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Ephedra intermedia alleviates ethanol-mediated gastric ulcer in rats by anti-inflammatory and antioxidant mechanisms

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Abstract

Ephedra intermedia has been used as a traditional therapy for various health purposes including gastric disorders. To validate its traditional use, we investigated the acute toxicity and the gastro-protective effects of *E. intermedia* leaf extract (EILE) in ethanol-induced gastric injury in rats. Sprague Dawley rats were randomly separated into 5 groups: normal control (A) and ulcer rats (B) received orally 0.5% carboxymethyl cellulose (CMC); C, rats ingested with omeprazole 20mg/kg; D and E, rats ingested with 250 and 500mg/kg EILE, respectively. After 60 min, groups B-E received an oral dose of absolute ethanol to initiate a gastric ulcer. After another 60 min, the rats were anesthetized and sacrificed. The toxicity trial showed the absence of any toxic signs in rats exposed to up to 5 g/kg EILE. Ethanol ingestion led to significant gastric tissue penetration indicated by decreased mucus content, reduced gastric pH, and severe mucosal lesions. Rats treated with omeprazole or EILE had significantly less stomach tissue damage, indicated by a suppressive effect on ethanol-mediated gastric grazes, improvement of gastric mucus and pH, decreased edema, and leukocyte extravasation into the submucosal layer. EILE treatment caused a significant up-regulation of the endogenous antioxidant enzymes (SOD and CAT) and a reduction in MDA levels. Moreover, EILE-treated rats showed increased expression of PAS (glycoprotein) in the gastric epithelium and fundic glands. The gastro-protective effects of *E. intermedia* could be attributed to its positive augmentation of various stomach factors making it a potentially viable source of potent pharmaceuticals for ulcer amelioration.

Keywords: Antioxidant enzymes, Acute toxicity, *Ephedra intermedia*, stomach ulcer, histology.

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Introduction

Gastric ulcer is considered the most abundant class of peptic ulcer with an incidence of 8-10% among nations worldwide [1]. The rate was found to be lower 2.4 and 6.1% in the Western and Chinese peoples, respectively [2]. The pathophysiology of this gastric disorder is initiated by many factors related to the inequity between antagonistic, amounts of gastric acid, and mucosal secretion, along with continuous blood flow, and prostaglandin availability [3]. Scientists declared many risk factors associated with the increased incidence of gastric ulcers, including alcoholism (inflammation), tobacco use, obesity, long-term stress (which causes Spartan damage and epithelial penetration) [4].

Ephedra species (*Ephedraceae* family) is a non-flowering seed plant with evergreen leaves including nearly 69 species distributed in the northern hemisphere [5,6]. *Ephedrae Herba* is the well-known herbaceous stem of *Ephedra sinica* Stapf, and Ephedra intermedia based on the records explained in the Chinese Pharmacopoeia 2020 edition [7]. *Ephedrae Herba* has been ingested for many purposes, including cold, cough, asthma, and absence of sweating [8]. Various fractions of *E. intermedia* exhibited considerable antimicrobial, antioxidant, substantial free radical scavenging activity, and cytotoxic potentials [9]. Ethnobotanical records of Chines herbal medicine show many therapeutic potentials of *E. intermedia* against kidney disorders, respiratory problems, allergies, cold symptoms, headaches, inflation, and gastrointestinal diseases [10]. Accordingly, experimental studies on this species revealed numerous biological potentials, including antioxidant, antibacterial, anti-inflammatory, anticancer, and degenerative disorders [11]. Such bioactivities were majorly correlated with its numerous phytochemical compounds belonging to sugars, phenolic compounds, cardiac glycoside, flavonoids, and alkaloids [12]. Recently, scientists also found a novel lignan, eplignan A (1), in addition to other known lignans (2–7) in the herbaceous stems of *E. intermedia*. Four of these compounds were found very effective in the positive modulation transforming growth factor β1 based on the in vitro study trial using BEAS-2 cell damage [13].

Alcohol overuse is a well-known cause of stomach disorders that will facilitate the development of peptic ulcers – especially when risk factors are present [14,15]. Ethanol can damage the epithelial layers of the stomach by modulating the gastric mucosal equilibrium, promoting the invasion of proactive cells, leukocyte infiltration, and increased inflammatory cytokines. Gastric layer penetration is considered as the initiative factor of the gastric ulcer and one of the histological features of this stomach injury. Chemical synthetics (proton pump inhibitors (PPI) have shown efficacy in the alleviation of gastric ulcer symptoms, However, long-term utilization of the PPI drugs (Omeprazole) to manage gastric ulcers may lead to a series of consequences including increased recurrence rate [16,17].

Moreover, alcoholism can lead to a quick erosion of the gastric mucosal layer, thereby facilitating the formation of gastric ulcers [18]. Increased levels of Alcohol can easily membranous damage and elevate the membrane permeability, which enhances the infiltration of leukocytes and neutrophils, causing peptic ulcers [19,20]. Ethanol has been utilized in the induction of functional modulation and characteristic changes in rats, such as augmentation of the secreted stomach acid and mucus content, which can lead to hemorrhagic and necrosis in the mucosal layers of the stomach, similar to those of humans suffering from peptic ulcers [21]. The initiative pathways of ethanol-induced peptic ulcer include a series of biological processes, including inflammation (mediated by inflammatory cytokines), oxidative damage (mediated by free radicles), and apoptosis [22,23]. Thus, ethanol-mediated peptic ulcer rat models were created to evaluate the therapeutic effects of interested medicinal agents for managing stomach ulcers. Most traditional therapeutic plants and their natural compounds which have been ingested for managing gastric ulcers are now shown to have great biological potentials, including free radical quenching, anti-inflammatory, and anti-apoptotic actions [24,25]. Moreover, researchers revealed the molecular pathway for preventing ethanol-induced stomach injury by linking it with the Mitogen-activated protein kinase and Nuclear factor kappa-

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light-chain-enhancer of activated B cells (NF-κB) mechanisms [26,27].

Therefore, searching for a new active ingredient with less antagonistic, increased biological potentials, and various bioactive components to ascertain its safety, is more affordable and has fewer drawbacks than chemical synthetics [28]. Phytochemicals originating from numerous plant species can have different therapeutic potentials against many human disorders [29]. Ethnobotanical studies revealed many different plant species that have been utilized for alleviating symptoms associated with gastric disease [30]. Many of these plant species including E. intermedia have not been explored to validate its traditional use. Therefore, the present works evaluate the toxic effect and gastro preventive roles of E. intermedia in ethanol-mediated gastric ulcers in rats by different histological, and biochemical assays.

Materials and Methods

Plant Collection

E. intermedia collected from the Safeen Mountain, shaqlawa district– Erbil, Iraq. The plant species were authentically sized (voucher number (378) by Prof. Dr. Abdullah Sh. Sardar.

Aerial parts were carefully washed with water, and air dried in a shadow place (20-25 ºC). Then, leaves were powdered until 710 um particles were formed. The plant powder (50 grams) homogenized (15 minutes) with 250 ml of ethanol. The mixture was transferred into dark glass container, after which the filtered (0.2 mm) supernatant dried at room temperature. [31].

Acute toxicity test

An acute toxicity trial was performed to validate the safety dosage of *E. intermedia* extract and to avoid the possible toxic effects of this plant. The study protocol followed the international guidelines for toxicity experiments on animals [32]. Sprague Dawley rats (36) weighing about 180-200 g provided by Animal House at Tishk international University. Rats were distributed into three groups and treated differently as revealed in Fig. 1.

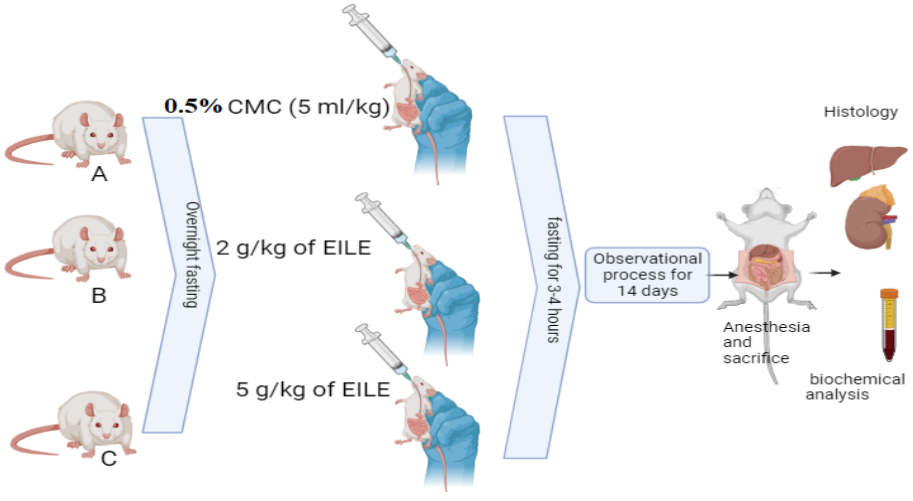

Figure 1. Shows schematic illustrations of the acute toxicity test for EILE. A, vehicle rats, received 0.5% CMC (5mg/kg); B, rats received orally 2 g/kg of EILE; and C, rats ingested with 5 g/kg of EILE.

Rats were fasting for overnight and sacrificed on day 15 by giving them an over dose of Ketamine (30 mg/kg, 100 mg/mL) and Xylazine (3 mg/kg, 100 mg/mL). Intercardia Blood samples were obtained for biochemical analysis and different organs (liver and kidney) were analyzed histopathologically by using H & E stain for any possible structural modifications [33].

Omeprazole.

The standard reference medicine (Omeprazole) was bought from local pharmacy. The omeprazole

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capsule was mixed with 0.5% (w/v) carboxymethyl cellulose (CMC) and delivered to rats through oral gavage at 20 mg/kg body weight (5 mL/kg) in accordance with the previous studies [21].

Gastroprotective experiment

Rats for Gastric Ulceration

The Sprague Dawley rats (180-250 g) were provided from Animal House Unite, Cihan University-Erbil. The animals were arbitrarily distributed into 5 wide mesh bottom cages (6 rats each) and were kept under standard diet (ad libitum) and drunk distal water for one week for adaptation purposes [34].

Ethanol-mediated Gastric Ulcer

The experimental animals were fastened (only food) for 24 hours before the supplementation. The rats were treated as follows: The vehicle rats (A) and ulcer controls (B) received orally 0.5% of CMC; reference rats (C) ingested with 20 mg/kg of omeprazole; D and E groups, rats ingested with 250 and 500 mg/kg of EILE, respectively. After 60 minutes, group A had 0.5% of CMC and group B-E ingested with 5ml/kg of absolute ethanol to induce gastric damage. After another 60 minutes, animals were sacrificed by using standard anesthesia injection (xylazine and ketamine), and the stomach specimens were obtained for different laboratory analysis [21].

Macroscopic Stomach Laceration Assessment.

Gastric mucosa layers were examined by using a stereomicroscope. The areas between hemorrhagic lesion were determined through a planimeter (10 × 10 mm2 = ulcer area (UA)) and a stereomicroscope (1.8x). The morphology of the ulcers was very comparable to the longitudinal axis of gastric tissues.

The ulcer bands were found by the determination of the numbers of small squares, 2 mm × 2 mm in each microscopic focus. The ulcer area in each gastric samples was estimated by using an equation as previously detailed.

$$UA\ (mm^2) = \text{Total no. of small squares} \times 4 \times 1.8$$

And the inhibition percentage (I%) for each treatment was by this equation:

$$(I\%) = [(UA\ control - UA\ treated) \div UA\ control] \times 100$$

Gastric Wall Mucus evaluation

The gastric layers were evaluated based on the previously developed methods. Briefly, different gastric glandular tissue sections were dissected and mixed with sucrose solution (alcian-blue solution (10 mL of 0.1% w/v) in a 0.16 M) and then buffered by sodium acetate (0.05 M) at pH 5 [35]. The gastric tissues were undergone a process of staining (alcian blue) for two hours and then washed by (sucrose) 10 mL of 0.25 M. Another staining process took place followed by washing with 0.5 M magnesium chloride for 2 h (with 30 min intervals). The produced blue extract was mixed with diethyl ether and centrifuged at 3000 rpm for 10 min, and the absorbance was found at 580 nm. The obtained absorbance was used to determine the concentration of alcian blue (mg) extract present in gastric glandular tissue (g tissue).

Assays for Bioactivities

The dissected stomach specimens were transferred into ice-cold saline, in addition to phosphate-buffered saline (PBS) and cocktail of a mammalian protease inhibitor and buffer (a 50 mM phosphate buffer, pH 7.4). The centrifugation of the mixture was performed at 4,000 rpm for 10 min (4 °C) and the separated supernatant was analyzed for endogenous antioxidant (CAT, SOD) and peroxidation indicator (MDA) contents (Cayman, USA). The tissue antioxidant estimations followed the standard procedures mentioned by the previous scientists on how tissue antioxidant can be estimated are available. The lipid peroxidation rate in the gastric tissues evaluated by the detecting amount of MDA levels in tissue homogenates by utilizing thiobarbituric acid technique.

The supernatant was transferred into a vial containing SDS (100 μL) and dye (4 mL) solutions and then the incubation took place for 1 h (100 °C), and the ice incubation for 10 minutes. After that, the congregation of the mixture (vials) performed at 1,600-×g (for 10 min, at 4°C). Within a period of 30 min, an amount (150 μL) of centrifuges mixture were

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transferred into a 96-well plate and the spectrophotometric absorbency was observed at 530– 540 nm [36].

Histological Investigations of the Stomach Mucosa

The gastric tissues were cute to form unified slices and transferred into a container of buffered formalin 10% for 18 h at 4◦C. The paraffinization and blocking of gastric slices were possible by using a tissue-processing machine (Leica, Germany). An equal segment (thickness of 5 μm) of the gastric tissues was produced (Leica Rotation Microtome, Germany) following the protocols mentioned by the previous researchers. The paraffinized tissues maintained on glass slides and colored by Hematoxylin and Eosin stains for microscopic observations [34].

Study of Mucosal Glycoproteins

The gastric mucus content of each stomach specimens was evaluated following the PAS technique. Briefly, gastric tissue sections (5-μm thickness) of the glandular portion fixed on glass slides and colored with periodic acid schiff (PAS) to observe the presence of glycoproteins under light microscope [16].

Statistical Analysis

The statistical findings are shown as mean ± S.E.M. The data calculation was performed in triplicate to ensure accuracy. Different statistical programs (SPSS one- ANOVA and graph pad prism version 9.0) are to compare different groups. Numbers were found significant at $P < 0.05$.

Results and Discussion

Acute toxicity

The present study demonstrated the absence of any changes in the behavior or physiology of rats exposed to 2 and 5 g/kg of EILE even after fourteen days of toxicity trial. Moreover, feed intake, body weight, and daily activity were very comparable between vehicle rats and EILE-ingested rats. The two observational processes have not detected any morbidity or mortality cases between experimental rats. Moreover, EILE ingestion has not caused any structural damage to the liver and kidney based on the histological examinations, which showed similar tissue structure and arrangements compared to that of vehicle rats (Fig. 2 A-C). The biochemical evaluation of serum samples from normal and supplemented rats showed the absence of any noticeable changes in examined parameters as shown in Table 1. The outcomes suggest the toxic dose of *E. intermedia* extract exceeds 5 g/kg.

Table 1. Effect of EILE on the serum biochemical.

Parameters	Vehicle	2 g/kg EILE-treated rats	5 g/kg EILE-treated rats
Sodium mmol/L	136.2±5.2	142.5±4.2	168.3±5.1
Potassium mmol/L	5.2±2.6	5.6±3.5	5.3±2.1
Carbon dioxide mmol/L	103±4.3	104.5±5.3	107.2±6.2
Anion gap mmol/L	34.20±2.1	35.22±1.3	38.4±2.1
Urea mmol/L	14.4±1.4	12.80±1.7	11.2±1.8
Creatinine mmol/L	5.80±1.4	6.10±1.8	7.12±2.3
Albumin g/L	32.10±2.1	28.20±2.8	27.3±3.4
Total bilirubin mmol/L	33.2± 1.8	33.1±2.0	35.3±3.0
Alkaline phosphatase U/L	167.3 ±3.9	161.41±3.2	168.30±3.5
Alanine aminotransferase U/L	79.23±3.5	81.34±4.3	84.39±2.4

Data shown as Mean ± SEM (n=6) and values within rows were found not- significant at p<0.05.

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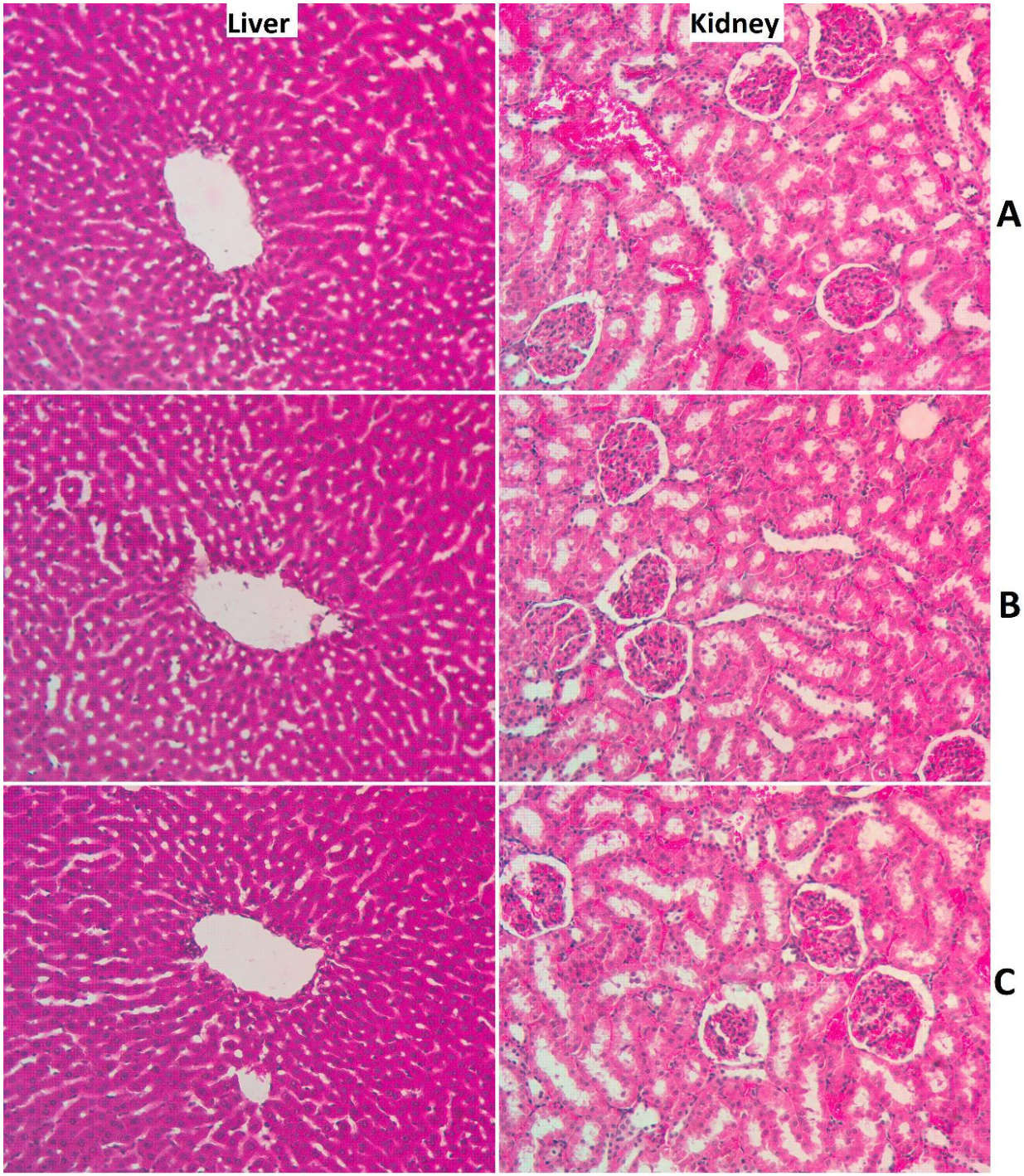

Figure 2. Histological views of kidney and liver of rats in acute toxicity trial. A, rats had only 0.5% CMC; B, 2 g/kg EILE-supplemented rats; C, 5 g/kg EILE -supplemented rats. Comparable tissue structure observed between vehicle group and EILE -treated rats (hematoxylin and eosin, 40x). There was no structure tissue damage in all three groups based on microscopic examination.

Effect of EILE against gastric ulcer

Gross morphology

The results have shown different levels of tissue penetrations and lesions among experimental rats. The vehicle rats had a normal bright appearance of the gastric mucosa with clear rugae muscles. Contrarily, ulcer control revealed numerous lesion areas and mucosal penetrations with clear ulcerative tissues. Rats treated with Omeprazole or *E.*

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intermedia extract showed less mucosal damage and noticeably fewer lesion areas compared to that ulcer controls (Fig. 3 A-E).

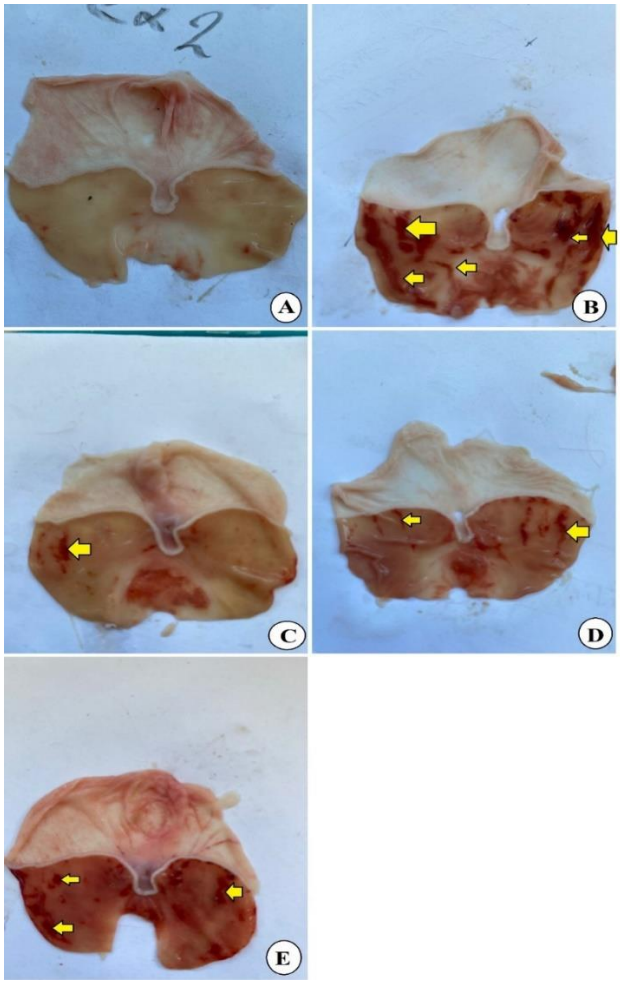

Figure 3. Effects of EILE on gastric mucosa of rats. A, vehicle rats received 0.5% of CMC; B, ulcerated rats had severed gastric lesions (yellow arrow); C, rats had 20 mg/kg omeprazole exhibited significantly lower gastric lesions compared to ulcer control and EILE-treated rats; D and E, rats received 250 and 500 mg/kg of EILE. EILE treatment (500 mg/kg) restrained gastric barriers against ethanol-mediated lesion.

As shown in Fig. 3A, ethanol ingestion caused severe and extensive hemorrhagic lesions in the gastric mucosa with disrupted surface areas. Pre-treatment of rats with E. intermedia extracts (250 and 500 mg/kg) lead to a significant reduction of ulcer areas and significantly lower gastric mucosal damage (more rugae muscles) compared to that of ulcer controls. Noticeably, rats who received a high dose (500 mg/kg) of *E. intermedia* extracts had more flat gastric mucosal folds and wider tissue surface area, denoting a higher protective effect of this dose compared to that of 250 mg/kg EILE ingestion. Moreover, the gross morphology was very similar between rats that received Omeprazole or 250 mg/kg EILE (Fig. 3A-E).

Effect of EILE on gastric mucus contents.

Normal control rats (A) had normal mucus amount without any observable gastric damage. Ulcer control rats (B) had the lowest gastric mucus content and the highest gastric ulcer areas compared to experimental and normal control rats. Rats receiving standard drugs (C) showed almost the same mucus content as normal controls and reduced gastric ulcer areas. Rats treated with EILE (D and E) showed significantly higher mucus amounts and lower ulcer areas than that of ulcerated control rats (Table 2).

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Table 2. Stomach content modulation after EILE ingestion in rats.

Animal group	Pre-feeding (5ml/kg)	Mucus Weight (g)	pH	Ulcer area $(mm)^2$	Inhibition (%)
A, Normal control	**0.5% CMC**	1.95 ± 0.32^{a}	6.16 ± 0.519^{a}	-	-
B, Ulcer control	**0.5% CMC**	0.72 ± 0.035^{e}	2.19 ± 0.22^{e}	609.0 ± 4.48^{d}	-
C, Omeprazole	**20 mg/kg omeprazole**	1.92 ± 0.044^{b}	6.15 ± 0.50^{b}	105.66 ± 3.38^{a}	82.65%
D, Low dose of EILE	**250 mg/kg**	1.54 ± 0.040^{d}	5.16 ± 0.057^{d}	154.76 ± 4.95^{c}	74.58%
E, High dose of EILE	**500 mg/kg EILE**	1.94 ± 0.34^{c}	6.45 ± 0.48^{c}	125.4 ± 2.28^{b}	79.40%

Values are revealed as Mean ± SEM (n = 6). Different superscript on values on the same column considered significant at $p<0.05$.

Histopathological Assessment of Stomach Lesions

The histopathological results from normal control rats revealed the usual criteria for gastric tissues represented by the presence of lamina propria and epithelium in the fundic mucosa. The surface epithelium layer revealed normal mucosal cells with narrow pits The fundic glands had all three common parts (base, neck, and isthmus) (Fig. 4A). The ulcer control rats showed extensive gastric tissue damage in the ulcer controls represented by numerous leukocyte infiltration from the submucosal layers (extravasation), edema, and necrotic penetration into the mucosa. Different levels of ulceration were observed including the cutoff of fundic mucosa from muscularis mucosa with the sloughing into necrotic lumen from fundic epithelium. The structural perturbation of the fundic glands was seen in all tissue slides obtained from ulcer controls. There were also numerous inflammatory cells and bulging of blood vessels as shown in Fig. 4B. Rats received Omeprazole or EILE had almost normal tissue structures, which were similar to that of normal control with the only exception of wider fundic glands and shorter pits. The mucosal surface has many oval-shaped nuclei and few vacuolated cytoplasm. Moreover, the parietal and chief cells were found in normal shape and higher values in compare to ulcer controls (Fig. 4A-E).

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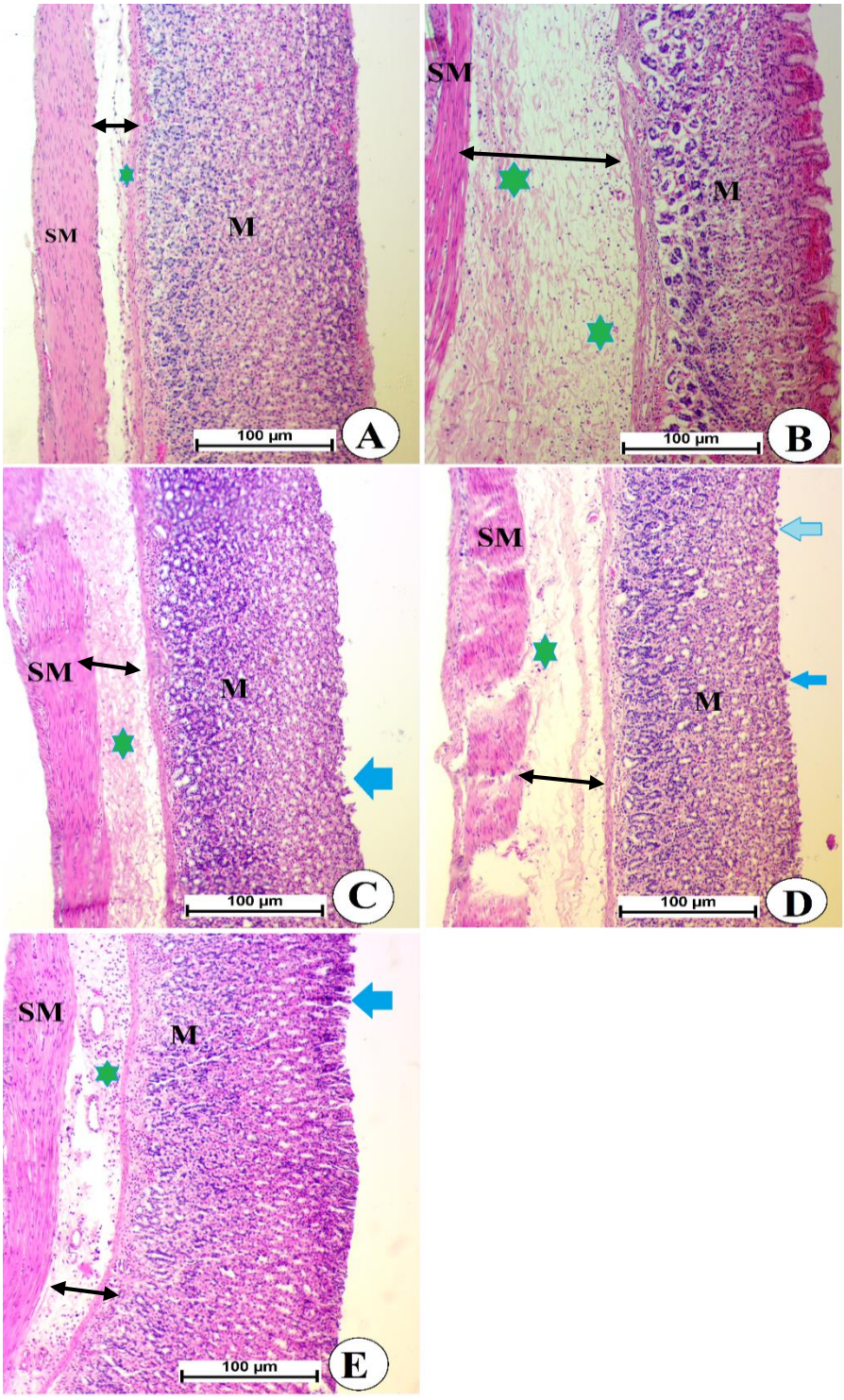

Figure 4. Influence of EILE on the stomach tissues in rats. A, normal controls; B, ulcerated rats, had sever mucosal disruption (blue arrow), leukocyte extravasation (green asterisk), and edema in the submucosa (double-headed black arrow); C, rats received 20 mg/kg Omeprazole experienced less gastric injury; D and E, rats received 250 and 500 mg/kg EILE revealed moderate penetration of gastric mucosal disruption (H & E stain, 10x). mucosal (M) and sub-mucosal (SM)

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PAS expression in gastric tissues

The gastric sections obtained from experimental rats showed different intensities of PAS expressions. Normal control rats had normal surface epithelial layers with thick continuous mucosal layers, which contained many fundic pits and fundic glands (basal, neck, and isthmus). Ulcer control rats revealed a light, thin, and intermittent intensity of PAS stain on their mucosal layers, denoting the lowest glycoprotein content in their gastric tissues. Moreover, ulcerative rats had fewer fundic pits and fundic glands based on the weak PAS expressions in their mucous film. Omeprazole or EILE-treated rats had very comparable results of PAS appearance in their gastric tissues with thick mucus films distributed on their epithelial layers, demonstrating increased glycoprotein concentrations in their stomach tissues (Fig. 5A-E).

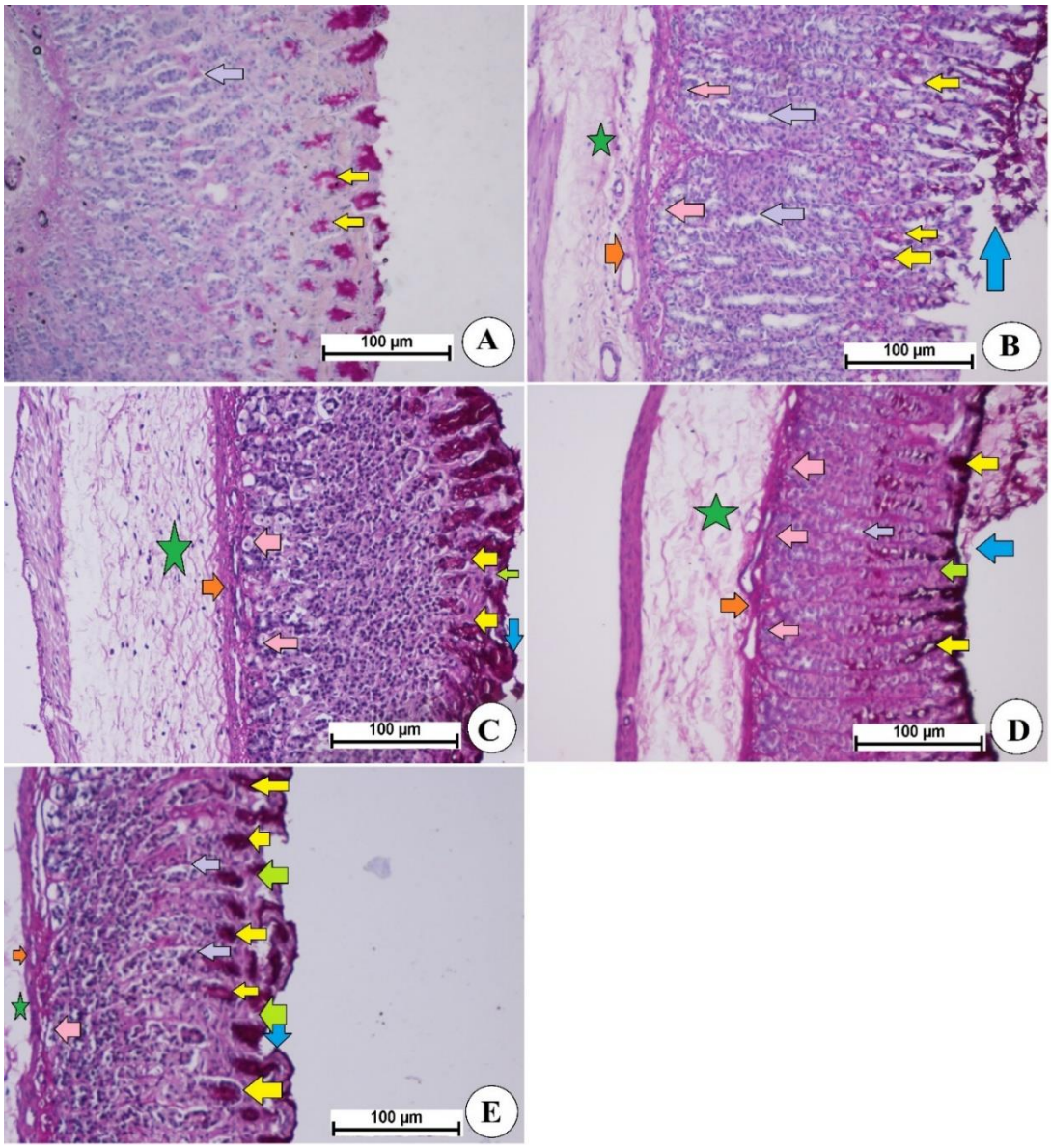

Figure 5. Photomicrographs of gastric sections in fundic mucosal layers in rats. A, normal controls exhibited increased PAS appearance; B, ulcer rats had a light intermittent intensity of PAS in fundic pits (yellow arrow) and fundic glands at basal (pink arrow), neck (gray arrow), and isthmus; C, reference rats (Omeprazole) had increased PAS intensity; D and E, rats treated with EILE had a very comparable expression of PAS as reference rats (PAS stain magnification 20×). Orange arrow, mucosal lesion; blue arrow, severe mucosal disruption; green asterick, leukocyte infiltration.

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Effect of E. intermedia on Antioxidant Activity

The antioxidant contents of gastric tissues obtained from rats exposed to different treatments were significantly varied (Fig. 6). In comparison, normal control rats had non-significant changes in the antioxidant and MDA contents in their gastric homogenate compared to treated groups. The ulcer rats experienced the lowest levels of antioxidants and the highest lipid peroxidation status compared to Omeprazole or EILE-treated rats (Fig. 6A-E). EILE supplementation caused a positive modulation of gastric antioxidants, which were significantly varied compared to ulcer controls (B). Rats ingested 500 mg/kg EILE had higher SOD (408±9.69 U/mg) and CAT (118.33±8.33) values than that (162±4.77 and 63.16±3.81) of ulcerated rats. The MDA content was significantly higher in ulcer controls and was non-significantly changed between treated groups. Expectedly, the investigational collections presented a significant reduction in MDA activity (Fig. 6). MDA values were statistically lower in 500 mg/kg EILE -treated rats (120.166±6.04) than that (216.33±11.05) in ulcer controls. Furthermore, Antioxidant and MDA values were very comparable between groups A, C, D, and E (Fig. 6).

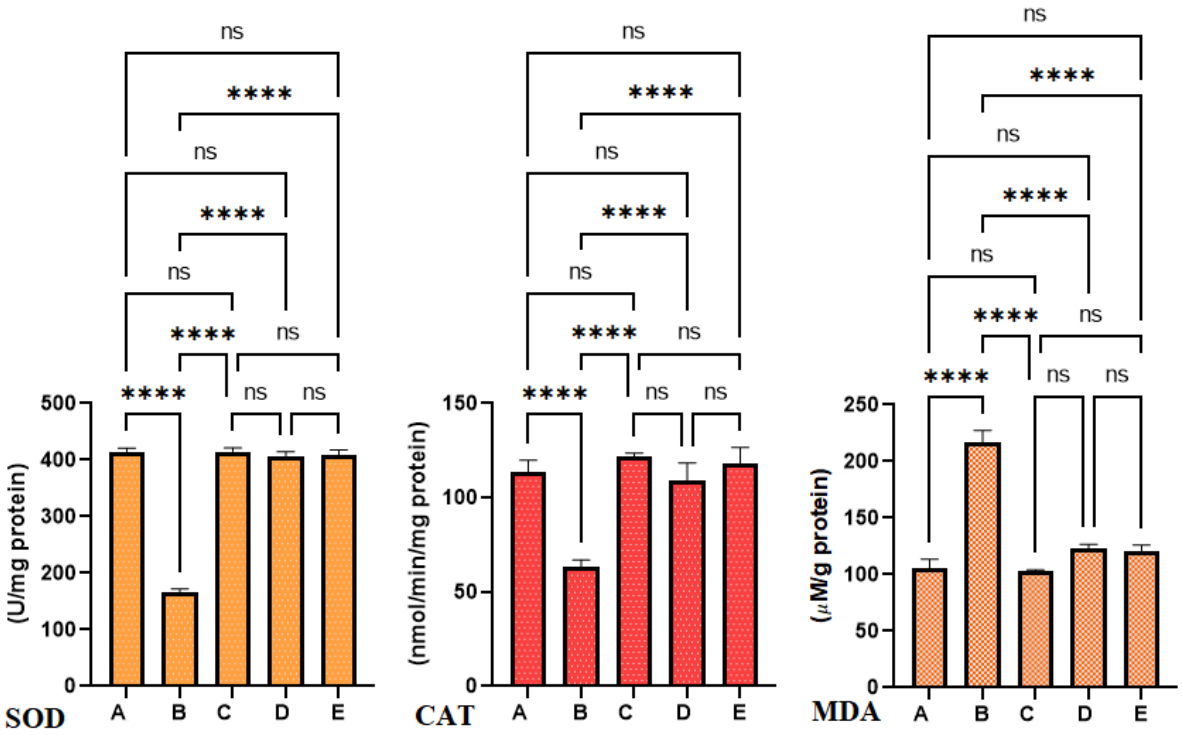

Figure 6. Modulation of antioxidants and MDA contents in gastric homogenates by EILE ingestion in rats. A, normal controls received 0.5% CMC; B, Ulcer control received only ethanol; C, rats received 20 mg/kg omeprazole); D and E, rats ingested with 250 and 500 mg/kg of EILE.

Discussion

The acute toxicity trial for any potential therapeutic agent has become a useful effective technique to reveal any possible side effects and to avoid toxic damage in animals. The present toxicity evaluation of *E. intermedia* extracts did not show any unwanted toxic signs and symptoms or mortality in rats exposed to 5 g/kg. Accordingly, the safety utilization of *E. intermedia and other Ephedra* species extracts Has been reported by different acute toxicity tests [37–39].

The mucus membrane is considered as first barrier defense line of the gastric tissues that can be easily damaged by alcohol ingestion. Increased Pepsin secretion, a stomach digestion enzyme, can also lead to gastric mucosal injury [40]. The homeostasis disruption between the protective and destructive factors of the stomach mucosa, which can be promoted by endogenous and exogenous aspects, will cause a series of gastric disorders including peptic ulcers [17,20].

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Alcohol as one of the exogenous factors can negatively modulate the defense systems of the stomach including mucus secretion and mucosal circulations, which will raise the gastric pH leading to gastric tissue necrosis. Alcohol (ethanol) overuse can cause hemorrhagic lesions through direct necrosis, lowering defense factors including down-regulation of bicarbonate and mucus production [41].

The chemical synthetics (Omeprazole), have anti-acid secretory (Blocking H2) actions and can strengthen the defense systems of the stomach including mucus (mucin-type glycoproteins) contents and gastric pH. In the present work, ethanol ingestion effectively induced the production of gastric injury represented by mucosal penetrations and leukocyte extravasation along with epithelial permeability for neutrophils. Moreover, gastric mucus content was significantly reduced in rats ingested only absolute ethanol, which was to previous reports [42].

Our data analysis revealed the rats that received *E. intermedia* extract had better gastric tissue profiles than that of ulcer controls represented by increased gastric mucus and gastric pH and significantly reduced ulcer areas, denoting the gastroprotective action of this plant. Up-surging the gastric mucus content has been always a target mechanism for most profitmaking anti-ulcer chemical synthetics (drugs) [17]. The protective effect of oral ingestion of EILE could be due to its positive modulation of gastric mucus, which plays significant defensive action against numerous necrotizing factors (ethanol) exposed to the stomach. Thus, a possible pathway of anti-ulcer action of EILE could be through increasing resistance of the mucosal barriers by a defense coating, which plays a major role in avoiding stomach injury and in the facilitation of the gastric epithelium [15,20]. Accordingly, the scientists revealed that preserved mucus levels on the glandular mucosa are one of the main protective factors to avoid gastric damage mediated by chemicals or stress factors [41]. Previously, researchers have shown the gastroprotective potentials of different *Ephedra* species by modulation of gastric defensive factors [43,44].

The histopathological results revealed that absolute alcohol ingestion leads to significant gastric tissue disruption and mucosal damage, increased permeability, and reduced mucus contents. The ulcer controls, treated only with ethanol, experienced different levels of gastric damage including increased inflammatory cells, mucosal vulnerability, and increased leukocyte permeability, which are all features of ethanol damage [21]. The pathogenic mechanism of ethanol-mediated stomach injury can be initiated directly or indirectly through the activation of various cellular processes mediated by free radicals, inflammatory cytokines, or lipoxygenase molecules. The present rat supplementation by *E. intermedia* extracts positively regulated the mucus content and other gastric defensive factors, which were reliable with the previous outcomes [34].

The pharmaceutical industry has provided many chemical synthetics to alleviate symptoms associated with gastric acidity and gastric disorders. Omeprazole is a well-known proton pump inhibitor utilized as an H_2 blocker that can reduce gastric acid secretions and alleviate digestion problems [20]. Moreover, Omeprazole is an effective drug for reducing the incidence of gastric ulcers and gastric reflux for short and long-period utilization [45]. In the present clinical practice, Omeprazole ingestion reduced gastric tissue damage represented by reduced ulcer index and higher ulcer inhibition percentages. In this context, *E. intermedia* ingestion led to similar outcomes of omeprazole ingestion in rats represented by less ulcer area, less mucosal damage, and leukocyte penetrations to the gastric wall. Similarly, researchers correlated the gastroprotective potentials of many medicinal plants with their positive modulation of gastric defense lines [21].

Scientists have shown that one of the early signs of initiating gastric ulcers is increased neutrophil extravasation into the gastric tissues. In other words, preventing neutrophil infiltration or lowering neutrophil permeation lowers the chances of developing gastric ulcer [46]. Accordingly, our study revealed that rats supplemented with *E. intermedia* leaf extract had lower neutrophil penetrations compared to ulcer controls. Another mechanism that could be correlated with the gastroprotective potentials of EILE, is the inhibitory

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effect of this plant on gastric muscle contraction, forming more flat gastric surfaces with fewer folds. Such biological action will lead to the relaxation of the gastric mucosa, which may protect the mucosal layers by increasing the mucosal surface area exposed to the destructive factors and lowering the volume of the stomach-irritating agents on the rugae crest [26,47].

The PAS staining procedure has shown a reliable technique to evaluate the amount of produced mucopolysaccharides (defensive factor against stress factors) by gastric cells. Increased production of gastric mucus, in case of the normal state, will cause an increased appearance of PAS stain when examined under a microscope. In this context, ulcer rats showed the minimum PAS expression due to their reduced gastric mucus content [48]. While EILE-treated rats showed increased expression of PAS stain in their gastric tissues based on the microscopic evaluations. Accordingly, scientists have shown significant potential of *Ephedra* species in the positive regulation of mucopolysaccharides [49,50]. Therefore, the outcome demonstrated that ethanol inhibited the gastric mucus (less PAS expression), but EILE supplementation (especially 500 mg/kg) up-regulated the mucus content covering the gastric mucosa (higher PAS expression), thereby reducing gastric injury and ulcer index by promoting first immune barrier of the stomach.

Oxidative stress as a molecular process can be result of an imbalance of ROS formation and elimination, consequently causing inflammation and weakening gastric defense barriers (SOD and CAT). Our data results show noticeable radical quenching potentials of EILE, possibly through the activation of anti-radical genes mediated by Nrf2. Accordingly, scientists have reported the anti-oxidant action of EILE through its inhibitory action on the expression of MDA and up-regulation of CAT, SOD, thereby decreasing the apoptotic and inflammatory process [51].

Gastric ulcers can be an outcome of many excited biological processes in the cell including lipid peroxidation and oxidative stress, which can be induced by the neutrophils through the continuous buildup of reactive oxygen species (superoxide anions) [21]. The present antioxidant evaluation showed different differences in the levels of endogenous antioxidant defense enzymes in gastric homogenates. Ethanol ingestion caused a significant reduction in antioxidant enzymes and a noticeable up-regulation of the MDA levels. MDA has been a well-known byproduct of the lipid peroxidation process, which will fluidity, rigidity, and permeability of the membrane and inhibit ionic interchange consequently leading to organelle damage. Gastric MAD was positively augmented and higher antioxidant enzymes were denoted in rats ingested with EILE (250 or 500 mg/kg) compared to that of ulcer controls. Similarly, numerous scientists have shown the antioxidant potential of *E. intermedia* in different experimental trials utilizing various doses [9]. Such biological action of this special plant has been majorly linked with their phytochemical contents, mainly alkaloids, phenolic, and flavonoids, cardiac glycosides, and anthraquinones [12,51].

Conclusion

Acute toxicity experiment established the absence of toxic effects of *E. intermedia* (up to 5 g/kg) in rats. The outcomes show efficient gastroprotective roles of *E. intermedia* extract in ethanol-mediated gastric ulcers in rats. Such biological actions were screened by different histological (H & E), PAS stain, and biochemical assays. The anti-ulcer actions of *E. intermedia* can be correlated with its positive augmentation of gastric mucus, gastric pH, and antioxidant defense enzymes in gastric tissues, which could be due to its phytochemical (lignan, eplignan) contents belonging to alkaloids, phenolic, and flavonoids, cardiac glycosides, and anthraquinones. The outcomes validate *E. intermedia* as a new source of pharmaceuticals against oxidative stress-mediated gastric ulcers.

Authors' Declaration

- Conflicts of Interest: None.

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- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee at Tishk international University (No.12, 5-11-2023).

Authors' Contribution Statement

A.A.J, M.A.A, conceptualization; M.A.A, A.A.J, Z.M.A, investigation; M.M.A, M.H.A, A.M.A, M.M.A, M.H.A, formal analysis and software; M.I.A, S.F.M, M.T.M, P.A.I, resources and validation; A.O.H, R.R.H, A.A.J, reviewing and editing; A.A.J, writing manuscript.

Reference

1. Woolf A, Rehman RB RR. Gastric ulcer. Treasure Isl. StatPearls Publ. 2023;
2. Fu S, Chen J, Zhang C, Shi J, Nie X, Hu Y, et al. Gastroprotective effects of Periplaneta americana L. extract against ethanol-induced gastric ulcer in mice by suppressing apoptosis-related pathways. Front. Pharmacol. Frontiers Media SA; 2021;12: 798421.
3. Sistani Karampour N, Arzi A, Rezaie A, Pashmforoosh M, Kordi F. Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats. Medicina . 2019; 55(3)64-73. https://doi.org/10.3390/medicina55030064
4. Fazalda A, Quraisiah A, Nur Azlina MF. Antiulcer effect of honey in nonsteroidal anti-inflammatory drugs induced gastric ulcer model in rats: a systematic review. Evidence-Based Complement. Altern. Med. Hindawi; 2018;2018. https://doi.org/10.1155/2018/7515692.
5. Veilleux-Lemieux D, Castel A, Carrier D, Beaudry F, Vachon P. Pharmacokinetics of ketamine and xylazine in young and old Sprague-Dawley rats. J. Am. Assoc. Lab. Anim. Sci. American Association for Laboratory Animal Science; 2013;52(5): 567–570.
6. Al-khateeb E, Al-Ani H, Al-Kadi K, Al-Obaidi E, Shalan N, Al-Rawi N. Investigation of the alkaloids of two Ephedra Spp. wildly grown in Iraq. Jordan J. Pharm. Sci. 2014;7(3) 191-198.
7. Zheng Q, Mu X, Pan S, Luan R, Zhao P. Ephedrae herba: A comprehensive review of its traditional uses, phytochemistry, pharmacology, and toxicology. J Ethnopharmacol. 2024;319(Pt 2):117273. https://doi.org/10.1016/j.jep.2023.117273
8. Kilpatrick K, Pajak A, Hagel JM, Sumarah MW, Lewinsohn E, Facchini PJ, et al. Characterization of aromatic aminotransferases from Ephedra sinica Stapf. Amino Acids. 2016; 48: 1209-1220. https://doi.org/10.1007/s00726-015-2156-1.
9. Nasar MQ, Zohra T, Khalil AT, Ovais M, Ullah I, Ayaz M, et al. Extraction optimization, Total Phenolic-Flavonoids content, HPLC-DAD finger printing, antimicrobial, antioxidant and cytotoxic potentials of Chinese folklore Ephedra intermedia Schrenk & CA Mey. Braz J Pharm Sci. 2023; 58, e20989. http://dx.doi.org/10.1590/s2175-97902022e20989
10. Tang S, Ren J, Kong L, et al. Ephedrae Herba: A Review of Its Phytochemistry, Pharmacology, Clinical Application, and Alkaloid Toxicity. *Molecules*. 2023; 28 (668) 1-22. http://dx.doi.org/10.3390/molecules28020663.
11. Almaimani G, Jabbar AAJ, Ibrahim IAA, Alzahrani AR, Bamagous GA, Almaimani RA, et al. Anise (Pimpinella anisum L.) attenuates azoxymethane-induced colorectal cancer by antioxidant, anti-inflammatory, and anti-apoptotic pathways in rats. Environ. Sci. Pollut. Res. 2024;31(3): 4439–4452. https://doi.org/10.1007/s11356-023-31349-z
12. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. Sci World J. *2017*(1), p.5873648. https://doi.org/10.1155/2017/5873648
13. Fan X, Cao Y, Zeng M, Wang R, Liu Y, Xu C, et al. A New Lignan from the Herbaceous stems of Ephedra intermedia Schrenket CA Meyer. Rec Nat Prod. 2023; 17(2):293-299.
14. Shin MS, Lee J, Lee JW, Park SH, Lee IK, Choi JA, et al. Anti-inflammatory effect of artemisia argyi on ethanol-induced gastric ulcer: Analytical, in vitro and in vivo studies for the identification of action mechanism and active compounds. Plants. 2021; 10(2): 332-345. https://doi.org/10.3390/plants10020332
15. Raish M, Shahid M, Bin Jardan YA, et al. Gastroprotective Effect of Sinapic Acid on Ethanol-Induced Gastric Ulcers in Rats: Involvement of Nrf2/HO-1 and NF-κB Signaling and Antiapoptotic Role. *Front Pharmacol* 2021; 12: 622815. https://doi.org/10.3389/fphar.2021.622815
16. Liu Y, Sui D, Fu W, Sun L, Li Y, Yu P, et al. Protective effects of polysaccharides from Panax ginseng on acute gastric ulcers induced by ethanol in rats. Food Funct. 2021; 12(6): 2741-2749.

Published Online First: August, 2024
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P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

https://doi.org/10.1039/d0fo02947e
17. Ahmed KA-A, Jabbar AAJ, M.Raouf MMH, et al. Wood calamint ameliorates ethanol-induced stomach injury in rats by augmentation of hsp/bax and inflammatory mechanisms. *J Mol Histol*. Epub ahead of print 2024. https://doi.org/10.1007/s10735-024-10211-7.
18. Nawahirsha S, Kurian G, Alexander T, Kurian S. Impact of alcohol on gastric mucosa in a population with high prevalence of Helicobacter pylori. Int. J. Adv. Med. 2021;8(6): 764.
19. Balogun SO, Damazo AS, de Oliveira Martins DT. Helicteres sacarolha A. St.-Hil. et al.: gastroprotective and possible mechanism of actions in experimental animals. J Ethnopharmacol. 2015; 166: 176-184. https://doi.org/10.1016/j.jep.2015.03.021
20. Saremi K, Rad SK, Tayeby F, Abdulla MA, Karimian H, Majid NA. Gastroprotective activity of a novel Schiff base derived dibromo substituted compound against ethanol-induced acute gastric lesions in rats. BMC Pharmacol Toxicol. 2019; 20(1)13-26. https://doi.org/10.1186/s40360-019-0292-z
21. Ben Ahmed Z, Yousfi M, Viaene J, Dejaegher B, Demeyer K, Heyden Y Vander. Four Pistacia atlantica subspecies (atlantica, cabulica, kurdica and mutica): A review of their botany, ethnobotany, phytochemistry and pharmacology. J. Ethnopharmacol. 2021;265: 113329. https://doi.org/10.1016/j.jep.2020.113329
22. Liang J, Dou Y, Wu X, Li H, Wu J, Huang Q, et al. Prophylactic efficacy of patchoulene epoxide against ethanol-induced gastric ulcer in rats: Influence on oxidative stress, inflammation and apoptosis. Chem. Biol. Interact. Elsevier; 2018;283: 30–37. https://doi.org/10.1016/j.cbi.2018.01.014
23. Mohan S, Hobani YH, Shaheen E, Abou-Elhamd AS, abdelhaleem A, Alhazmi HA, et al. Girinimbine from curry leaves promotes gastro protection against ethanol induced peptic ulcers and improves healing via regulation of anti-inflammatory and antioxidant mechanisms. Food Funct. 2020; 11(4): 3493-3505. https://doi.org/10.1039/D0FO00053A
24. Simões S, Lopes R, Campos MCD, Marruz MJ, da Cruz MEM, Corvo L. Animal models of acute gastric mucosal injury: Macroscopic and microscopic evaluation. Anim Model Exp Med. 2019; 2(2): 121-126. https://doi.org/10.1002/ame2.12060
25. Zhou D, Yang Q, Tian T, Chang Y, Li Y, Duan LR, et al. Gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats: Involvement of the Nrf2/HO-1 signaling and anti-apoptosis role. Biomed Pharmacother. 2020; 126-page. https://doi.org/10.1016/j.biopha.2020.110075
26. Gong G, Rigetu Zhao, Zhu Y, et al. Gastroprotective effect of cirsilineol against hydrochloric acid/ethanol-induced gastric ulcer in rats. *kjpp* 2021; 25: 403–411. https://doi.org/10.4196/kjpp.2021.25.5.403
27. Akanda MR, Kim IS, Ahn D, Tae HJ, Nam HH, Choo BK, et al. Anti-Inflammatory and Gastroprotective Roles of Rabdosia inflexa through Downregulation of Pro-Inflammatory Cytokines and MAPK/NF-κB Signaling Pathways. Int J Mol Sci . 2018; 19(2) 584-599. https://doi.org/10.3390/ijms19020584
28. Jabbar AAJ, Alamri ZZ, Abdulla MA, Salehen NA, Ibrahim IAA, Hassan RR, et al. Boric Acid (Boron) Attenuates AOM-Induced Colorectal Cancer in Rats by Augmentation of Apoptotic and Antioxidant Mechanisms. Biol Trace Elem Res. 2024;202(6):2702-2719. https://doi.org/10.1007/s12011-023-03864-0
29. Jan R, Khan M, Asaf S, Lubna, Asif S, Kim K-M. Bioactivity and therapeutic potential of kaempferol and quercetin: new insights for plant and human health. Plants. MDPI; 2022;11(19): 2623. https://doi.org/10.3390/plants11192623.
30. Khémiri I, Bitri L. Effectiveness of Opuntia ficus indica L. inermis seed oil in the protection and the healing of experimentally induced gastric mucosa ulcer. Oxid Med Cell Longev. 2019. https://doi.org/10.1155/2019/1568720
31. Ibeh LN, Ijioma SN, Emmanuel O, et al. Psidium guajava leaf extract improves gastrointestinal functions in rats and rabbits: an implication for ulcer and diarrhoea management. *Biomarkers Biochem Indic Expo response, susceptibility to Chem* 2021; 26: 737–746. https://doi.org/10.1080/1354750X.2021.
32. Guideline O. 423: Acute oral toxicity-acute toxic class method. OECD Guidelines for the Testing of Chemicals. Paris: OECD Publishing; 2001. 1–14 p.
33. Ahmed KA-A, Jabbar AAJ, Galali Y, M. Al-Qaaneh A, Akçakavak G, Salehen NA, et al. Cumin (Cuminum cyminum L.) seeds accelerates wound healing in rats: Possible molecular mechanisms. Ski. Res. Technol. John Wiley & Sons, Ltd; 2024;30(5): e13727. https://doi.org/10.1111/srt.13727
34. Shady NH, Abdullah HS, Maher SA, Albohy A, Elrehany MA, Mokhtar FA, et al. Antiulcer Potential of Psidium guajava Seed Extract Supported by Metabolic Profiling and Molecular Docking. Antioxidants. 2022; 11(7)1230. https://doi.org/10.3390/antiox11071230
35. Zhang J, Yin Y, Xu Q, et al. Integrated serum pharmacochemistry and investigation of the anti-gastric ulcer effect of Zuojin pill in rats induced by ethanol. *Pharm Biol* 2022; 60: 1417–1435. https://doi.org/10.1080/13880209.2022.2098345.
36. Vijayan DK, Sreerekha PR, Dara PK, et al. Antioxidant defense of fish collagen peptides attenuates oxidative stress in gastric mucosa of experimentally ulcer-induced rats. *Cell Stress Chaperones* 2022; 27: 45–54. https://doi.org/10.1007/s12192-021-01245-x.
37. Soni MG, Carabin IG, Griffiths JC, Burdock GA. Safety of ephedra: lessons learned. Toxicol Lett. 2004; 150(1): 97-110.

Published Online First: August, 2024
https://doi.org/10.21123/bsj.2024.10952
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

https://doi.org/10.1016/j.toxlet.2003.07.006.

38. Hamoudi M, Amroun D, Boutefnouchet S, Bensouici C, Kaoula S, Harzallah D, et al. Phytochemical screening, in vitro antioxidant and enzyme inhibitory properties, and acute toxicity of extracts from the aerial parts of Ephedra nebrodensis, a source of bioactive compounds. Comb Chem High Throughput Screen. 2022; 25(6): 1058-1071. https://doi.org/10.2174/1386207324666210303094339.
39. Belazougui K, Mesrouk S, Mohammedi H, Akcha S, Aïnouz L, Mecherara-Idjeri SF. Phytochemical analysis, mineral composition, Assessment of antioxidant properties and cytotoxic potential of Ephedra alata. subsp. Alenda secondary metabolites. Food Biosci. 2023; 53: 102657. https://doi.org/10.1016/j.fbio.2023.102657
40. Taha MME, Abdelwahab SI, Mariod A, Abdulla MA, Nordin N, Jayapalan JJ, et al. Stem bark methanolic extract of Bauhinia thonningii (Schum) demonstrates gastroprotective properties against in vivo ethanol-induced injury. Funct. Food Sci. 2023;3(4): 26–40. https://doi.org/10.31989/ffs.v3i4.1048
41. Omar H, Nordin N, Hassan darvish P, Hajrezaie M, Azizan Syahadah AH, Fadaeinasab M, et al. Methanol leaf extract of actinodaphne sesquipedalis (Lauraceae) enhances gastric defense against ethanol-induced ulcer in rats. Drug Des Devel Ther. 2017; 11. https://doi.org/10.2147/DDDT.S120564
42. Zaghlool SS, Abo-Seif AA, Rabeh MA, et al. Gastro-Protective and Anti-Oxidant Potential of Althaea officinalis and Solanum nigrum on Pyloric Ligation/Indomethacin-Induced Ulceration in Rats. *Antioxidants (Basel, Switzerland)*; 8. Epub ahead of print October 2019. https://doi.org/10.3390/antiox8110512.
43. Hikino H, Kiso Y, Ogata M, Konno C, Aisaka K, Kubota H, et al. Pharmacological Actions of Analogues of Feruloylhistamine, an Imidazole Alkaloid of Ephedra Roots1. Planta Med. 1984; 50(06): 478-480.
44. Pirbalouti AG, Amirmohammadi M, Azizi S, Craker L. Healing effect of hydro-alcoholic extract of Ephedra pachyclada Boiss. in experimental gastric ulcer in rat. Acta Pol Pharm Res. 2013; 70(6): 1003-1009.
45. Ofusori AE, Moodley R, Jonnalagadda SB. Antiulcerogenic effects of Celosia trigyna plant extracts on ethanol-induced gastric ulcer in adult Wistar rats. J. Tradit. Complement. Med. 2020;10(6): 586–593. https://doi.org/https://doi.org/10.1016/j.jtcme.2019.11.004
46. Dhiyaaldeen SM, Alshawsh MA, Salama SM, Alwajeeh NSI, Al Batran R, Ismail S, et al. Potential activity of 3-(2-Chlorophenyl)-1-phenyl-propenonein accelerating wound healing in rats. Biomed Res. Int. 2014;2014. https://doi.org/10.1155/2014/792086
47. Gwaram NS, Musalam L, Ali HM, Abdulla MA, Shaker SA. Synthesis, spectral characterization and biological activity of Zn(II) complex with 2′-[1-(2-hydroxyphenyl) ethylidene]benzenesulfanohydrazide. Arab J Chem. 2016; 9: S1197-S1207. https://doi.org/10.1016/j.arabjc.2012.01.002
48. Saremi K, Rad SK, Khalilzadeh M, et al. In vivo acute toxicity and anti-gastric evaluation of a novel dichloro Schiff base: Bax and HSP70 alteration. *Acta Biochim Biophys Sin (Shanghai)*; 52. Epub ahead of print 2020. https://doi.org/10.1093/abbs/gmz140.
49. Ben Lamine J, Boujbiha MA, Dahane S, Cherifa A Ben, Khlifi A, Chahdoura H, et al. α-Amylase and α-glucosidase inhibitor effects and pancreatic response to diabetes mellitus on Wistar rats of Ephedra alata areal part decoction with immunohistochemical analyses. Environ. Sci. Pollut. Res. Springer; 2019;26: 9739–9754. https://doi.org.10.1007/s11356-019-04339-3
50. Lim J, Lee H, Ahn J, Kim J, Jang J, Park Y, et al. The polyherbal drug GGEx18 from Laminaria japonica, Rheum palmatum, and Ephedra sinica inhibits hepatic steatosis and fibroinflammtion in high-fat diet-induced obese mice. J Ethnopharmacol. 2018; 225: 31-41. https://doi.org/10.1016/j.jep.2018.06.034
51. Ataei Azimi A, Delnavaz Hashemloian B, Amirinia A. The comparison of alkaloid, phenolic content and antioxidant activity of wild Ephedra intermedia (Schrenket) and in vitro culture of it's explants. J Plant Process Funct. 2017; 6(21): 143-152.

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Ephedra intermedia يخفف قرحة المعدة الناجمة عن الإيثانول في الفئران عن طريق آليات مضادة للالتهابات ومضادات الأكسدة

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الخلاصة

تم استخدام *Ephedra intermedia* كعلاج تقليدي لأغراض صحية مختلفة بما في ذلك اضطرابات المعدة. للتحقق من صحة استخدامه التقليدي، قمنا بدراسة السمية الحادة والتأثيرات الوقائية المعدية لمستخلص أوراق *Ephedra intermedia* (EILE) في إصابة المعدة الناجمة عن الإيثانول في الفئران. تم فصل الفئران سبراغ داولي بشكل عشوائي إلى 5 مجموعات: مجموعة السيطرة الطبيعية (A) ومجموعة الفئران المقرحة (B) تلقت عن طريق الفم 0.5 ٪ كاربوكسي ميثيل السليلوز (CMC)؛ مجموعة (C) الفئران التي تناولت أوميبرازول 20 ملغم/كغم؛ المجموعتين D وE، تناولت الفئران 250 و500 ملغم/كغم من EILE على التوالي. بعد 60 دقيقة، تلقت المجموعات B-E جرعة فموية من الإيثانول المطلق لاحداث قرحة المعدة. وبعد 60 دقيقة أخرى، تم تخدير الفئران والتضحية بها. أظهرت تجربة السمية عدم وجود أي علامات سمية في الجرذان المعرضة لتناول 2 و5 غم / كغم من EILE عن طريق الفم. وكشفت تجربة الوقاية المعدية عن اختراق كبير لأنسجة المعدة في السيطرة على القرحة، كما يتضح من انخفاض محتوى المخاط، وانخفاض درجة الحموضة في المعدة، واخداث تمزقات شديدة في الطبقة المخاطية. اظهرت الفئران التي عولجت بالأوميبرازول أو EILE تأثيرا قليلا جدا من تلف أنسجة المعدة، مما يشير إلى تأثير منخفض بشكل ملحوظ على جروح المعدة بوساطة الإيثانول، كما يتضح من تحسن مخاط المعدة ودرجة الحموضة، وخفض مساحات القرحة، وانخفاض الوذمة، وتسرب الكريات البيض إلى الطبقة تحت المخاطية. في جناسة المعدة، أدى علاج EILE إلى زيادة كبيرة في إنزيمات مضادات الأكسدة (SOD وCAT) وخفض محتوى MDA بشكل ملحوظ. علاوة على ذلك، أظهرت الفئران المعالجة بـ EILE زيادة في التعبير عن صبغة PAS في ظهارة المعدة والغدد القاعدية (القاعدة والرقبة والبرزخ). يمكن أن تعزى التأثيرات الوقائية المعوية لـ *Ephedra intermedia* إلى زيادتها الإيجابية لعوامل المعدة المختلفة، مما قد يؤكد صحتها كمضاف علاجي مستقبلي لقرحة المعدة بعد التقييم الدوائي.

الكلمات المفتاحية: الإنزيمات المضادة للأكسدة، السمية الحادة، *Ephedra intermedia* , قرحة المعدة، علم الانسجة.