mg/kg of the

P. decaisnei extract (G5) had a significantly higher inhibition % (83.5%) of ulcer area compared to 76.72% for 200 mg/kg of the *P. decaisnei* extract treated rats (G4) (Table 1).

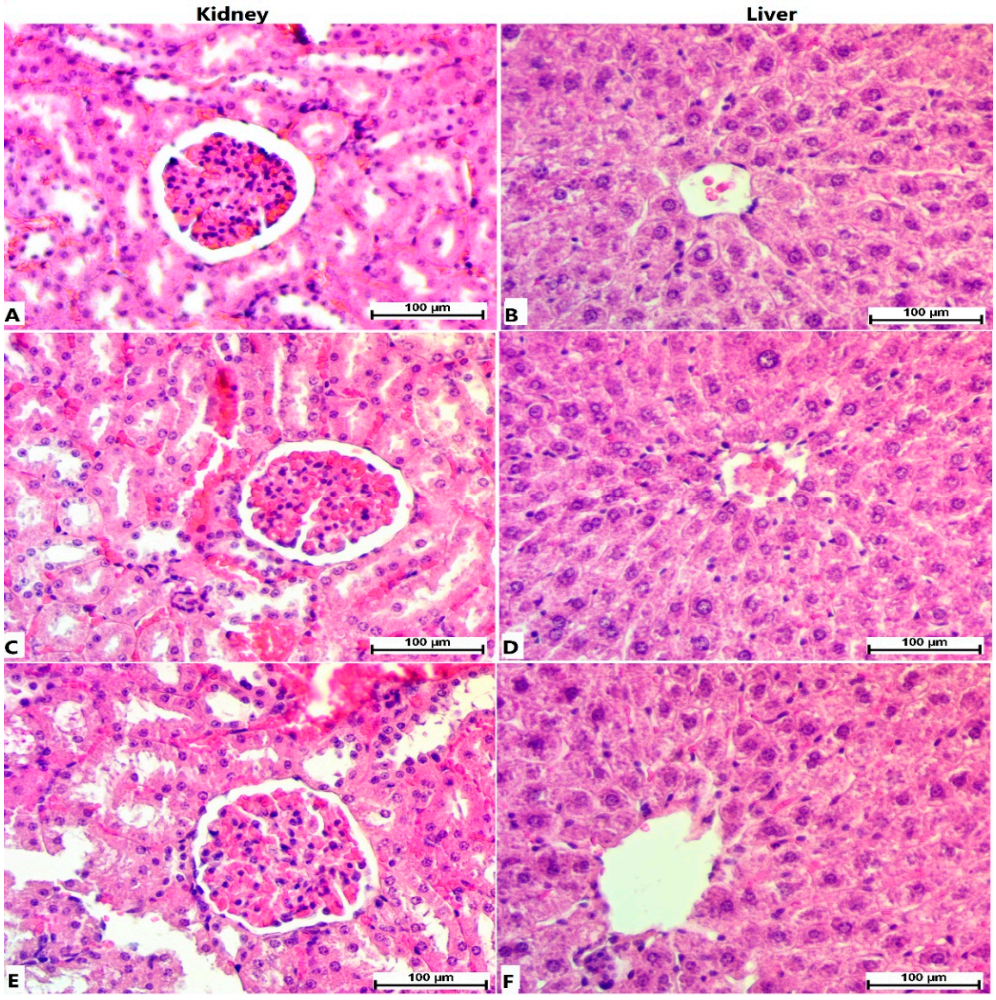

Figure 2. Histological portions of the kidney and liver of rats tested for acute toxicity. Rats administered with 5 mL/kg of the vehicle (0.5% CMC) (**A**,**B**). Rats administered with the PDE, 200 mg/kg (**C**,**D**). Rats administered with the PDE, 400 g/kg (**E**,**F**). No significant changes were observed in the structures of the livers and kidneys between the treated and control groups (H & E staining, 40×).

Table 1. Protective effects of *P. decaisnei* on some stomach parameters of the rats with ethanol-induced gastric ulcers.

Animal Groups	Pre-Feeding (5 mL/kg)	Mucus Weight (g)	pH	Ulcer Area (mm^2)	Inhibition (%)
G1	0.5% CMC	1.75 ± 0.65^{a}	6.10 ± 0.55^{a}	-	-
G2	0.5% CMC	0.67 ± 0.32^{b}	2.9 ± 0.8^{d}	655.78 ± 8.55^{d}	-
G3	20 mg/kg omeprazole	1.77 ± 0.48^{a}	6.2 ± 0.60^{a}	98.33 ± 5.43^{a}	85.0% ^{a}
G4	200 mg/kg PDE	1.52 ± 0.33^{c}	5.22 ± 0.5^{c}	152.66 ± 7.30^{c}	76.72% ^{b}
G5	400 mg/kg PDE	1.76 ± 0.58^{a}	5.8 ± 0.6^{b}	108.16 ± 4.89^{b}	83.5% ^{a}

Data expressed as mean value ± SEM (n = 6). Numbers shown with different superscriptions within the column mean they are statistically variant, based on Tukey's honesty test at $p < 0.05$. PDE, *Papver decaisnei* extract.

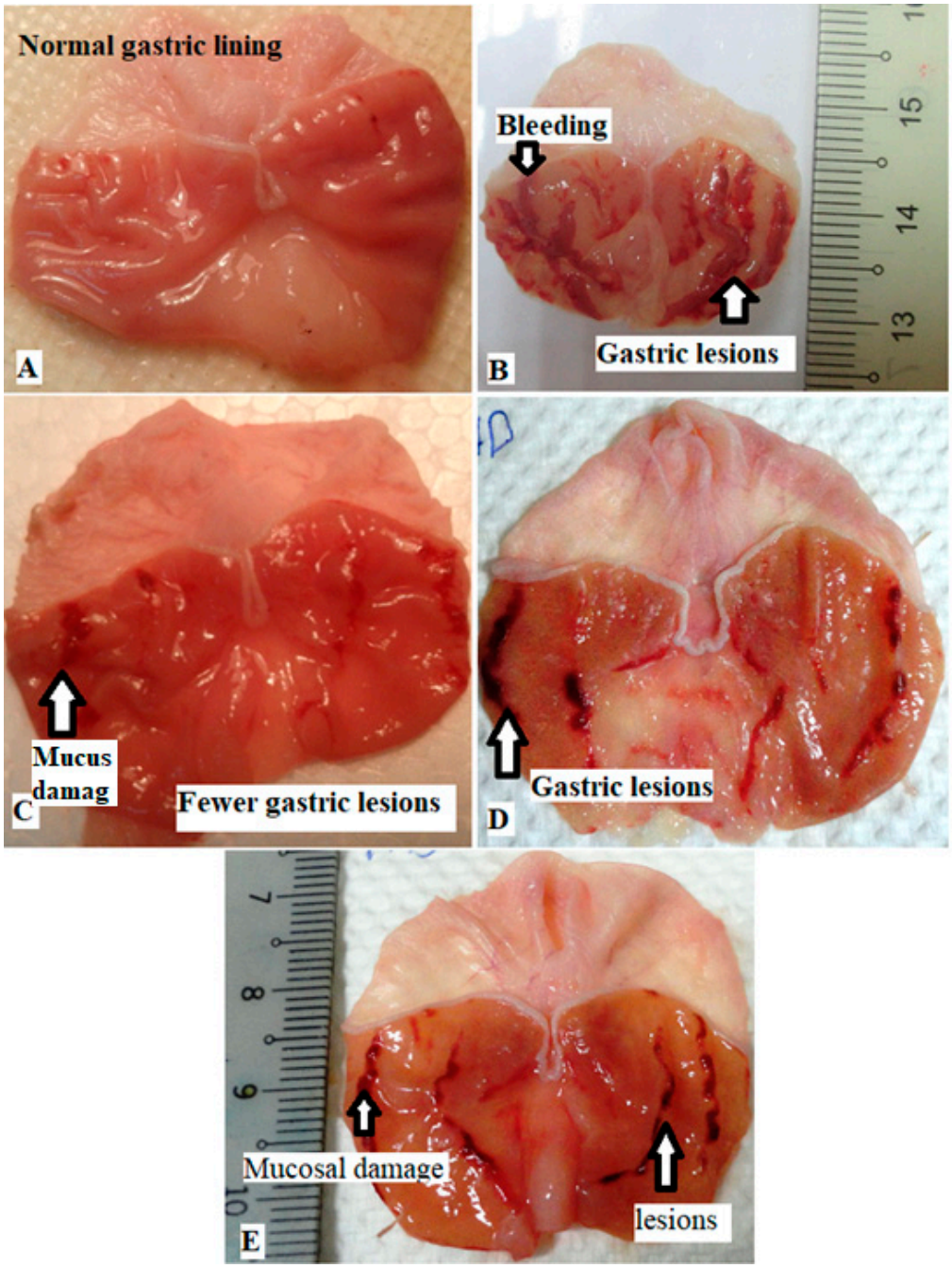

Figure 3. Influences of the PDE on the gross appearance of the stomach mucosa obtained from the ethanol-induced ulcerogenic rats. (**A**), normal control; (**B**), ulcer control; (**C**), reference rats (omeprazole, 20 mg/kg); (**D**), rats received 200 mg/kg of the PDE; (**E**), rats received 400 mg/kg of the PDE.

3.5. H & E Stain

The rats with no pretreatment had severe lesions with submucosal damages after the ethanol gavage. Furthermore, the ulcer control showed more intense edema with leukocytes in the subcutaneous tissues than that of the plant treated groups. While, the rodents treated with *P. decaisnei* had a better defense gastric epithelium with less gastric damage and less edema inflammation in the submucosa (Figure 4).

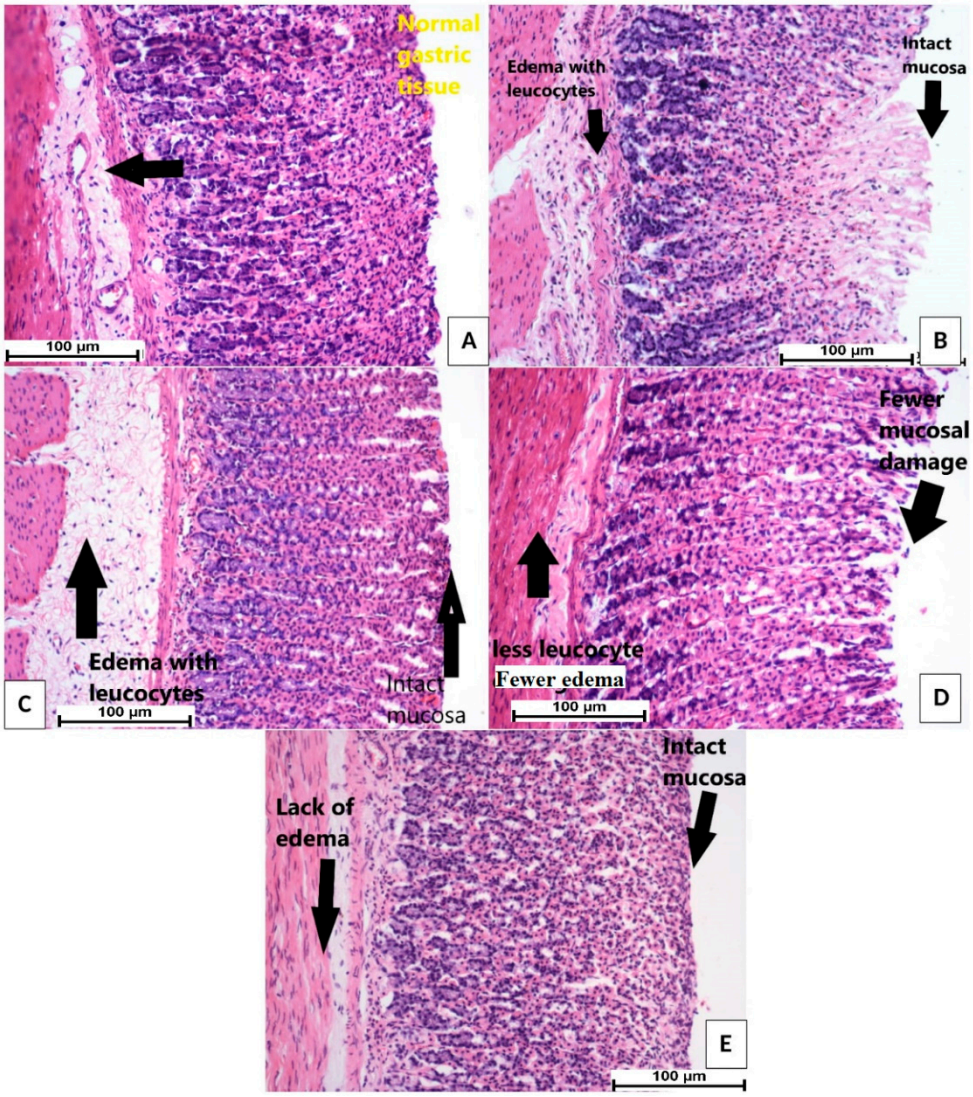

Figure 4. Influences of the PDE on the microscopic views of the stomach tissues obtained from ethanol-induced ulcerogenic rats. (**A**), normal control exhibited normal mucosal structure based on the microscopic views; (**B**), ulcer control had severe stomach injuries in their mucosal linings with multiple lesions areas; (**C**), reference rats (omeprazole, 20 mg/kg) experienced mild mucosal injury with the presence of edema and leukocytes in the submucosal areas; (**D**), rats administered 200 mg/kg of the PDE had moderate mucosal injuries with multiple; (**E**), rats administered 400 mg/kg of the PDE had mild mucosal damage with a lack of edema based on the microscopic views (H & E stain, magnification 20×).

3.6. PAS Stain

The present results showed that the rats treated with *P. decaisnei* had higher PAS staining concentrations of the gastric glycoprotein than those of the negative (ulcer) control (Figure 5). The ulcer controlled rats showed significantly greater gastric tissue disruption with severe inflammatory edema and leukocytes than those of the herbal treated rats.

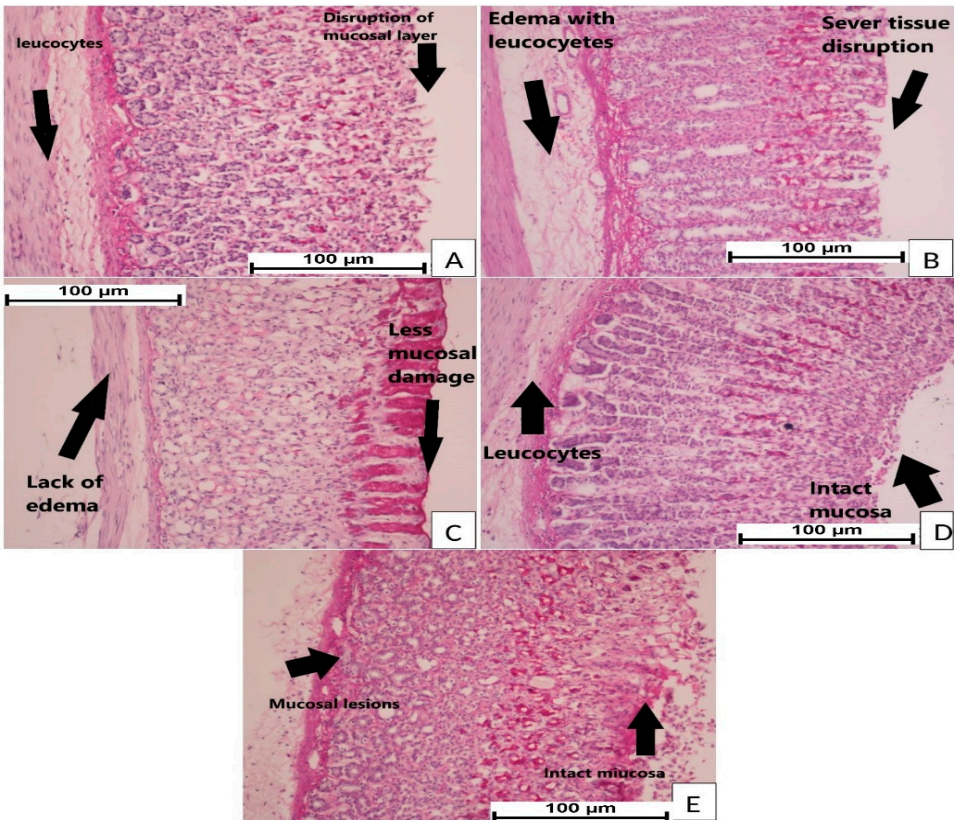

Figure 5. Influences of the PDE on the microscopic views of the gastric mucosa stained with PAS glycoprotein in the ethanol-induced ulcerogenic rats. (**A**), normal control had a normal appearance of the stomach mucosa; (**B**), ulcer control showed sever stomach tissue damage with mild magenta PAS stain; (**C**), reference rats (omeprazole, 20 mg/kg) had non-significant stomach tissue disturbances with a high PAS staining concentration; (**D**), rats received 200 mg/kg of the PDE had mild to moderate stomach tissue damage with fewer leukocytes in the submucosal areas; (**E**), rats received 400 mg/kg of the PDE showed mild gastric mucosal injury with fewer lesions and moderate expressions of the PAS stain (PAS stain magnification 20×).

3.7. Immunohistochemical Staining

The results have shown that the omeprazole or *P. decaisnei* treated rats had higher HSP70 and Bax gastric protein expressions than those of the negative (ulcer) control (G2). The analysis of the HSP70 protein expressions have shown antigens with a strong brown color in rats administered with omeprazole (G3) or *P. decaisnei* (G4 and G5) (Figure 6). Furthermore, the Bax protein expressions were significantly higher in the negative (ulcer) control (G2) than those of the rats pre-ingested omeprazole (G3) or *P. decaisnei* (G4 and G5). The measurement of the HSP70 and Bax proteins staining intensity in the gastric tissues was made via applying the Image J software. (Figure 7).

3.8. P. decaisnei Effects on the Gastric Antioxidants

The biochemical analysis has shown that the negative (ulcer) control had significantly lower SOD, CAT, and PGE2 enzyme actions than those of rats who pre-ingested omeprazole (G3) or *P. decaisnei* (G4 and G5) (Figure 8). The rats treated with *P. decaisnei* showed a significant restoration of SOD, CAT, and PGE2 activities to the standard values. The rats administered with *P. decaisnei* extracts at 400 mg/kg showed notably higher levels of SOD (14.27 ± 1.22) and CAT (35.19 ± 1.36) than those (SOD, 12.15 ± 1.38; CAT, 31.425 ± 1.96); (SOD, 9.08 ± 0.68; CAT, 19.97 ± 1.19) of the *P. decaisnei* 200 mg/kg and ulcer control groups, respectively. The malondialdehyde value was notably lower (23.64 ± 2.19) in rats that ingested *P. decaisnei* 400 mg/kg (G5), compared to those 86.65 ± 6.0 and 29.45 ± 3.17 mg/dL

of the G2 and G4 groups, respectively. The positive (normal) and standard drug (omeprazole) administered rats had non-significant differences in their gastric antioxidant status. The *P. decaisnei* 400 mg/kg treated rats had significantly higher prostaglandin E2 levels (30.66 ± 1.50,) compared to those 12 ± 1.41, 7.16 ± 1.19, and 21.66 ± 1.75 of the G1, G2, and G4 groups, respectively (Figure 8).

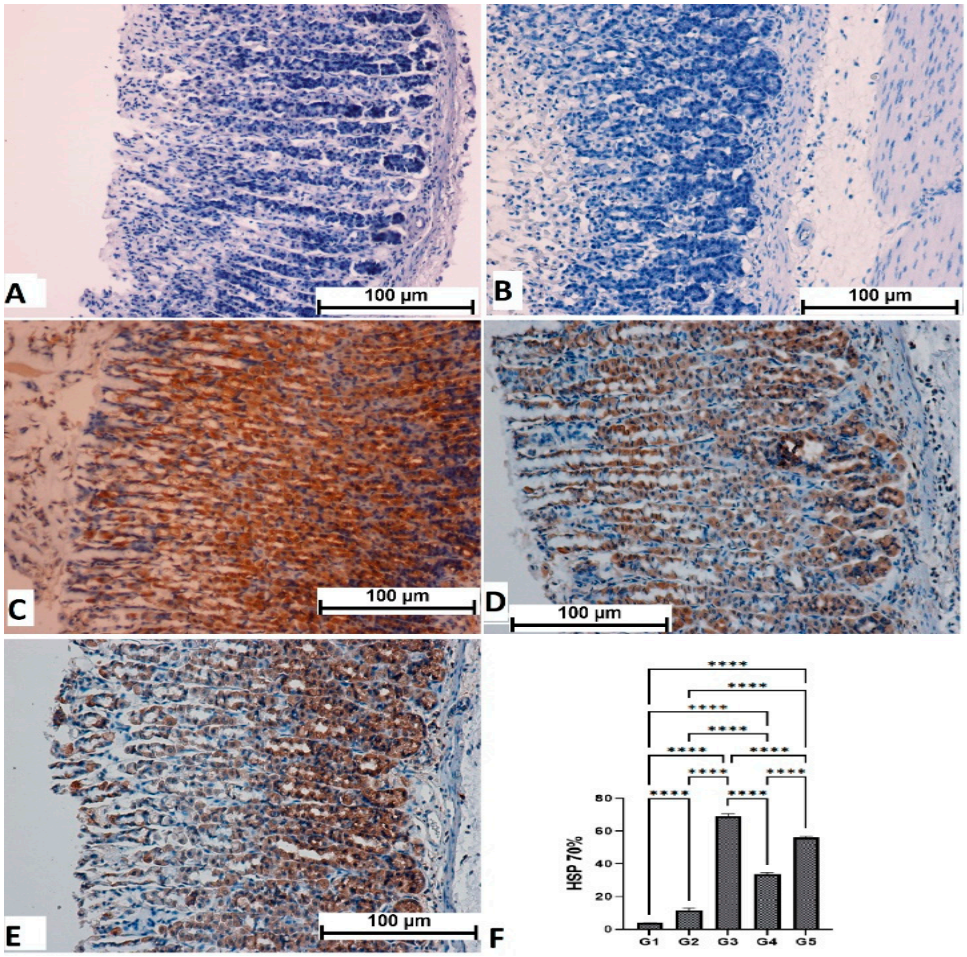

Figure 6. Influences of the PDE on the microscopic views of the stomach mucosa based on the HSP70 protein representation (**A**–**E**) and quantitative statistics (**F**) of the HSP70 protein expression by the stomach mucosa obtained from the ethanol-induced ulcerogenic rats. (**A**), normal control; (**B**), ulcer control; (**C**), reference rats (omeprazole, 20 mg/kg); (**D**), rats received 200 mg/kg of the PDE; (**E**), rats received 400 mg/kg of the PDE. Normal control rats had a normal structure of the stomach mucosa and a low representation of the HSP70 protein in their stomach tissues (**A**,**F**). Ulcer control rats showed severe stomach tissue injury with significantly very low HSP70 protein representations in their stomach tissues compared to other treated groups (**B**,**F**). Reference rats experienced a mild stomach tissue injury with an increased expression of the HSP70 protein in their gastric mucosa (**C**,**F**). Rats administered 200 mg/kg of the PDE had moderate stomach lesions with increased representations of HSP70 in their stomach tissues (**D**,**F**). Rats administered 400 mg/kg of the PDE showed a mild stomach tissue injury with increased representations of HSP70 protein, compared to the ulcer controlled rats (**E**,**F**). (HSP70 stain, magnification 20×). Values expressed as means ± SEM. The antigen area is observed in a brown color. The significant difference in the HSP 70% protein expressions are presented as $p < 0.0001$ (****).

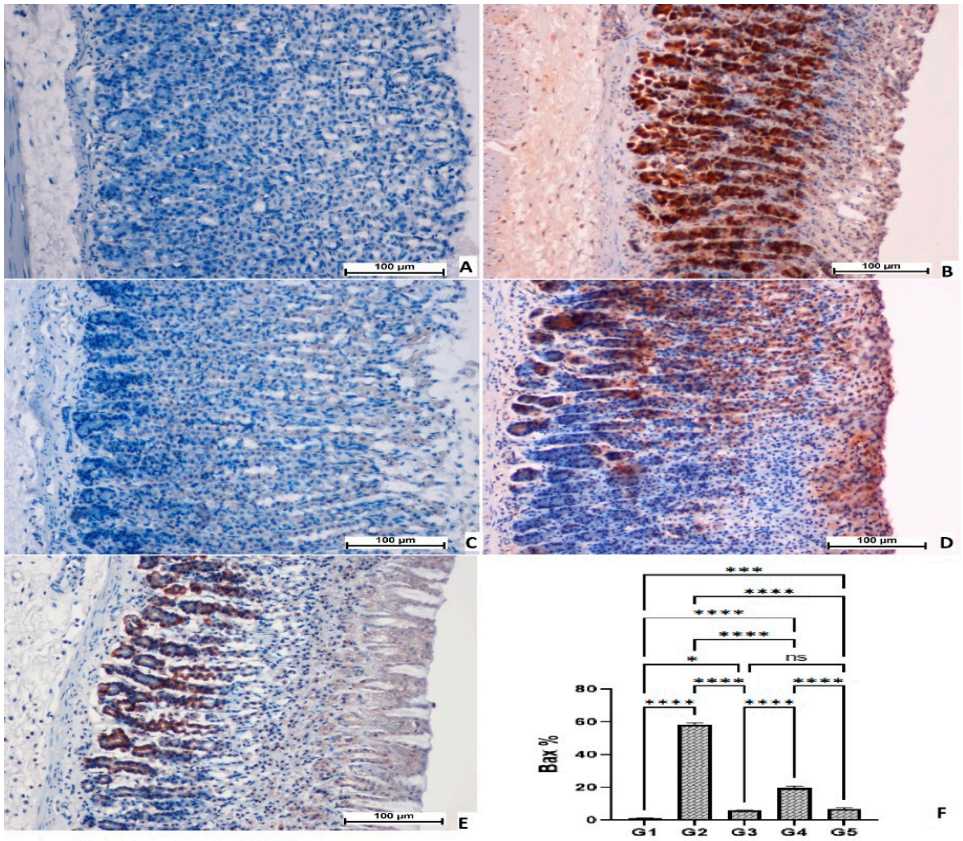

Figure 7. Influences of the PDE on the microscopic views of the stomach mucosa based on the Bax protein representation (**A**–**E**) and quantitative statistics (**F**) of the Bax protein expression by the stomach mucosa obtained from the ethanol-induced ulcerogenic rats. (**A**), normal control; (**B**), ulcer control; (**C**), reference rats (omeprazole, 20 mg/kg); (**D**), rats received 200 mg/kg of the PDE; (**E**), rats received 400 mg/kg of the PDE. Normal control rats had a normal appearance of their stomach tissues and significantly the lowest representation of the Bax protein in their gastric tissues (**A**,**F**). Ulcer control rats experienced severe stomach tissue damages with increased representations of the Bax protein in their gastric mucosa (**B**,**F**). Reference rats had a mild stomach tissue injury with significantly decreased representations of the Bax protein in their mucosal linings, compared to the ulcerogenic rats (**C**,**F**). Rats administered 200 mg/kg of the PDE had a moderate gastric tissue injury with significantly reduced representations of the Bax protein, compared to the ulcerogenic rats (**D**,**F**). Rats administered 400 mg/kg of the PDE showed a gastric mucosal injury and significantly lower representations of the Bax protein than those of the ulcerogenic and low dose treated rats (**E**,**F**). Non-significant changes observed in the quantitative measurements of the Bax protein expression between the C and E rat groups. (Bax stain, magnification 20×). Values expressed as means ± SEM. Antigen areas observed in a brown color. The significant levels in the Bax protein expressions are presented as ns, non-significant; *, $p < 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$.

3.9. P. decaisnei Effects on Malondialdehyde (MDA) in Gastric Homogenates

The data results have shown that the experimental negative (ulcer) rats (G2) had significantly higher MDA in the gastric tissue homogenates than those of all of the other examined rats. These oxidative stress markers were expressively decreased in the gastric homogenate of the rats treated with the *P. decaisnei* extracts (G4 and G5). There were non-significant changes found between the MDA levels of the normal control and the omeprazole groups (G1 and G3) (Figure 8).

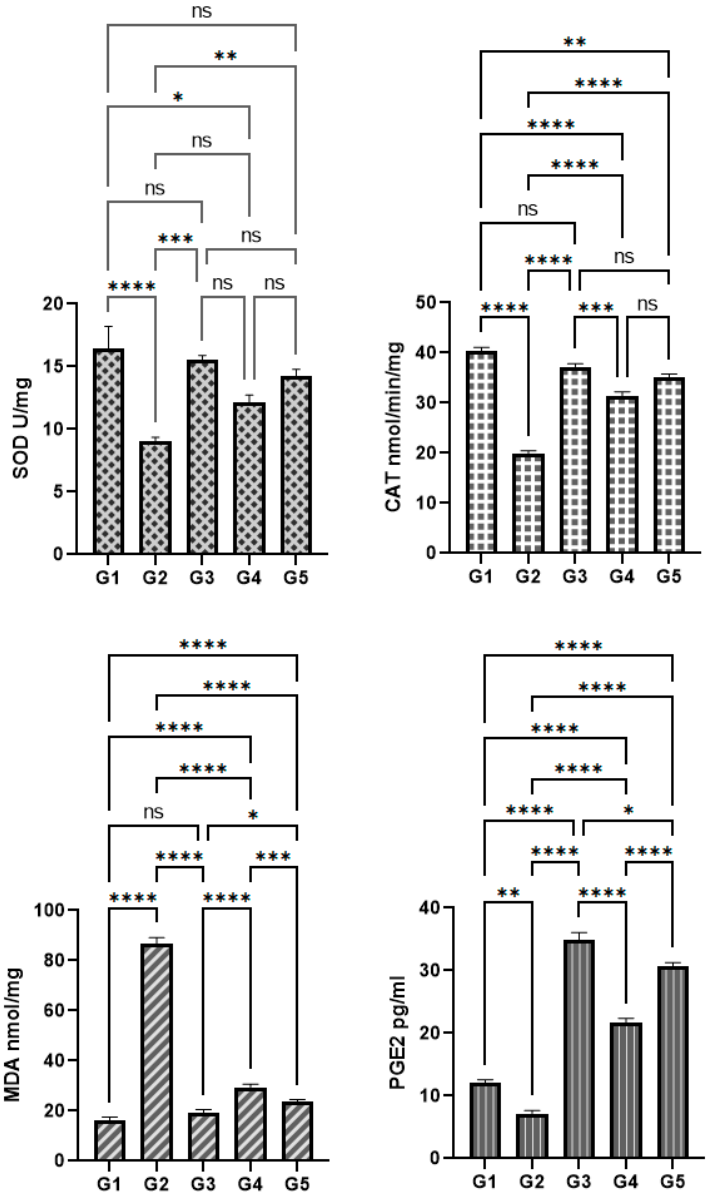

Figure 8. Influences of the PDE on SOD, CAT, PGE2, and MDA levels in stomach tissue homogenates of the ethanol-induced ulcerogenic rats. G1, normal control; G2, ulcer control; G3, reference rats (omeprazole, 20 mg/kg); G4, rats received 200 mg/kg of the PDE; G5, rats received 400 mg/kg of the PDE. Data are expressed as means ± SEM (n = 6). The significant levels are presented as *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

3.10. Effects of P. decaisnei extracts on the Serum Cytokine Enzymes

The data analysis of the cytokine enzymes examination revealed the superiority of the rats treated with omeprazole or *P. decaisnei* (Figure 9). In which, the rats ingested omeprazole or *P. decaisnei* had notably lower TNF-α and IL-6 and higher IL-10 than those of the negative group (Figure 9). The TNF-α values of G1 (129.16–4.83 P.pg/mL) and G5 (132.66–4.412 P.pg/mL) were lower than those (519.16–9.217 and 249–6.06 P.pg/mL) for G2 and G4, respectively. Furthermore, The IL-6 values of G5 (151.16–5.77 Pg/mL) were lower than those (340.83–5.34 and 145.83–3.488 Pg/mL) of the G2 and G4, respectively, while they had not significantly changed, compared to G 1 (154.33–2.804 Pg/mL). The IL-10 numbers were significantly restored in G4 (204.33–44.98 Pg/mL), compared to those (105.66–5.39 and 175.166–52.96, and 181.166–5.56 Pg/mL) of G2, G3, and G4, respectively, while not significantly different, compared to G1 (236.166–3.816). In contrast, the cytokine enzymes

were negatively affected in the ulcer group compared to the rats treated with omeprazole or *P. decaisnei.*

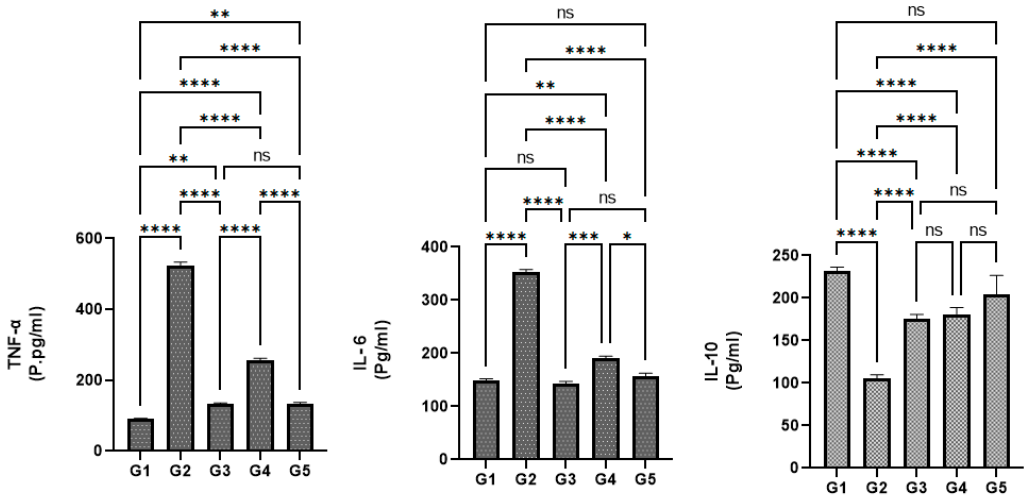

Figure 9. Influences of the PDE on TNF a, IL 6, and IL10 levels in the serum specimens of the ethanol-induced gastric ulcerogenic rats. G1, normal control; G2, ulcer control; G3, reference rats (omeprazole, 20 mg/kg); G4, rats received 200 mg/kg of the PDE; G5, rats received 400 mg/kg of the PDE. Data are expressed as means ± SEM (n = 6). The significant levels are presented as *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

4. Discussion

The acute toxicity test did not propose any sign of toxicity or death in the 400 mg/kg *P. decaisnei* -treated rats. The test showed the safety of this plant in the 400 mg/kg administration. Similarly, researchers and toxicologists have reported the safety of *P. rhoeas* in the 2 g/kg dosage administration in experimental rat models [18,19]. Furthermore, biochemical research on *P. somniferum* reported the safety of 2000 mg/kg body weight/day on rats with no adverse effects on the hematological and biochemical parameters [20].

Until now, the main causes of gastric ulcers are not completely clear, but scientists agreed that most cases are the outcomes of an imbalance between aggressive factors and holding the mucosal integrity via the endogenous antioxidant defense system [21]. These gastric defensive mechanisms are disrupted by alcohols (ethanol) and necrotizing agents that produce gastric mucosal lesions [22]. These mucosal layer damages by ethanol began with microvascular damage (disruption of the vascular endothelium) that leads to uprising vascular permeability, edema production, and epithelial wall disruption. Ethanol could lead to necrotic lesions in the mucosal layers via its direct gastric impairments, decreasing the bicarbonate secretion and the gastric mucus reduction [23].

The presented data revealed that ethanol decreased the gastric mucosal thickness, reduced the gastric mucus, and the prostaglandin levels in the gastric tissue homogenates. Consequently, ethanol caused a greater ulcer area formation, and more acid secretion, which leads to desquamation of the gastric epithelium with ulceration and hemorrhage between the injured gastric glands. Similar results were found by the previous researchers [24].

The current study showed that the experimental rats treated with *P. decaisnei* had flattened gastric linings and more surface area in the gastric epithelia, and fewer gastric damages in contrast to the ulcer controls. Accordingly, previous research on several medicinal herbs or natural compounds reported their efficiency in flattening gastric mucosa layers and their anti-ulcer actions against ethanol-induced ulcers in rats [25]. Furthermore, a research study has reported the anti-ulcer actions of fresh roots of *Papaver rhoeas* in 670 mg/kg in ethanol-induced ulcer rats [26]. Furthermore, investigators have stated that chelerythrine (CHE), a quaternary benzo[c] phenanthridine alkaloid naturally exist in the Papaveraceae family, as an anti-ulcer agent after showing its efficacy in 1, 5,

10 mg/kg versus ethanol-induced ulcer rats [27]. The researchers also reported the anti-ulcer actions of an isoquinolinic alkaloid called N-methylcorydaldine, that was first isolated from *Papaver bracteatum*, after showing its (10–100 µg/mL) potentials in inhibiting gastric H+/K+-ATPase activity (percentage: 71.43 (IC50: 60.9 µg/mL)) and significantly reduced the plasma gastrin level (96.8 ± 8.9 pg/mL) in ethanol-induced gastric ulcer rats [28]. Furthermore, O-methylarmepavine and isocorydine [(6aS)-1,2,10-trimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline-11-ol] were another two isolated alkaloids from the genus *Papaver* that were reported as anti-ulcer agents after showing their efficiency reducing gastric H+/K+-ATPase activity and plasma gastrin levels in the ethanol-induced ulcerogenic rats [29].

The gastroprotection efficacy of *P. decaisnei* against ethanol-induced stomach damage could be correlated with its efficacy in the reduction of inflammatory cell permeation in the layers of the submucosa. Accordingly, a research study reported the *Papaver nudicaule* as anti-inflammatory herbal medicine, after presenting its efficacy against lipopolysaccharide-induced inflammation through decreasing the nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-κB) and the signal transducer and activator of transcription 3 (STAT3) signaling pathways [29].

The present study shows training displayed via histological experiments, in the gastric layers of ulcer controls, severe hemorrhagic gastric damages with increased leucocytes (neutrophils), penetration, and edema were found. While *P. decaisnei* in pretreated rats before ethanol consumption had fewer gastric damages with lower gastric lesions and absence of edema as presented by the comparison with the stomach mucosa. In accordance with our findings, several herbal medicines enriched with phenolic and alkaloid contents, were reported as anti-ulcer against ethanol-induced ulcer rats. Previously, researchers have shown the gastroprotective properties of *Rondeletia odorata* [30], *Calpurnia aurea* [25], *P. brateatum* [28], and they have linked the plants' pharmacological actions (anti-ulcer, anti-inflammatory, and antioxidant) with the plants' phytochemical contents (phenolics, flavonoids, and alkaloids) via the possible mechanism of the downregulation of the cytokine enzymes (IL-1β, IL-6, and TNF-α) and the upregulation of endogenous antioxidant enzymes (SOD, CAD, and PGE). This consequently results in smaller ulcer areas, greater gastric juice volume, fewer or absent of edema, and leukocytes infiltration in the gastric submucosa through the reduction of stomach acidity (up-rising gastric pH).

The PAS staining application shows the potentials of carmine staining of the stomach tissues that releases mucopolysaccharides. The mucosal secretion is considered as a defense mechanism of the gastric mucosal tissues, tissues with higher mucus secretions will show a higher intensity of the PAS stain. The data outcomes showed that the rats pretreated with *P. decaisnei* had a higher intensity of the PAS stain in the gastric tissues compared to the negative group. Accordingly, a previous study on medicinal plants reported the same results in the experimental rat trials [31].

The current study showed that absolute ethanol caused oxidative stress by increasing the reactive oxygen species (ROS) production, which has been correlated with the decrease in the expression of the HSP70 protein and enhances the proapoptotic expression. Oxidative stress has been known for its destruction of proteins (DNA) and lipids, leading to oxidative stress, cell damage, and tissue injuries [6]. The HSP70 proteins are well known for their protective efficacy against oxidative stress that occurred in the gastric epithelium induced by ethanol (alcohol). Furthermore, the HSP70 protein prevents the deformed or half denaturized proteins to aggregate and mediates the protein refolds. The *P. decaisnei* pretreated rats showed an upregulation of the HSP70, which lead to increased protection of the gastric epithelial layers. Similar results were found by multiple investigators as they showed that the upregulation of the HSP70 was significantly correlated with the gastric defense system against absolute ethanol, which could be explained by decreasing the ROS-mediated gastric oxidative stress [32,33]. The increased HSP70 expression in the *P. decaisnei* treated groups could be due to its phytochemical contents (phenolics, flavonoids, and alkaloids) that were reportedly stated as antioxidants [34,35].

Oxidative stress has been known as a serious cause of inflammation, which is defined as an imbalance in the ROS (superoxide, hydroxyl free radicals, and hydrogen peroxide), which also lowers antioxidant enzyme productions that fight free radicals [32]. Antiradical enzymes such as the phase 2 antioxidant induced enzymes and the cell protective genes (glutamate-cysteine ligase catalytic (GCLC), heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO1)) were activated via the nuclear factor erythroid-2-related factor 2 (Nrf2), which has an important role in cell protection from free radicals and inflammations [36–38]. Nrf2 is dimerized with the Maf small-sized proteins in the nucleus and joins cis-regulatory with the antioxidant response-element to stimulate the transcriptional expression. This action could be down-regulated by numerous genes, such as NQO1 and HO-1, as scientists have clarified [38]. Furthermore, the NF-κB signaling considered as a suppressor of the antioxidant actions due to its inhibitory effects on the Nrf2-Keap1 mechanism via the p65 and the Keap1 interactions [39]. Thus, establishing a new anti-inflammatory Nrf2 signaling is crucial to enhance the cells' antioxidant defense systems, due to its potentials in the upregulation of cell protection genes (HO-1, NQO1, and GCLC) [38]. The current study showed the significant antioxidant potentials of *P. decaisnei*, which could be through the activation of the antioxidant genes mediated by Nrf2. Similarly, a recent study has reported that the isoquinoline alkaloids profile and the antioxidant potentials of *P. nudicaule* via the mechanism of the Nrf2-mediated stimulation of antiradical genes. While the notable antioxidant actions of the *P. rhoeas* extracts were explained via the upregulation of NQO1, without Nrf2 mediation, possibly via suppressing NF-κB [40].

The human Bax pro-apoptotic protein is encoded by the BAX gene and is a well-known member of the Bcl-2 family, that is involved in the regulation of the programmed cell death (P53-mediated apoptosis) via the interaction and the increased permeability of the mitochondria, leading to a cytochrome c loss [41]. Studies have shown that many factors including absolute ethanol could lead to the development of apoptosis of the gastric epithelial overexpression of pro-apoptotic proteins, such as the Bax protein reduction of the anti-apoptotic bodies (Bcl-2) [42]. The *P. decaisnei* pretreatment in rats lead to the Bax protein downregulation and the increased HSP70 protein appearance based on the stomach tissue homogenate analysis compared to the ulcer controls. Accordingly, a previous study has found the efficacy of medicinal plants to decrease the Bax protein and increase the HSP70 protein expression possibly because the plant's biological actions (antioxidants) that can defend the stomach mucosa against oxidative stress-related diseases induced by absolute ethanol [43].

The present investigation shows that *P. decaisnei* pretreated rats significantly decreased the MDA values and increased the antioxidants (SOD, CAT, and PGE2) in the gastric tissue homogenate against the oxidative stress-induced via ethanol gavage. The presented data also suggest *P. decaisnei* as a strong anti-inflammatory agent as the plant-treated rats had preserved the gastric PGE2 contents against the absolute ethanol-induced ulcers. Similar results were published by numerous researchers as they showed the efficacy of the medicinal herbs or their isolated natural compounds to decrease the levels of endogenous enzymes (SOD, CAT, and PGE2) and decreasing the MDA levels based on the stomach tissue homogenate analysis of the alcohol-induced gastric ulcer rats [44–48]. The increased levels of the endogenous antioxidant enzymes are crucial in the gastroprotection against the oxidative pressure induced by the ROS ethanol-induced stomach damage. The antioxidant actions of *P. decaisnei* could be related with it is phytoconstituents [alkaloids (62.03%), phenolics (55.43%), fatty acids (42.51%), esters (32.08%), terpenoids (25.59%), and phytosterols (15.68%)], as explained in detail in our recently published in-vitro study [11]. Similarly, many studies have stated those phytochemicals are scavengers of free radicals [46–48]. Furthermore, a recent study has shown the significant antioxidant actions of *P. somniferum* and correlated this biological activity with the plant's high phenolic contents [49].

Cytokine enzymes (TNF-α, IL-6, and IL-10) are potent inflammatory mediators concealed by macrophages known to have a significant role in the ethanol-induced gastric injury and penetration of the polymorphonuclear neutrophils in the submucosal layer [50].

The present study showed that rats administered with absolute ethanol without pre-treatment had higher TNF-α and IL-6 and lower IL-10 levels than those of the reference and plant-treated rat groups. The *P. decaisnei* pretreatment significantly decreased the proinflammatory TNF-α and IL-6 levels while increasing the anti-inflammatory IL-10 in the blood plasma compared to the ulcer controls. Furthermore, the pre-treatment of rats with *P. decaisnei* statistically lowered the TNF-α IL-6 augmented IL-10 level comparable to rats pretreated with omeprazole. Studies have shown that the pro-inflammatory IL-6 initiates the site inflammation in the lymphocytes, neutrophils, and monocytes. Furthermore, researchers have shown that ulcer-related oxidative stress can be controlled through regulations of pro-inflammatory cytokines (TNF-α and IL-6) [51]. *P. decaisnei* exerts a cytoprotective action through the alleviation of inflammation-related-oxidative stress by the downregulation of the pro-inflammatory TNF-α and IL-6 levels and the upregulation of anti-inflammatory IL-10 enzymes. Similarly, scientists have shown the efficacy of herbal medicine in improving inflammatory cytokines [52].

The anti-ulcer mechanisms of *P. decaisnei* could be explained by its efficacy to scavenge and reduce free radicals, which decrease the oxidative stress occurrence and consequently leads to smaller ulcer areas and more gastric mucus and a higher gastric pH initiating gastric repair and anti-inflammatory defense system.

5. Conclusions

The presented data results on the gastroprotective role of *P. decaisnei* showed the effectiveness of the herbal medicine against alcohol-induced gastric ulcers in a rat trial as found by the histopathological and gross investigations. The significant upregulation of the gastric mucus secretion, increased the surface area of the epithelial tissues of the stomach, improving the stomach pH values, reducing the edema inflammation, and lowering the disruption of the coat wall of the gastric submucosa (improving communicating junctions and immunomodulatory systems). The investigation of the stomach epithelial homogenate revealed that *P. decaisnei* has efficiently raised the SOD, CAT, and PGE2, and significantly lowered the MDA values. Furthermore, the *P. decaisnei* treated rats experienced the upsurging of the HSP70 and the downregulation of the expressed Bax proteins in the mucosal slices from rats' stomachs. The prophylactic risk factors play an important role because of the gastric mucosal antioxidants and anti-inflammatory facilitators. The bioactivity mechanisms of *P. decaisnei* can be correlated with its antioxidant and anti-inflammatory actions through free radical quenching and reducing the formation of reactive single O2 possibly via stimulating the expression of the antioxidant response element–based genes mediated by Nrf2, which protects the stomach mucosal linings from the risk factors of oxidative stress induced by ethanol and facilitates the restoration of gastric tissues and mucosal linings. To obtain those outcomes, the present study faced several obstacles including animal space limitations, sample size, and facility shortage. Therefore, future research is suggested in the broader form via advanced facilities for more approval on the obtained bioactivities of *P. decaisnei.*

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