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Original Research

Gender Differences in the Effects of Esomeprazole on Ethanol-Induced Acute Gastric Injury in Mice

Zhi-hui Yi[1,†], Yao Wu[2,†], Yang Yu[2], Feng Li[2], Tao Zeng[2], Yu Zeng[1], Linling Lv[1], Li-hong Wan[2,*], Ling Zhu[2,*]

[1]Department of Gastroenterology, West China Hospital, Sichuan University, 610041 Chengdu, Sichuan, China
[2]Department of Pharmacology, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, 610041 Chengdu, Sichuan, China
*Correspondence: wanlihong1976@sina.com (Li-hong Wan); zhuling529@163.com (Ling Zhu)
[†]These authors contributed equally.
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Abstract

Background: A gastric ulcer is a common gastrointestinal disorder, particularly associated with alcohol abuse, leading to acute ulcers. Proton pump inhibitors, such as esomeprazole, are effective for treating acute alcoholic ulcers. However, their therapeutic effects vary, and the underlying reasons for these differences remain unclear. In this study, we investigated gender differences in the effects of esomeprazole on ethanol-induced acute gastric injury in mice. We evaluated the potential different gastroprotective effects of esomeprazole by modulating the receptor-interacting protein kinase1 (RIPK1) and nuclear factor kappa-B (NF-κB) pathways. **Methods**: The effects of esomeprazole on ethanol-induced acute gastric injury *in vivo* were analyzed. Macroscopic observation and pH measurement, histological analysis, and pepsin activity were used to assess the gastroprotective effects of esomeprazole. Serum levels of interleukin (IL-6) and tumor necrosis factor alpha (TNF-α) in female mice were measured by enzyme-linked immunosorbent assay (ELISA). Immunohistochemistry (IHC) assay and western blotting analysis were used to evaluate the anti-inflammatory effects and underlying mechanisms of esomeprazole. **Results**: Ethanol-induced acute gastric injury in mice, including ulcer area, ulcer index score, ulcer depth, bleeding and inflammation, pH, and pepsin, was reversed by esomeprazole pretreatment *in vivo*. Esomeprazole exhibited significant gastroprotective effects on alcohol-induced ulcers in mice, whether administered by injection or gavage, at both high and low doses. Notably, there were gender differences in the treatment effect. The improvement in these indicators was more pronounced in female mice, especially in response to the esomeprazole enteric capsule 3.03 mg/kg. We found that esomeprazole can reverse the elevation of serum inflammatory factors such as IL-6 caused by alcoholic gastric ulcer. Esomeprazole notably reduced the inflammatory injury score in gastric tissue, with decreased RIPK1 and NF-κB protein levels, accompanied by increased Schiff's periodic acid staining and enhanced caspase 8 expression. The anti-necrosis, anti-inflammation effect of esomeprazole in ethanol-induced acute gastric injury is partly via RIPK1 and NF-κB-dependent. **Conclusions**: Esomeprazole has excellent gastroprotective and anti-inflammatory effects in ethanol-induced acute gastric injury by modulating RIPK1and NF-κB in a dependent manner. The improved response demonstrated in female mice may be attributed to the protective effects of estrogen on the gastrointestinal tract.

Keywords: gender differences; esomeprazole; ethanol-induced acute gastric injury

1. Introduction

Gastric ulcer is a common gastrointestinal disorder characterized by mucosal damage and a complex pathogenesis involving multiple factors, such as Helicobacter pylori infection, acid secretion dysregulation, NSAID use, alcohol abuse, and genetic susceptibility. Epidemiological studies highlight significant gender disparities in gastric ulcer (gastrointestinal disorders) incidence, clinical manifestations, and treatment outcomes [1–4]. In terms of incidence, males exhibit a higher prevalence, whereas females may display distinct pathological features linked to hormonal fluctuations. Higher male prevalence may correlate with smoking and alcohol consumption, while female susceptibility could involve psychosocial factors, necessitating gender-stratified prevention approaches, etc. Furthermore, clinical observations suggest gender-specific differences in therapeutic responses include adverse reactions (e.g., to proton pump inhibitors (PPI) or antibiotics) and ulcer recurrence rates [5]. Proton pump inhibitors such as esomeprazole are effective drugs for treating acute ulcers. In terms of treatment, omeprazole may be more effective for female patients with gastric ulcers [5–7]. Different studies have found that the efficacy of PPI in treating ulcer bleeding varies, and long-term use may also cause varying degrees of lung damage [6,7]. The differences in the effectiveness of PPIs in alcoholic ulcers remain unclear. These differences prompt us to consider whether the therapeutic effect of PPI is gender related, and what may be the underlying mechanisms of these differences?

Therefore, the study of gender differences in ulcers has important clinical significance and scientific value; however, systematic investigations into these disparities remain limited, and the underlying mechanisms are poorly understood. The mechanism in gastric mucosal protection and repair requires further exploration, particularly their interactions with inflammation, oxidative stress, and *H. pylori* infection. Clarifying gender differences and inflammation mechanisms could inform personalized treatment strategies, such as some special drug therapy for females or adjusted drug dosages for males [8,9]. The sexual dimorphism in necroptotic signaling has been evidenced in kidney ischemia-reperfusion injury. Female mice, which sustain milder kidney injuries, exhibit delayed and attenuated necroptotic signaling, involving molecules such as receptor-interacting protein kinase1 (RIPK1), receptor-interacting protein kinase 3 (RIPK3), and mixed lineage kinase domain-like protein (MLKL) [10]. By comparing therapeutic outcomes between genders in mice, this study aims to provide a theoretical basis for gender-specific clinical treatment of proton pump inhibitors. Therefore, further investigation into the treatment differences and mechanism of gastric ulcers related to gender is of great significance for optimizing clinical treatment plans and improving proton pump inhibitor therapeutic efficacy.

2. Material and Methods

2.1 Experimental Design and Ethanol-Induced Acute Gastric Injury Model in Mice

In accordance with the Committee on the Ethics of Animal Experiments of Sichuan University (Permit Number: 20240611004), a total of 60 Kunming mice (18–22 g, 6–8 week-old, 30 male and 30 female) were purchased from DaShuo Biological Technology Co., Ltd. (Chengdu, China) and housed under standard conditions (room temperature: 23 °C ± 1 °C; relative humidity: 55% ± 5%) with a 12-h day/night cycle (lights on at 8:00 am). After acclimation to the housing conditions for 5 days, all mice were randomly divided into six groups (10 mice per group, half male and half female): control group, ethanol group, esomeprazole injection (Chia Tai Tianqing Pharmaceutical Group Co., Ltd., lot no. 201230148, Jiangsu, China) high dose (EHI, 6.06 mg/kg, ip), low dose (ELI, 3.03 mg/kg, ip) and esomeprazole enteric capsule (Chia Tai Tianqing Pharmaceutical Group Co., Ltd., lot no. 19062501, Jiangsu, China) high dose (EHO, 6.06 mg/kg, orally), low dose (ELO, 3.03 mg/kg, orally) treatment groups. The esomeprazole enteric capsule was dissolved in 0.9% sodium chloride. The dose selection for esomeprazole was determined based on animal equivalent doses, in accordance with the recommended daily human doses [11,12].

All treatment groups were pre-treated with the corresponding dose of esomeprazole, once a day for 5 days. The control group and the ethanol group were given the same volume of saline. Two hours after the last pre-treatment, 100% ethanol 0.2 mL was administered orally to induce acute gastric injury, according to our preliminary experiments [12]. The control group received saline 0.2 mL orally. After 1 h from induction, all mice were euthanized with pentobarbital sodium (20 mg/mL, 150 mg/kg, intraperitoneally).

2.2 Macroscopic Observation and pH Value Measurement

The gastric tissues of all mice were removed and opened along the greater curvature. Gastric ulcerative lesions were assessed by two blinded observers and photographed. On the mucosal side of the stomach, according to the diameter of gastric ulcer, the ulcer index (UI) was scored as follows: 0: no lesion; 1: ulcer area with the longest diameter $\leq$1 mm; 2: the longest diameter is $>$1 mm and $\leq$2 mm; 3: the longest diameter is $>$2 mm and $\leq$3 mm; 4: the longest diameter is $>$3 mm and $\leq$4 mm; 5: the longest diameter $>$4 mm [13].

The gastric juice pH value was determined with pH test paper.

Then, the gastric tissues were cut into 2 halves. One was kept in 10% Neutral formalin solution for further histological studies, and one-half was kept at –80 °C.

2.3 Histological Analysis and Immunohistochemistry (IHC) Assay

After fixation in 10% Neutral formalin solution for 24 h, gastric tissues were embedded in paraffin and cut into 4 µm sections. These sections were stained with hematoxylin and eosin (HE) and Schiff's periodic acid (PAS) for histological evaluation. The morphology of the gastric tissue was observed blindly using a light microscope (DM250, LEICA, Germany), and gastric injury was graded from 0 (normal) to 4 (severe). Ulcer depth, bleeding, and inflammation were scored, respectively. Finally, the gastric injury score was obtained by adding the individual scores for each type.

PAS stain was used to assess the mucus production in the gastric tissue.

For the IHC assay, the 4-µm sections were incubated with primary receptor-interacting protein kinase1 (RIPK1) antibodies (dilution 1:50, Proteintech, USA) overnight at 4 °C, washed three times with phosphate-buffered saline (PBS), and then stained with secondary antibody (goat anti-rabbit IgG labeled by horseradish peroxidase (HRP)) for 4 hours. 3,3'-diaminobenzidine (DAB) was used for IHC signal detection after washing three times with PBS, then hematoxylin counterstaining for 2 min. Observe the images using an optical microscope (DM250, LEICA, Germany) and perform semi-quantitative analysis blindly through ImageJ software.

2.4 Pepsin Activity

Gastric tissues were homogenized with the extracting solution and centrifuged at 4 °C, 10,000 rpm for 10 min, and

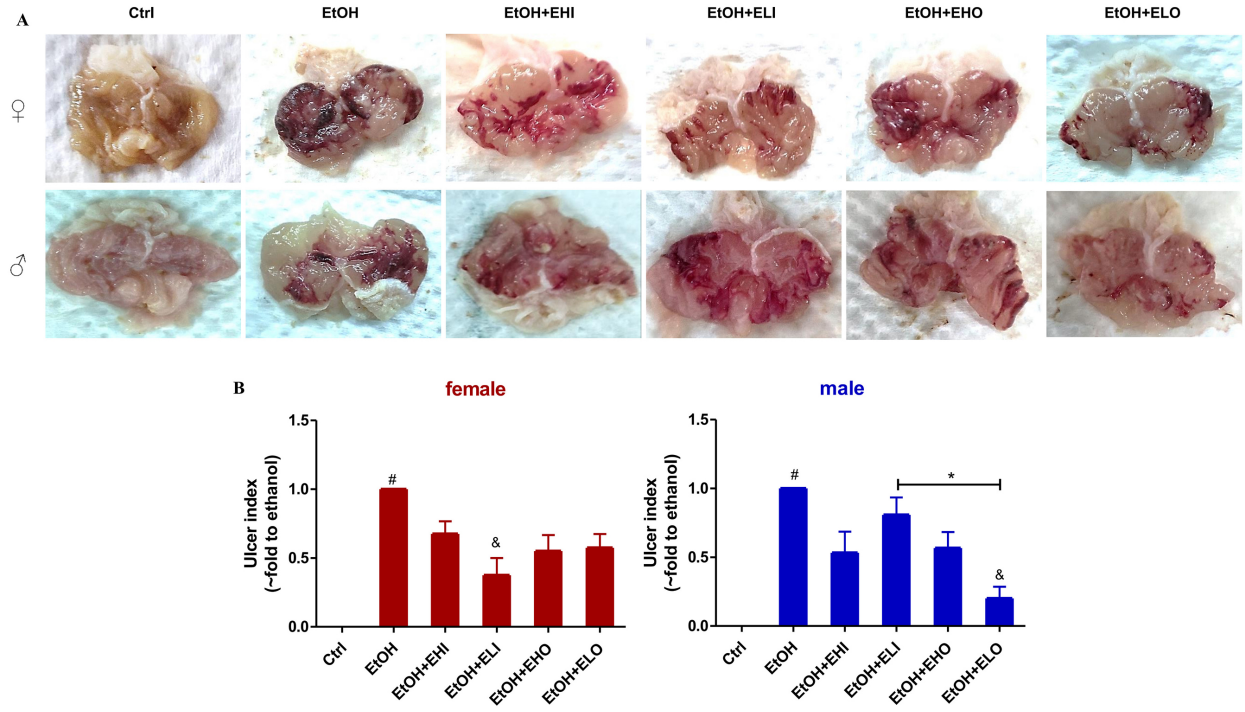

Fig. 1. Effects of esomeprazole on the EtOH-induced gastric injury in mice. Representative images of the mouse stomach from (A) control, EtOH, EtOH + esomeprazole 6.06 mg/kg i.p (EtOH + EHI), EtOH + esomeprazole 3.03 mg/kg i.p (EtOH + ELI), and EtOH + esomeprazole 6.06 mg/kg gavage (EtOH + EHO), EtOH + esomeprazole 3.03 mg/kg gavage (EtOH + ELO). (B) The ulcer index was compared between mice of different genders. Significant differences were observed after administration of esomeprazole at the dose of 3.03 mg/kg i.p in female mice and 3.03 mg/kg p.o in male mice. $^{\#}$ $p < 0.05$, as compared to control mice, $^{\&}$ $p < 0.05$, as compared to EtOH-group, * $p < 0.05$, as compared to EtOH + ELO-group. Results are expressed as mean ± SEM for n = 5 mice per gender group.

the protein concentrations in supernatant (Cpr) were measured by BCA at 562 nm. Pepsin activity from gastric tissues was determined by ultraviolet spectroscopy. The opticle density (OD) values were detected at 275 nm using a spectrophotometer. Pepsin activity (U•mg prot^{-1}) = 1.31 × ($A_{test} - A_{control}$)/Cpr.

2.5 Enzyme-Linked Immunosorbent Assay (ELISA) Analysis

Blood samples of mice were centrifuged at 3000 g for 15 min at 4 °C, then serum was collected, and the concentrations of IL-6 and TNF-α were measured using commercial ELISA kits (Boster, Wuhan, China) according to the manufacturer's protocol. The absorbance was measured at 450 nm using an iMark microplate reader (BIO-RAD).

2.6 Western Blotting

Total proteins were extracted using radioimmunoprecipitation assay (RIPA) Lysis Buffer (Beyotime Institute of Biotechnology, China), and the protein concentrations were measured with a BCA protein assay kit (Beyotime Institute of Biotechnology, China). Equal amounts of total protein (40 µg/10 µL) were loaded onto 8% SDS-PAGE gels at 120 v for 60 min, electro-transferred to polyvinylidene difluoride (PVDF) membranes, blocked in 5% non-fat milk at room temperature for 2 h, and incubated with the diluted primary antibodies, including Caspase8 (Cell Signalling Technology, 4790S, 1:1000), RIPK1 (Proteintech, 17519-1-AP, 1:1000), and NF-κB (Cell Signalling Technology, 3033S, 1:1000) at 4 °C overnight in turn. After washing three times with Tween-20 (TBST), the membranes were probed with horseradish peroxidase-conjugated secondary antibody (Anti-rabbit IgG, Cell Signalling Technology, 7074P2, 1:2000 or Anti-mouse IgG, Cell Signalling Technology, 7076P2, 1:2000) at room temperature for 2 h. β-actin (Cell Signalling Technology, 3700S, 1:1000) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cell Signalling Technology, 97166S, 1:1000) was the loading control. The protein was detected by chemiluminescence reagent (GE Healthcare), and the protein band density was quantified using Image J software (version 1.53, National Institutes of Health, Bethesda, MD, USA).

2.7 Statistics

All values were expressed as mean ± SEM, and statistically analyzed by one-way analysis of variance (ANOVA) with Bonferroni correction (GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA)). $p < 0.05$ was considered statistically significant.

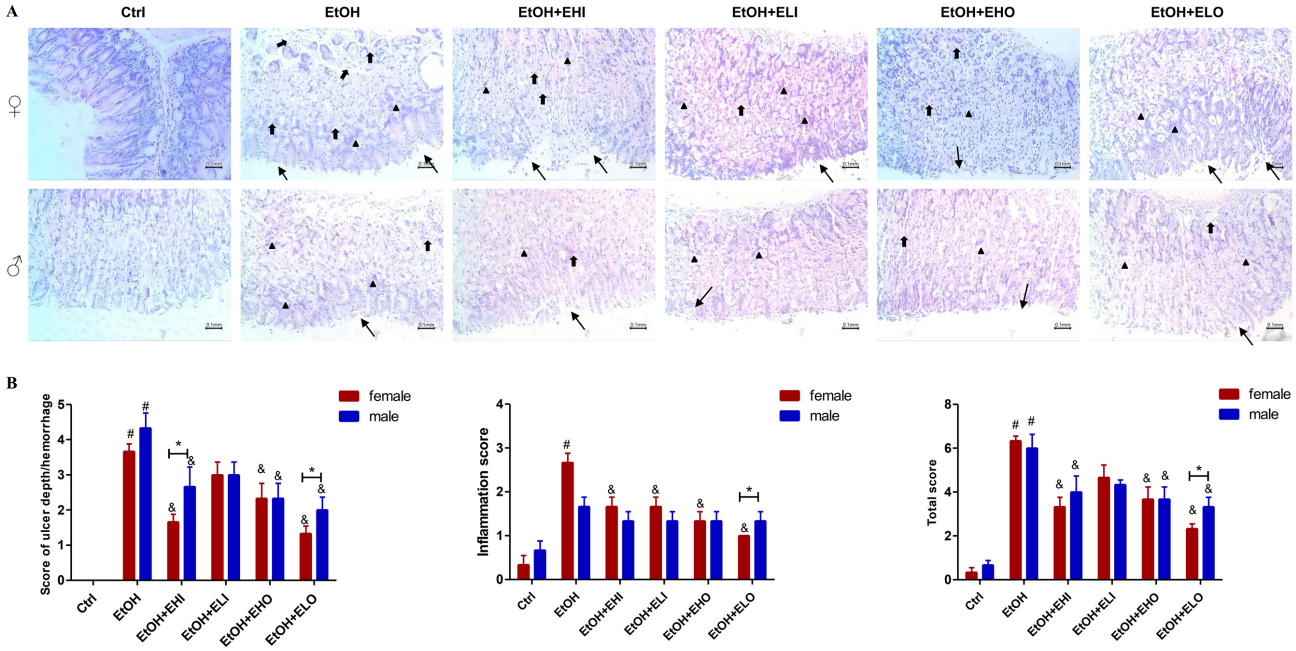

Fig. 2. Gastroprotective effect of esomeprazole on the EtOH-induced gastric injury in mice by histopathological evaluation. (A) Representative images of histopathological evaluation of the damage in the gastric mucosa stained with hematoxylin and eosin (HE) stains (×20) from control, EtOH, EtOH + esomeprazole 6.06 mg/kg i.p (EtOH + EHI), EtOH + esomeprazole 3.03 mg/kg i.p (EtOH + ELI), and EtOH + esomeprazole 6.06 mg/kg gavage (EtOH + EHO), EtOH + esomeprazole 3.03 mg/kg gavage (EtOH + ELO). Scale bar: 0.1 mm. The slender arrow indicates mucosal damage, with altered villus morphology and disordered, shrunken cellular arrangement in the villi; the triangle, short, thick arrow denotes inflammation and bleeding, along with red blood cells. (B) Gastric injury scores include ulcer depth, bleeding, and inflammation score, were compared. $^{\#}$ $p < 0.05$, as compared to control mice; $^{\&}$ $p < 0.05$, as compared to EtOH-group; * $p < 0.05$, as compared to the male group. Results are expressed as mean ± SEM for n = 5 mice per gender group.

3. Results

3.1 Gender Different Effects of Esomeprazole on the EtOH-Induced Gastric Injury in Mice

To investigate the gender difference of the gastroprotective effect of esomeprazole, the mouse acute ethanol (EtOH)-induced model of gastric injury was established by intragastric administration of 100% EtOH. First, the gastric mucosal injury was observed in gross tissues, and the ulcer index (UI) was used to evaluate the extent of gastric injury. As shown in Fig. 1A, no visible lesions developed in the control group, but 100% EtOH markedly resulted in gastric mucosa ulcer and bleeding in female and male mice, manifested by an increased UI compared with the control group ($p < 0.05$, Fig. 1B). Interestingly, esomeprazole injection and enteric capsule demonstrated different degrees of gastroprotective effects. In female mice, pre-treatment with ELI at 3.03 mg/kg (clinical equivalent dose 20 mg) significantly attenuated gastric mucosal injury as shown by decreased UI compared with EtOH group ($p < 0.05$, Fig. 1B), whereas in male mice, similar reduction in UI occurred in pre-treatment with ELO at 3.03 mg/kg ($p < 0.05$, Fig. 1B).

Next, histopathological evaluation of the damage in the gastric mucosa was performed using HE. In the control group, the gastric mucosa of mice was intact, and there was no inflammatory cell infiltration and mucosal hemorrhage (Fig. 2A). 100% EtOH induced an extensive disruption of the gastric mucosal layer with a marked presence of ulcerative plaque, gland atrophy, and hemorrhage, inflammatory cell infiltration in the mucosal and submucosal layer, with an increased gastric injury score compared with the control group (Fig. 2B). As compared to the EtOH group, two dose forms of esomeprazole significantly reduced EtOH induced gastric mucosal injury with a significant inhibition in ulcer/hemorrhage score and total score, except for the ELI group (left and right panel, Fig. 2B). However, there was obviously inhibition in inflammatory cell infiltration only in female mice (middle panel, Fig. 2B). Therefore, as shown in the right panel of Fig. 2B, we found pre-treatment with ELO at 3.03 mg/kg (clinical equivalent dose 20 mg) significantly prevented the presence of ulcerative plaque, hemorrhage, and inflammatory cell infiltration, especially in female mice ($p < 0.05$). Furthermore, we compared the different dose form of esomeprazole at the dose of 3.03 mg/kg (clinical equivalent dose 20 mg) in female mice and found that both ulcer/hemorrhage scores and inflammation scores were lower in ELO than those in ELI ($p < 0.05$, Fig. 3), suggesting there was a good response to esomeprazole enteric capsule 3.03 mg/kg in female mice.

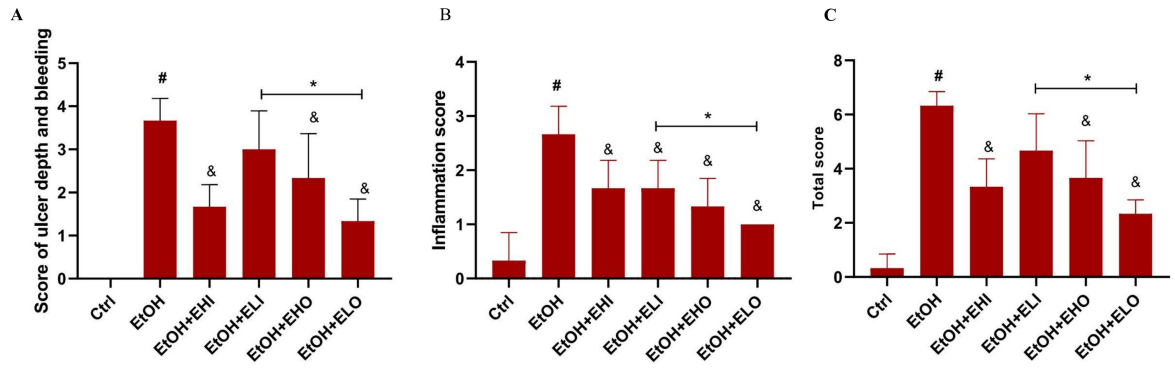

Fig. 3. Gastroprotective and anti-inflammatory effects of esomeprazole in the EtOH-induced gastric injury in female mice. (A) Gastric injury scores include ulcer depth, bleeding, (B) inflammation score and (C) total score were compared from control, EtOH, EtOH + esomeprazole 6.06 mg/kg i.p (EtOH + EHI), EtOH + esomeprazole 3.03 mg/kg i.p (EtOH + ELI), and EtOH + esomeprazole 6.06 mg/kg gavage (EtOH + EHO), EtOH + esomeprazole 3.03 mg/kg gavage (EtOH + ELO). * $p < 0.05$, significant differences were observed after administration of esomeprazole at the dose of 3.03 mg/kg i.p and 3.03 mg/kg p.o in female mice; $^{\#}$ $p < 0.05$, as compared to control mice; $^{\&}$ $p < 0.05$, as compared to EtOH-group. Results are expressed as mean ± SEM for n = 5 mice per group.

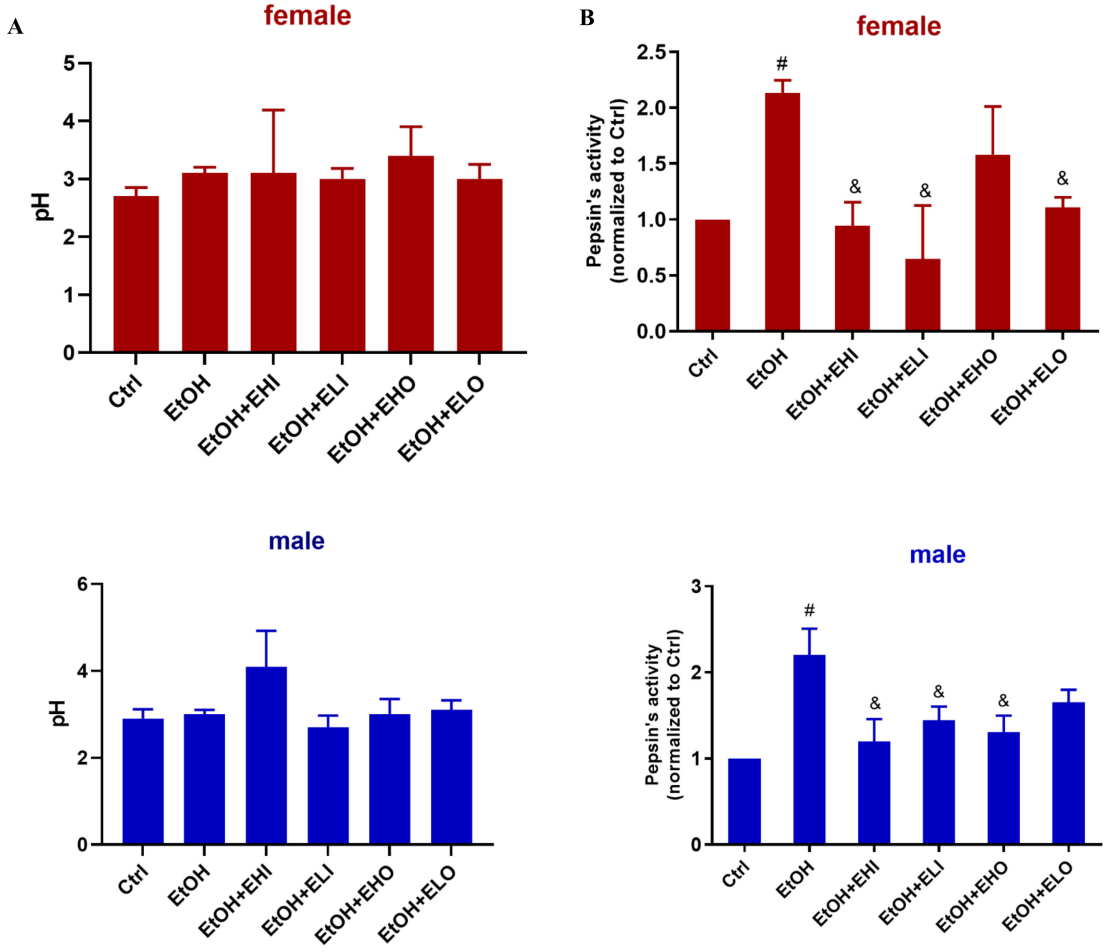

Fig. 4. Effects of esomeprazole on gastric acid secretion in the EtOH-induced gastric injury in mice. (A) pH value with pH test paper from control, EtOH, EtOH + esomeprazole 6.06 mg/kg i.p (EtOH + EHI), EtOH + esomeprazole 3.03 mg/kg i.p (EtOH + ELI), and EtOH + esomeprazole 6.06 mg/kg gavage (EtOH + EHO), EtOH + esomeprazole 3.03 mg/kg gavage (EtOH + ELO). (B) Pepsin activity was compared. Significant differences were observed after administration of esomeprazole EtOH + EHI, EtOH + ELI, and EtOH + ELO group in female mice, EtOH + EHI, EtOH + ELI, and EtOH + EHO in male mice. $^{\#}$ $p < 0.05$, as compared to control mice; $^{\&}$ $p < 0.05$, as compared to EtOH-group. Results are expressed as mean ± SEM for n = 5 mice per gender group.

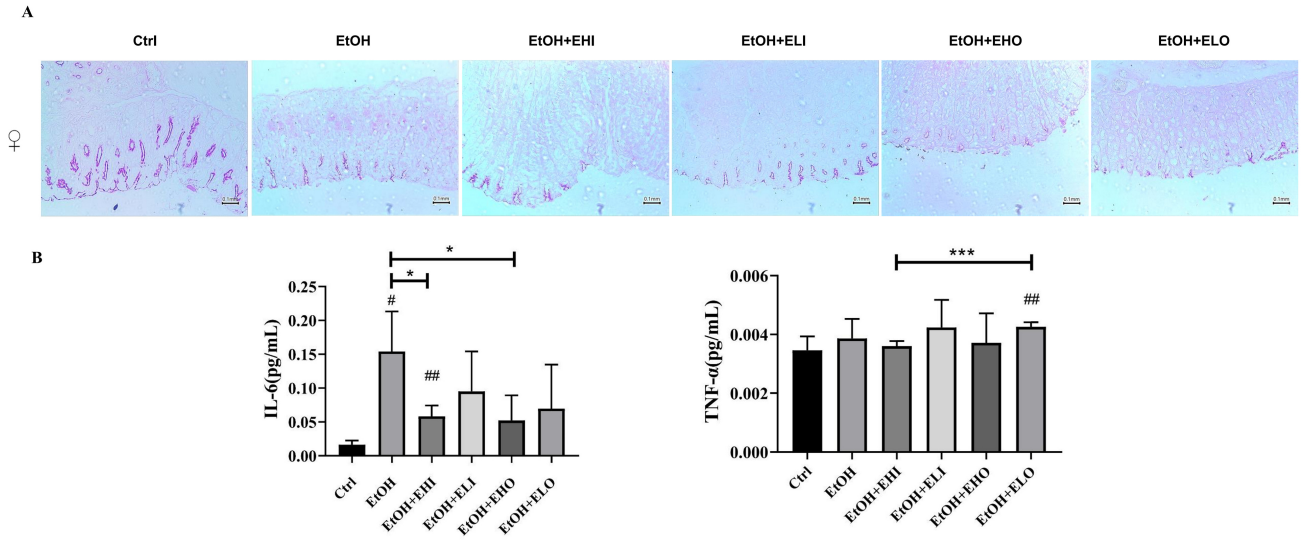

Fig. 5. Effects of esomeprazole on serum inflammatory factors and gastric mucosal glycoprotein expression in the EtOH-induced gastric injury in female mice. (A) Representative PAS staining images of the mouse stomach from control, EtOH, EtOH + esomeprazole 6.06 mg/kg i.p (EtOH + EHI), EtOH + esomeprazole 3.03 mg/kg i.p (EtOH + ELI), and EtOH + esomeprazole 6.06 mg/kg gavage (EtOH + EHO), EtOH + esomeprazole 3.03 mg/kg gavage (EtOH + ELO). PAS staining of the gastric mucus was weaker in the EtOH group than in the control group; mucosal glycoprotein expression increased in all esomeprazole groups. Scale bar: 0.1 mm. (B) Serum concentrations of inflammatory factors IL-6 and TNF-α in mice were quantified with an enzyme-linked immunosorbent assay (ELISA) kit from control, EtOH, EtOH + esomeprazole 6.06 mg/kg i.p (EtOH + EHI), EtOH + esomeprazole 3.03 mg/kg i.p (EtOH + ELI), and EtOH + esomeprazole 6.06 mg/kg gavage (EtOH + EHO), EtOH + esomeprazole 3.03 mg/kg gavage (EtOH + ELO). $^{\#}$ $p < 0.05$, as compared to control mice; $^{\#\#}$ $p < 0.05$, as compared to control mice; * $p < 0.05$, as compared to EtOH-group; *** $p < 0.05$, as EtOH-ELO compared to EtOH-EHI group. n = 5 mice per female group.

3.2 Gender Different Effect of Esomeprazole on the Inhibition of Gastric Secretion

To examine the gender different effect of esomeprazole on acid secretion and pepsin activity, we measured the pH value with pH test paper first and found the pH values of the EtOH group in both female and male mice were little difference compared with that of the control group ($p > 0.05$, Fig. 4A). Interestingly, pretreatment with esomeprazole slightly increased the pH value in female mice, but slightly decreased it in male mice ($p > 0.05$, Fig. 4A). However, EtOH-enhanced pepsin activity was significantly inhibited by pretreatment with esomeprazole in female and male mice ($p < 0.05$, Fig. 4B). In detail, in female mice low dose of esomeprazole injection demonstrated the best efficacy, and in male mice, two dose forms of esomeprazole showed similar efficacy, except for ELO (Fig. 4B). These data also indicated that there was a gender difference in the effect of esomeprazole on the inhibition of gastric secretion.

3.3 PAS in Female Mice

Considering the gender difference in esomeprazole efficacy, we chose female mice as the animal model in the subsequent experiment. PAS staining was used to evaluate the content of gastric mucosal glycoprotein. As we expected, the PAS staining of the gastric mucus was weaker in the EtOH group than that in the control group, which was significantly increased in all esomeprazole pretreatment groups (Fig. 5A).

3.4 Anti-Inflammatory Effect of Esomeprazole Contributes to Preventing EtOH-Induced Gastric Injury in Female Mice

Our studies demonstrated that the score of the EtOH-induced gastric injury tissues in female mice was significantly attenuated following esomeprazole pretreatment ($p < 0.05$, Fig. 3). The histological analysis results showed that the gastric injury score, including ulcer depth, bleeding, and inflammation score were significantly increased in the EtOH group compared to the normal control group ($p < 0.05$, Fig. 3). After pretreatment with esomeprazole, these scores decreased to varying degrees in both the injection and gavage groups, as well as the high-dose and low-dose groups. Interestingly, we found that compared to the low-dose injection group, the low-dose gavage group was more effective in improving gastric inflammation damage ($p < 0.05$, Fig. 3). Plasma IL-6 and TNF-α concentrations, two effective biomarkers for inflammation, were measured by ELISA. As shown in Fig. 5B, compared to the control group, the EtOH group significantly increased plasma IL-6 concentrations in mice ($p < 0.05$), and TNF-α showed no obvious change; and ELISA results also showed that esomeprazole significantly de-

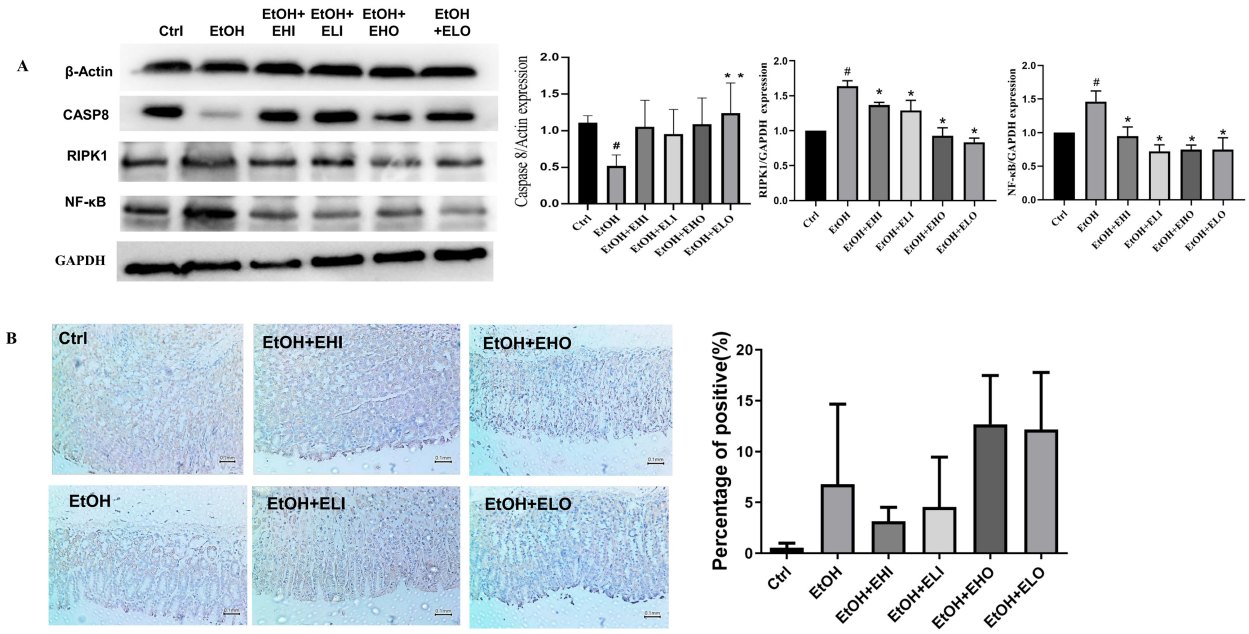

Fig. 6. Anti-inflammatory effects of esomeprazole expression via receptor-interacting protein kinase1 (RIPK1)/NF-κB pathway in the EtOH-induced gastric injury in mice. (A) Representative Western blotting images of CASP-8, RIPK1, and NF-κB in the mouse stomach. GAPDH was used as an internal standard (left panel); quantitative analysis from different groups by Image J software (2.1.0/1.53c) (right panel). (B) Representative IHC images of RIPK1 staining in the EtOH-induced gastric injury mice (n = 5) from control, EtOH, EtOH + esomeprazole 6.06 mg/kg i.p (EtOH + EHI), EtOH + esomeprazole 3.03 mg/kg i.p (EtOH + ELI), and EtOH + esomeprazole 6.06 mg/kg gavage (EtOH + EHO), EtOH + esomeprazole 3.03 mg/kg gavage (EtOH + ELO). Scale bar: 0.1 mm. $^{\#}$ $p <$ 0.05, as compared to control mice; * $p < 0.05$, as compared to EtOH group; ** $p < 0.05$, as compared to EtOH group. n = 5 mice per group.

creased IL-6 concentrations ($p < 0.05$). Next, as expected, WB analysis showed that the important factors, including RIPK1 and NF-κB levels in the inflammatory transmission pathway, were significantly increased in EtOH-treated mice, whereas notably ameliorated by 6.06, 3.03 mg/kg esomeprazole pretreatment (Fig. 6A). Our data also showed that EtOH markedly decreased caspase-8 expression in the gastric tissue, while EtOH +esomeprazole could increase anti-inflammation protein caspase level in contrast to EtOH mice (Fig. 6A). In addition, we found esomeprazole pretreatment (injection groups) suppressed RIPK1 expression by IHC assay, that consistent with previous studies (Fig. 6B).

4. Discussion

In the present study, we demonstrated that esomeprazole, a proton pump inhibitor, exerts significant gastroprotective effects in ethanol-induced gastric injury. The result of our study indicated that it has a good therapeutic effect on ethanol-induced gastric ulcers in mice, with a significant gender difference, and the therapeutic effect is better in female mice. Moreover, our study has shown that the gastroprotective mechanism of esomeprazole is related to its anti-inflammatory, anti-necrosis action, which is partly associated with the RIPK1 and NF-κB signaling pathway.

Esomeprazole, a widely used proton pump inhibitor, is well-known for its ability to reduce gastric acid secretion by inhibiting the H^+/K^+-ATPase enzyme in parietal cells. This mechanism has made it a cornerstone in the treatment of acid-related disorders such as gastroesophageal reflux disease and peptic ulcers. However, the findings from the mentioned research open new avenues for understanding its potential therapeutic applications beyond acid suppression.

As a result, the EtOH + esomeprazole group had less severe inflammation. This observation that esomeprazole can effectively treat ethanol-induced gastric ulcers in mice suggests that it may have additional protective mechanisms in the gastrointestinal tract. Ethanol is a well-known irritant that can cause severe gastric mucosal damage, leading to ulcer formation. Our research found that the effect of pH on alcoholic ulcers was not significant, but esomeprazole still had a good anti-ulcer effect, which was not significantly related to changes in pH; and its effect had significant gender differences, so we analyzed whether it is related to other mechanisms to play a protective role against ulcers. The fact that esomeprazole shows a better therapeutic effect in female mice indicates that there might be gender-specific differences in the pathophysiology of ethanol-induced ulcers or in the pharmacodynamics of esomeprazole. Hormonal differences between male

and female mice could potentially influence the susceptibility to gastric injury and the response to treatment. For example, estrogen has been demonstrated to exert protective effects on the gastrointestinal tract through estrogen-related receptor alpha, which potentially plays a crucial role in regulating immune homeostasis by maintaining a balanced pro-inflammatory and anti-inflammatory response, thus contributing to the preservation of epithelial integrity [14]. This might contribute to the enhanced therapeutic effect observed in female mice. Histological observations have found that esomeprazole can significantly reduce tissue inflammation, ulcer necrosis area, and depth; and esomeprazole can reverse the elevation of serum inflammatory factors such as IL-6 caused by alcoholic gastric ulcer in female mice. Although our research found that there was no change in TNF-α level, we analyzed that this may be related to the time of our experimental observation. A blood sample was drawn one hour after the model was established, and the change might not be significant at that time because TNF-α is a rapidly and transiently increasing inflammation-associated biomarker, peaking at 3 h and returning to baseline within 24 h [15], while the influence of IL-6 lasts longer, thus showing an obvious change. Therefore, we speculate that its gender differences may be related to its anti-inflammatory, anti -necrosis effects.

Moreover, the association of esomeprazole's gastroprotective effect with its anti-inflammatory action is intriguing. Inflammation and necroptosis play a crucial role in the development and progression of gastric ulcers [16]. Members of the receptor-interacting protein kinase (RIPK) family are important regulators of inflammation and cell death [17–19]. RIPK1 complex regulates necroptosis, which is a highly controlled programmed cell death and works against pathogen-mediated infections, and morphologically features cellular organelle swelling followed by plasma membrane rupture [20–22]. The classical necroptosis is initiated with TNF-α and activates RIPK1 under the deactivation of caspase-8 [22]. Researchers reveal that coordinated NF-κB inflammatory and RIPK1 necroptosis signaling pathways within dying cells orchestrate efficiency and immunity [23]. Also, cell death could cause inflammation in different tissues, regulate RIPK1 kinase activity-mediated apoptosis by NF-κB-dependent and -independent functions, which are critical for averting chronic tissue injury and inflammation in the gut (intestine) [23–26]. Some drugs play beneficial roles in alcohol disease via regulating multiple signaling transduction pathways include RIPK1/3 and NF-κB [27]. So, inhibition of RIPK3-dependent necroptosis by caspase-8 was identified as a key mechanism preventing inflammation in epithelial barriers and gut [18,28,29]. The RIPK1 signaling pathway is a key regulator of inflammation, and its activation can lead to the production of pro-inflammatory cytokines that exacerbate tissue damage. By modulating this pathway, esomeprazole could compromise the increase of the protein levels of important inflammation pathway transcription factor (NF-κB) and necroptotic markers (RIPK1), whereas notably upregulating Caspase-8 *in vivo*, indicating esomeprazole may be able to reduce inflammation by anti-necroptosis role and promote gastric mucosal healing. This finding suggests that the therapeutic effects of esomeprazole might not be solely due to its acid-suppressing properties but also involve its ability to modulate inflammatory responses.

In conclusion, the research findings highlight the potential of esomeprazole as a therapeutic agent for ethanol-induced gastric ulcers, particularly in female mice. Further studies are needed to elucidate the exact mechanisms underlying its anti-inflammatory effects and its interaction with the RIPK1and NF-κB pathway. This could lead to the development of new treatment strategies for gastrointestinal disorders, especially those with an inflammatory component.

5. Limitations

However, there are many limitations in the present study. Firstly, the use of pH test paper is relatively imprecise. Secondly, this study did not evaluate sex hormones, estrous cycles, or sex-dependent pharmacokinetics, and the intentional focus on females in subsequent experiments introduces systematic bias, thereby weakening the claim of truly gender-dependent mechanisms. In future studies, we will conduct a thorough assessment of sex hormone levels and investigate gender-dependent mechanisms in male mice.

6. Conclusions

Esomeprazole has excellent gastroprotective and anti-inflammatory effects in ethanol-induced acute gastric injury, especially in female mice, by modulating the RIPK1 and NF-κB-dependent pathways.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

LZ, ZHY, and LHW conceived the idea, and designed the study; LZ wrote and revised the manuscript; YW, YY and FL contributed to most of the animal and molecular biology experiments; TZ, YZ, and LLL assisted in completing animal experiments and participated in data analysis. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All animal experiments should comply with the ARRIVE guidelines. All methods were carried out in accordance with relevant guidelines and regulations. The study was approved by the Animal Ethics Committee of Sichuan University (NO. 20240611004). The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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Conflict of Interest

The authors declare no conflict of interest.

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