

Modulation of NF- κ B and oxidative stress pathways in ethanol-induced gastric injury by a multi-component herbal formula: An in vivo study

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Abstract

Background. Chronic alcohol consumption can damage the gastric mucosa and increase the risk of severe gastrointestinal disorders, including gastritis, peptic ulcers, and potentially gastric cancer. Modern research has demonstrated that Erchen decoction possesses anti-inflammatory, antioxidant, and gastric mucosal protective properties and has been applied in the treatment of various inflammatory conditions.

Objectives. This study aimed to investigate the effects of Erchen decoction on chronic ethanol-induced gastric injury.

Materials and methods. Seventy-two male Sprague Dawley rats were randomly assigned to the following groups: Con group (control), Mod group (model), OXY group (oxymatrine, 5 mL/kg), ECDH group (high-dose Erchen decoction, 10 mL/kg), ECDM group (medium-dose Erchen decoction, 5 mL/kg), and ECDL group (low-dose Erchen decoction, 2.5 mL/kg). Gastric histopathological changes were evaluated using hematoxylin and eosin (H&E) staining and transmission electron microscopy (TEM). Proliferation of gastric mucosal cells was assessed using immunohistochemical detection of proliferating cell nuclear antigen (PCNA). Serum levels of tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured using enzyme-linked immunosorbent assay (ELISA) and biochemical analysis. Western blot (WB) was used to analyze protein expression associated with inflammatory pathway activation.

Results. Compared with the Mod group, Erchen decoction significantly alleviated gastric injury and increased PCNA expression in the gastric mucosa. Its therapeutic efficacy decreased in a dose-dependent manner, with weaker effects observed at lower doses. Erchen decoction significantly reduced serum levels of TNF- α , IL-1 β , and MDA while enhancing SOD activity; however, these effects were less pronounced in the ECDL group than in the ECDM and ECDH groups. Importantly, Erchen decoction markedly decreased the expression of toll-like receptor 4 (TLR4), nuclear factor kappa-B (NF- κ B), NOD-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein (ASC), cleaved caspase-1, IL-1 β , and interleukin-18 (IL-18).

Conclusions. Erchen decoction alleviates ethanol-induced gastric injury by reducing inflammation, attenuating oxidative stress, preserving cellular structural integrity, and regulating the NF- κ B signaling pathway.

Key words: ethanol-induced gastric injury, gastric mucosal protection, NF- κ B signaling pathway, oxidative stress, TLR4/NLRP3 inflammasome

Highlights

- Erchen decoction attenuates chronic ethanol-induced gastric injury by improving gastric mucosal pathology, promoting gastric epithelial cell proliferation, reducing inflammatory cytokines (TNF- α , IL-1 β) and malondialdehyde (MDA), and enhancing superoxide dismutase (SOD) antioxidant activity.
- Erchen decoction suppresses key inflammatory signaling pathways, including TLR4/NF- κ B/NLRP3 inflammatory activation, by downregulating toll-like receptor 4 (TLR4), nuclear factor kappa-B (NF- κ B), NOD-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein(ASC), cleaved caspase-1, interleukin (IL)-1 β , and IL-18 expression.
- Regulation of the NF- κ B signaling pathway represents a central mechanism underlying the gastroprotective effects of Erchen decoction in chronic alcohol-induced gastric injury.
- Erchen decoction shows potential as a therapeutic strategy for the prevention and treatment of chronic alcohol-related gastric mucosal damage.

Background

Chronic ethanol-induced gastric injury is a common digestive system disorder. Chronic alcohol consumption can damage the gastric mucosa and increase the risk of developing severe gastrointestinal conditions such as gastritis, peptic ulcers, and potentially gastric cancer.^{1–3} Alcohol exerts a direct effect on the gastric mucosa, leading to oxidative stress and inflammation, which can impair its protective function and contribute to tissue damage. In addition, the inflammatory process is mediated by interactions among multiple cytokines and signaling pathways.^{4–6} Among these, the nuclear factor kappa-B (NF- κ B) pathway plays a central role in regulating the inflammatory response.^{7–9} Nuclear factor kappa-B is a transcription factor with a wide range of biological functions and regulates the expression of numerous inflammation-related genes. Under physiological conditions, NF- κ B remains inactive in the cytoplasm.^{10–12} External stimuli, including bacterial or viral infections and inflammatory signals, can activate NF- κ B, promoting its translocation to the nucleus and the subsequent initiation of the inflammatory response.^{13–15}

Despite advancements in conventional therapies such as proton pump inhibitors (e.g., omeprazole) and histamine 2 (H2) receptor antagonists, their prolonged use is associated with adverse effects, including gastrointestinal disturbances, and incomplete efficacy in mitigating ethanol-related mucosal injury.¹⁶ Therefore, it is important to explore novel therapeutic approaches for alleviating chronic alcohol-induced gastric injury.

Erchen decoction, a classic traditional Chinese medicine (TCM) formula, consists of *Pinelliae rhizoma*, *Citri exocarpium rubrum*, *Poria*, and *Zingiberis rhizoma recens*, and contains 123 active components, including baicalein, stigmasterol, quercetin, and β -sitosterol.¹⁷ Erchen decoction has traditionally been used to treat headaches and chest tightness attributed to exogenous wind-cold, internal injury, and dampness retention. It has also been widely used in the management of nonalcoholic fatty liver disease (NAFLD) and

chronic obstructive pulmonary disease.^{18,19} The therapeutic application of Erchen decoction in various inflammatory diseases is largely attributed to its anti-inflammatory and antioxidant properties.^{17,20} In recent years, with the increasing focus on the modernization of TCM, the potential therapeutic effects of Erchen decoction in chronic alcohol-induced gastric injury have attracted growing attention. Therefore, this study was undertaken to investigate the therapeutic effects of Erchen decoction in a rat model of chronic ethanol-induced gastric injury and to further explore its underlying mechanisms, thereby addressing existing mechanistic gaps in the current research on its effects in chronic gastric injury.

Advances in microscopy, particularly transmission electron microscopy (TEM), have revolutionized the morphological sciences by providing unprecedented levels of magnification and resolution for the examination of biological samples. Transmission electron microscopy enables detailed visualization of the ultrastructure and organization of cellular components, including the cytoskeleton, membrane systems, and organelles, thereby elucidating structural–functional relationships within cells and tissues.²¹ In the context of chronic alcohol-induced gastric injury, TEM has proven to be an invaluable tool for elucidating pathological alterations in gastric mucosal cells, including mitochondrial damage, endoplasmic reticulum stress, and disruption of cellular junctions. These ultrastructural changes are critical for understanding the underlying mechanisms of alcohol-induced gastric injury. Therefore, in this study, hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), TEM, biochemical assays, and western blot (WB) analysis were used to comprehensively evaluate and characterize the therapeutic effects and underlying mechanisms of Erchen decoction in alcohol-induced gastric injury.

Objectives

The study aims to investigate the effects of Erchen decoction on chronic ethanol-induced gastric injury.

Materials and methods

Animals

Seventy-two healthy male Sprague Dawley rats, aged 8 weeks and weighing approx. 170 g, were obtained from Henan Skbers Biotechnology Co., Ltd. (Zhengzhou, China). The rats were housed in a controlled environment at a room temperature of 20–24°C and relative humidity of 60–80%, with free access to food and water. The standard rat chow was purchased from Henan Huanyu Hekang Biotechnology Co., Ltd. (Zhengzhou, China). It consisted primarily of corn, soybean meal, bran meal, fish meal, macronutrients (vitamins), micronutrients (amino acids), mineral supplements (e.g., ferrous sulfate), edible oil, and salt (0.5% each). This study received ethical approval (No. GENINK-20230029; issued on October 8, 2023) from Tianjin Jinke Bona Biotechnology Co., Ltd. (Tianjin, China). All experiments were conducted in accordance with the Chinese Government's Guidelines for the Care and Use of Laboratory Animals to minimize animal pain and discomfort.²²

Laboratory reagent

Erchen decoction (Q/BJT 0002S-2020; *Pinelliae rhizoma* 15 g, *Citri exocarpium rubrum* 15 g, *Poria* 9 g, *Glycyrrhizae radix et rhizoma praeparata cum melle* 4.5 g, 7 pieces of *Zingiberis rhizoma recens*, 1 *Mume fructus*; Bozhou Bojiatang Biotechnology Co., Ltd., Bozhou,

China); oxymatrine (OXY) solution (1ST40311; Tianjin Alta Technology Co., Ltd., Tianjin, China); H&E staining solution (BA4041–BA4024; Zhuhai BaSO Biotechnology Co., Ltd., Zhuhai, China); anti-proliferating cell nuclear antigen (PCNA) antibody (AF0261; Shanghai Beyotime Biotech Inc., Shanghai, China); serum biochemical factor enzyme-linked immunosorbent assay (ELISA) kits (A001-3-2, A003-1-2; Nanjing Jiancheng Bioengineering Institute, Nanjing, China); Bradford protein quantification kit (P0006; Shanghai Beyotime Biotech Inc.); anti-interleukin-18 (IL-18) antibody (1:2,000; 10663-1-AP; Proteintech, Rosemont, USA); anti-interleukin-1 beta (IL-1 β) antibody (1:2,000; 16806-1-AP; Proteintech); anti-caspase-1 antibody (1:1,000; 24232S; Cell Signaling Technology (CST), Danvers, USA); anti-cleaved caspase-1 antibody (1:1,000; 89332S; CST); anti-toll-like receptor 4 (TLR4) antibody (1:1,000; 66350-1-Ig; Proteintech); anti-NF- κ B antibody (1:1,000; 10745-1-AP; Proteintech); anti-NOD-like receptor pyrin domain-containing protein 3 (NLRP3) antibody (1:2,000; 68102-1-Ig; Proteintech); and anti-apoptosis-associated speck-like protein containing a CARD (ASC) monoclonal antibody (1:5,000; 10500-1-AP; Proteintech).

Grouping

As shown in Table 1, the study design involved randomly dividing 72 rats into 6 groups: a control group (Con group; n = 12), a model group (Mod group; n = 12), a high-dose Erchen decoction group (ECDH group; n = 12), a medium-dose Erchen decoction group (ECDM group;

Table 1. Overview of experimental design

Group	Number of rats	Ethanol/saline regimen	Additional treatment	Dose and duration	Total study duration	Outcome assessments
Control (Con)	12	8 g/kg normal saline by gavage, once daily for 12 weeks	none	–	12 weeks (plus 1 week acclimatization)	At the end of week 12, rats were fasted overnight (approx. 15 h), then euthanized. Gastric tissues were harvested for: histopathology (H&E staining), IHC (PCNA), TEM, biochemical assays (SOD activity, MDA level, TNF- α , IL-1 β), and WB (inflammatory/apoptotic proteins: TLR4, NF- κ B, NLRP3, ASC, and caspase-1).
Model (Mod)	12	8 g/kg ethanol by gavage, once daily for 12 weeks	none	–	12 weeks (plus 1 week acclimatization)	
High-dose Erchen decoction (ECDH)	12	8 g/kg ethanol by gavage, once daily for 12 weeks	Erchen decoction (high dose: 10 mL/kg)	10 mL/kg/day, administered during the final 4 weeks of ethanol exposure	12 weeks (plus 1 week acclimatization)	
Medium-dose Erchen decoction (ECDM)	12	8 g/kg ethanol by gavage, once daily for 12 weeks	Erchen decoction (medium dose: 5 mL/kg)	5 mL/kg/day, administered during the final 4 weeks of ethanol exposure	12 weeks (plus 1 week acclimatization)	
Low-dose Erchen decoction (ECDL)	12	8 g/kg ethanol by gavage, once daily for 12 weeks	Erchen decoction (low dose: 2.5 mL/kg)	2.5 mL/kg/day, administered during the final 4 weeks of ethanol exposure	12 weeks (plus 1 week acclimatization)	
Oxymatrine (OXY)	12	8 g/kg ethanol by gavage, once daily for 12 weeks	oxymatrine solution (5 mL/kg)	5 mL/kg/day, administered during the final 4 weeks of ethanol exposure	12 weeks (plus 1 week acclimatization)	

H&E – hematoxylin and eosin; IHC – immunohistochemistry; PCNA – proliferating cell nuclear antigen; TEM – transmission electron microscopy; SOD – superoxide dismutase; MDA – malondialdehyde; TNF- α – tumor necrosis factor alpha; IL-1 β – interleukin-1 beta; WB – western blot; TLR4 – toll-like receptor 4; NF- κ B: nuclear factor kappa-B; NLRP3 – NOD-like receptor thermal protein domain-associated protein 3; ASC – apoptosis-associated speck-like protein.

n = 12), a low-dose Erchen decoction group (ECDL group; n = 12), and an oxymatrine group (OXY group; n = 12). After a 1-week acclimatization period, the Con group received 8 g·kg⁻¹ normal saline. Administration was performed 3 times daily for 12 weeks. Other groups received 8 g·kg⁻¹ ethanol by gavage for 12 weeks.²³

The Erchen decoction groups and the OXY group were treated daily during the final 4 weeks of ethanol exposure (weeks 9–12). The ECDH group received 10 mL·kg⁻¹ Erchen decoction, the ECDM group received 5 mL·kg⁻¹ Erchen decoction, and the ECDL group received 2.5 mL·kg⁻¹ Erchen decoction. The OXY group received 5 mL·kg⁻¹ OXY solution daily. The above dosages were calculated based on the adult daily dose (1 dose per day, 200 g per dose) and converted to the equivalent rat dose using a standard human body weight of 70 kg.²⁴ No significant toxicity of Erchen decoction has been reported in previous studies.^{25,26} According to common clinical dosages and relevant literature, the dosage used in this study complies with established safety standards for TCM.⁴ Animals were fasted overnight for 15 h on the final day of the experiment prior to anesthesia induction and blood sample collection.

Hematoxylin and eosin staining analysis

Rat gastric histopathology was assessed using H&E staining. To preserve tissue structure, gastric tissue samples were immersed in 4% paraformaldehyde for 24 h. After fixation, the samples were dehydrated through a graded ethanol series (70–100%) for approx. 1 h, followed by clearing in xylene. Following dehydration and clearing, the samples were embedded in paraffin and sectioned at a thickness of 4–5 μm. The sections were then dewaxed in xylene, rehydrated through decreasing concentrations of ethanol, and stained with H&E solutions for 3–5 min and 1–2 min, respectively. After staining, the sections were dehydrated again, cleared in xylene, and mounted with sealing medium and a coverslip. Pathological changes, including edema, hemorrhage, epithelial shedding, and inflammatory cell infiltration, were evaluated using light microscopy (model DP26; Olympus Corp., Tokyo, Japan).

Immunohistochemistry analysis

Gastric tissue samples were immersed in 4% formaldehyde for 24 h, dehydrated through a graded series of ethanol solutions, and subsequently embedded in paraffin. The paraffin blocks were sectioned at a thickness of 4–5 μm and mounted onto glass slides. The sections were dewaxed in xylene and rehydrated through decreasing concentrations of ethanol. For antigen retrieval, the sections were heated under high pressure in citrate buffer for 20 min.

The samples were subsequently incubated in a blocking solution containing 5% normal serum and 1% bovine

serum albumin (BSA) for 30 min to reduce nonspecific binding. The sections were then incubated overnight at 4°C with a primary antibody against proliferating cell nuclear antigen (PCNA). After washing, the sections were treated with a secondary antibody for 1 h. The chromogenic reaction between the enzyme-labeled secondary antibody and its substrate (typically 5–10 min) was used to visualize antibody binding. Subsequently, hematoxylin was used for nuclear counterstaining, followed by clearing in xylene, and the sections were mounted with a coverslip. PCNA expression in the nuclei was observed and quantitatively analyzed under a light microscope (model DP26; Olympus Corp.) to assess cell proliferation in gastric tissue.

Transmission electron microscopy observation

Gastric tissue was excised and cut into 1 mm³ pieces, immediately fixed in 2.5% glutaraldehyde followed by 1% osmium tetroxide for 2 h, and then treated with 1% tannic acid for 30 min. The samples were subsequently dehydrated through a graded ethanol series (50–100%), with each step lasting 15 min.

After dehydration, the tissue was infiltrated with acrylic resin by incubation in a mixture of resin and ethanol for 2 h, followed by pure resin overnight, and then subjected to thermal polymerization and embedding in a mold. The cured resin blocks were subsequently sectioned into ultrathin sections (60–90 nm), mounted on copper grids, and stained with lead uranyl acetate for 10 min to enhance contrast. The sections were then placed in the TEM sample holder, and images were acquired for quantitative and qualitative analysis (model HT7800; Hitachi Ltd., Tokyo, Japan).

Serum biochemical detection

The manufacturer's instructions were followed for reagent preparation and microplate handling using the pre-made ELISA kits. Diluted serum samples and standards were added to the microplate wells, incubated for 1–2 h, and then washed with wash buffer. An enzyme-conjugated secondary antibody was added and incubated, followed by washing and the addition of substrate solution. After 15–30 min, a stop solution was added to terminate the reaction. Absorbance was measured at 450 nm to determine the concentrations of inflammatory markers.

For the determination of superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels, serum samples were added to the prepared reaction mixtures, and absorbance or fluorescence intensity was measured based on the ability of SOD to inhibit substrate oxidation and on the formation of the colored product generated by the reaction of MDA with thiobarbituric acid. Finally, SOD activity and MDA concentrations were calculated by comparison with the standard curve.

Western blot analysis

Sample proteins were extracted using radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitors, followed by centrifugation at 4°C for 10 min to remove insoluble material. A Bradford protein quantification kit (P0006; Shanghai Beyotime Biotech Inc.) was used to determine protein concentration and ensure equal loading onto the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gel.

The samples were mixed with 5 volumes of loading buffer, boiled for 5 min to achieve denaturation, and then separated by electrophoresis on a 10% SDS–PAGE gel. The separated proteins were transferred onto a polyvinylidene difluoride (PVDF) or nitrocellulose membrane, followed by wet transfer at 100 V for 1–2 h. After blocking in Tris-buffered saline with Tween (TBST) containing 5% skim milk for 1 h, the membranes were incubated with specific primary antibodies, followed by horseradish peroxidase (HRP)-conjugated secondary antibodies.

The membranes were incubated with primary antibodies overnight and with secondary antibodies for 1 h. Finally, an enhanced chemiluminescence (ECL) detection system (GD50202; Monad Biotech. Co. Ltd., Wuhan, China) was used to visualize the signals, and the expression levels of each target protein were quantitatively analyzed based on band intensity.

Statistical analyses

We used IBM SPSS Statistics for Windows v. 22 (IBM Corp., Armonk, USA). Data (n = 3 or n = 9) are presented as the min–max range or as the median (1st and 3rd quartile (Q1, Q3)). The data were derived from 3 or 9 independent

replications of a given experiment, and only the aggregate scores in Tables 2,3 represent the sum of scores for sub-indicators. For multiple comparisons involving variability across all groups, the nonparametric Kruskal–Wallis test (n = 3 or n = 9) was used for between-group comparisons, followed by Dunn’s test for planned pairwise comparisons.

When the global p-value in a Kruskal–Wallis test is non-significant, pairwise comparisons are not required. However, between-group comparison tests were not performed if any group had all values equal to a constant (n = 3). In cases where only 1 group had constant values (n = 3), 2 groups were directly compared using a one-sample non-parametric Wilcoxon signed-rank test. When both groups showed no variability (n = 3), standard statistical tests were not applied and the results were reported descriptively. In contrast, when both groups showed variation (n = 3), comparisons were performed using the Mann–Whitney U test. The p-values were corrected using the Bonferroni method for both multiple Kruskal–Wallis tests and multiple Dunn’s tests. Statistical significance was defined as p < 0.05.

Results

Erchen decoction regulates pathological injury of gastric tissue

A comparison of the therapeutic effects of different doses of Erchen decoction based on macroscopic images of ethanol-induced gastric injury in rats showed that gastric tissue in the Con group exhibited no obvious lesions, with uniform coloration and intact structure. The scores for gastric epithelial erosion, ulceration, and hemorrhage were nearly 0, indicating no ethanol-induced damage. In contrast, gastric

Table 2. Score of ethanol-induced gastric injury in rats

Group	Gastric epithelial erosion	Gastric epithelial ulcer	Gastric epithelial hemorrhage focus	Aggregate score
Con	0	0	0–1	0–1
Mod	5	0–5	0–5	10–13*
OXY	0	0	2–4	2–4
ECDH	0	2	1–5	3–7
ECDM	0–5	0–2	2–5	5–9
ECDL	3–5	2–4	2–4	9–11

The lower the score, the less severe the injury.

Gastric epithelial erosion: All samples in Con group scored 0, all samples in Mod group scored 5, and all samples in group OXY and ECDH group scored 0, so no standard statistical test was applied for comparison between these 2 groups due to absence of variability; One-sample nonparametric Wilcoxon signed-rank test: $Z_{Mod\ vs\ ECDM} = 0.000$, $p_{Mod\ vs\ ECDM} = 0.317$; $Z_{Mod\ vs\ ECDL} = 0.000$, $p_{Mod\ vs\ ECDL} = 0.317$.

Gastric epithelial ulcer: one-sample nonparametric Wilcoxon signed-rank test: $Z_{Con\ vs\ Mod} = 3.000$, $p_{Con\ vs\ Mod} = 0.180$; $Z_{OXY\ vs\ Mod} = 3.000$, $p_{OXY\ vs\ Mod} = 0.180$; $Z_{ECDH\ vs\ Mod} = 4.000$, $p_{ECDH\ vs\ Mod} = 0.593$. Mann–Whitney U test: $U_{Mod\ vs\ ECDM} = 2.000$, $p_{Mod\ vs\ ECDM} = 0.400$; $U_{Mod\ vs\ ECDL} = 4.500$, $p_{Mod\ vs\ ECDL} > 0.999$.

Gastric epithelial hemorrhage focus: Kruskal–Wallis test: $H = 5.430$, $df = 5.000$, $Adj.p = 0.732$. Aggregate score: Kruskal–Wallis test: $H = 15.492$, $df = 5.000$, $Adj.p = 0.016$. Dunn’s test: $Z_{Con\ vs\ Mod} = -13.667$, * $Adj.p_{Mod\ vs\ Con} = 0.010$; $Z_{OXY\ vs\ Mod} = 10.333$, $Adj.p_{OXY\ vs\ Mod} = 0.085$; $Z_{ECDH\ vs\ Mod} = 7.333$, $Adj.p_{ECDH\ vs\ Mod} = 0.455$; $Z_{Mod\ vs\ ECDM} = 5.167$, $Adj.p_{Mod\ vs\ ECDM} > 0.999$; $Z_{Mod\ vs\ ECDL} = 0.500$, $Adj.p_{Mod\ vs\ ECDL} > 0.999$.

*represents statistical significance compared with Con group. Sample n = 3; p-value was adjusted using Bonferroni correction for multiple Kruskal–Wallis tests (n = 2) and multiple Dunn’s post hoc tests (n = 5).

Adj.p – adjusted p-value; Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – middle-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group; Z – z-score/standard score; p – probability value; U – U-statistic; H – H-index; df – degrees of freedom.

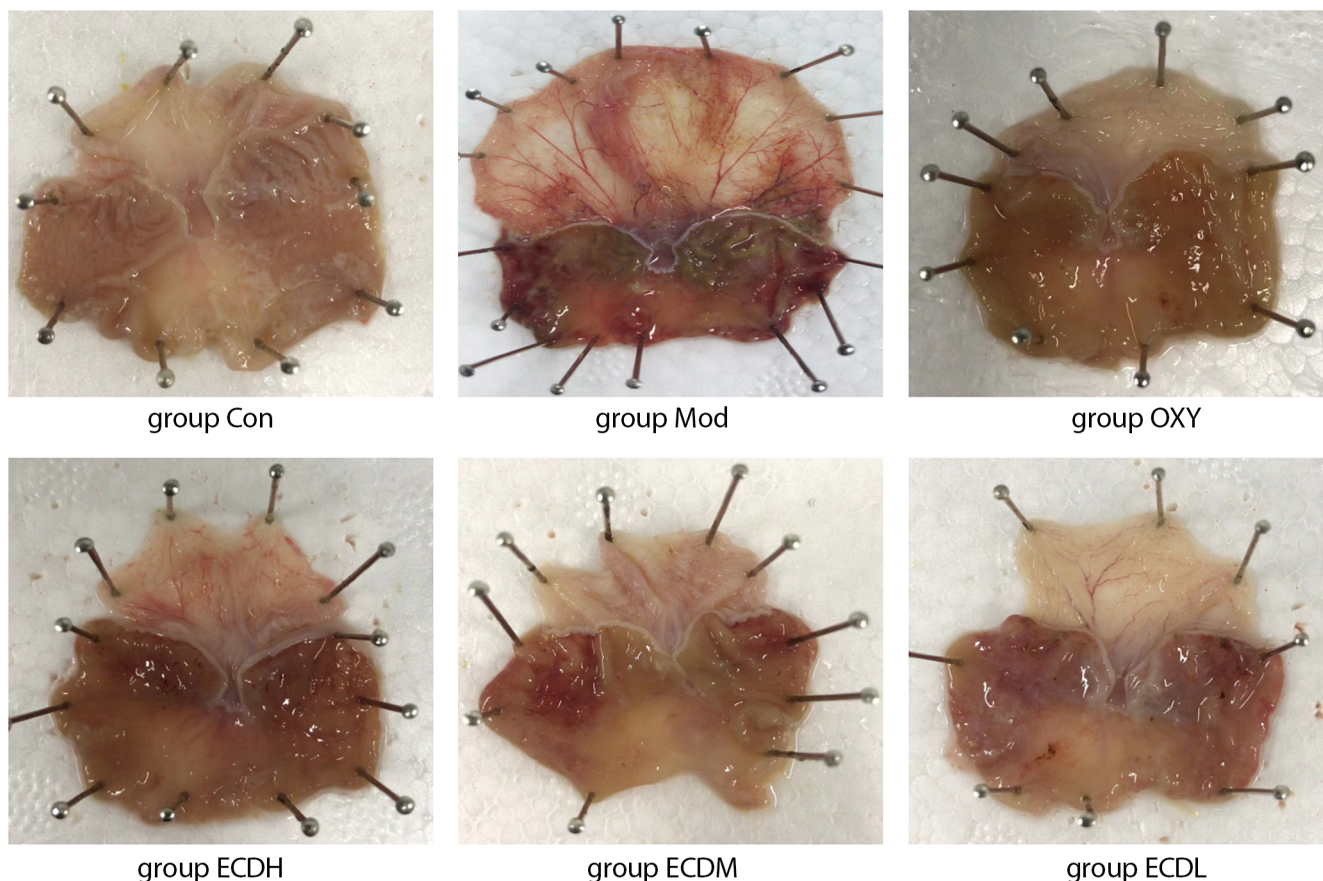


Fig. 1. Gastric tissue status in different treatment groups (n = 3)

Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group.

tissue in the Mod group displayed marked red hemorrhagic areas, including epithelial erosion, ulceration, and focal bleeding lesions. The Mod group had significantly higher aggregate scores than the Con group (Dunn's test: $Z_{\text{Mod vs Con}} = -13.667$, adjusted $p_{\text{Mod vs Con}} = 0.010$), indicating severe damage induced by 50% ethanol. In the OXY group, gastric tissue damage was mild, hemorrhage was reduced, and the aggregate gastric injury score was lower than that in the Mod group, suggesting that OXY treatment may attenuate ethanol-induced injury. The severity of gastric tissue damage varied among the Erchen decoction dosage groups, with the ECDH group exhibiting the mildest damage, followed by the ECDM group. Compared with the ECDH and ECDM groups, the ECDL group exhibited more severe damage; however, relative to the Mod group, the ECDL group showed improvement, with lower scores for gastric mucosal and epithelial injury. These results indicate that Erchen decoction protects against ethanol-induced gastric injury in a dose-dependent manner (Fig. 1, Table 2).

Erchen decoction regulates gastric injury in a dose-dependent manner

The results demonstrated the gastric tissue status across different treatment groups (Fig. 2, Table 3). In the Con

group, the overall gastric architecture was essentially normal: the mucosal layer was intact, the glands were neatly arranged, no epithelial shedding, atrophy, edema, or congestion was observed, and no abnormalities were detected in the submucosa or muscularis. Compared with the Con group, the Mod group exhibited an irregular mucosal layer, disorganized glandular arrangement, mucosal epithelial atrophy, edema and congestion, and submucosal edema accompanied by inflammatory cell infiltration.

The Mod group exhibited an abnormal overall gastric architecture, with a significantly higher aggregate score than the Con group (Dunn's test: $Z_{\text{Mod vs Con}} = -14.667$, adjusted $p_{\text{Mod vs Con}} = 0.005$). Compared with the Mod group, both the OXY and ECDH groups showed improvement in ethanol-induced gastric injury. The general organization of the gastric tissue was largely preserved, with a slightly irregular mucosal layer, relatively well-organized glands, mild mucosal epithelial swelling and hyperplasia with congestion, and limited inflammatory cell infiltration in the submucosa. A reduction in the dose of Erchen decoction was associated with a gradual decrease in therapeutic efficacy against ethanol-induced gastric injury.

Table 3. Score of gastric injury in rats

Group	Mucosa cell edema	Mucosal cell bleeding	Mucosal cell shedding	Inflammatory infiltration	Aggregate score
Con	0	0	0–1	0	0–1
Mod	3	3–4	1–2	1–2	8–11*
OXY	1	1	0–1	1	3–4
ECDH	1	1	0–1	1	3–4
ECDM	1–2	2–3	0–1	0–1	4–5
ECDL	2–3	2–3	1–2	1	6–8

The lower the score, the less severe the injury.

Mucosa cell edema: All samples in Con group scored 0, all samples in the Mod group scored 3, and all samples in the OXY and ECDH groups scored 1, so no standard statistical test was applied for comparison between these 2 groups due to absence of variability; One-sample nonparametric Wilcoxon signed-rank test: $Z_{Mod\ vs\ ECDM} = 0.000$, $p_{Mod\ vs\ ECDM} = 0.102$; $Z_{Mod\ vs\ ECDL} = 0.000$, $p_{Mod\ vs\ ECDL} = 0.157$.

Mucosal cell bleeding: One-sample nonparametric Wilcoxon signed-rank test: $Z_{Con\ vs\ Mod} = 6.000$, $p_{Con\ vs\ Mod} = 0.102$; $Z_{OXY\ vs\ Mod} = 6.000$, $p_{OXY\ vs\ Mod} = 0.102$; $Z_{ECDH\ vs\ Mod} = 6.000$, $p_{ECDH\ vs\ Mod} = 0.102$. Mann–Whitney U test: $U_{Mod\ vs\ ECDM} = 0.500$, $p_{Mod\ vs\ ECDM} = 0.100$; $U_{Mod\ vs\ ECDL} = 1.000$, $p_{Mod\ vs\ ECDL} = 0.200$.

Mucosal cell shedding: Kruskal–Wallis test: $H = 7.942$, $df = 5.000$, $Adj.p = 0.318$.

Inflammatory infiltration: One-sample nonparametric Wilcoxon signed-rank test: $Z_{Con\ vs\ Mod} = 6.000$, $p_{Con\ vs\ Mod} = 0.102$; $Z_{OXY\ vs\ Mod} = 3.000$, $p_{OXY\ vs\ Mod} = 0.157$; $Z_{ECDH\ vs\ Mod} = 3.000$, $p_{ECDH\ vs\ Mod} = 0.157$. Mann–Whitney U test: $U_{Mod\ vs\ ECDM} = 0.500$, $p_{Mod\ vs\ ECDM} = 0.100$. One-sample nonparametric Wilcoxon signed-rank test: $Z_{Mod\ vs\ ECDL} = 3.000$, $p_{Mod\ vs\ ECDL} = 0.157$. Aggregate score: Kruskal–Wallis test: $H = 15.883$, $df = 5.000$, $Adj.p = 0.014$. Dunn’s test: $Z_{Con\ vs\ Mod} = -14.667$,

* $Adj.p_{Mod\ vs\ Con} = 0.005$; $Z_{OXY\ vs\ Mod} = 10.500$, $Adj.p_{OXY\ vs\ Mod} = 0.075$; $Z_{ECDH\ vs\ Mod} = 9.333$, $Adj.p_{ECDH\ vs\ Mod} = 0.150$; $Z_{Mod\ vs\ ECDM} = 6.167$, $Adj.p_{Mod\ vs\ ECDM} = 0.765$; $Z_{Mod\ vs\ ECDL} = 2.333$, $Adj.p_{Mod\ vs\ ECDL} > 0.999$.

*represents statistical significance compared with Con group. Sample $n = 3$; p-value was adjusted by Bonferroni correction for multiple Kruskal–Wallis tests ($n = 2$) and multiple Dunn’s tests ($n = 5$). $Adj.p$ means adjusted p-value.

$Adj.p$ – adjusted p-value; Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group; Z – z-score/standard score; p – probability value; U – U-statistic; H – H-index; df – degrees of freedom.

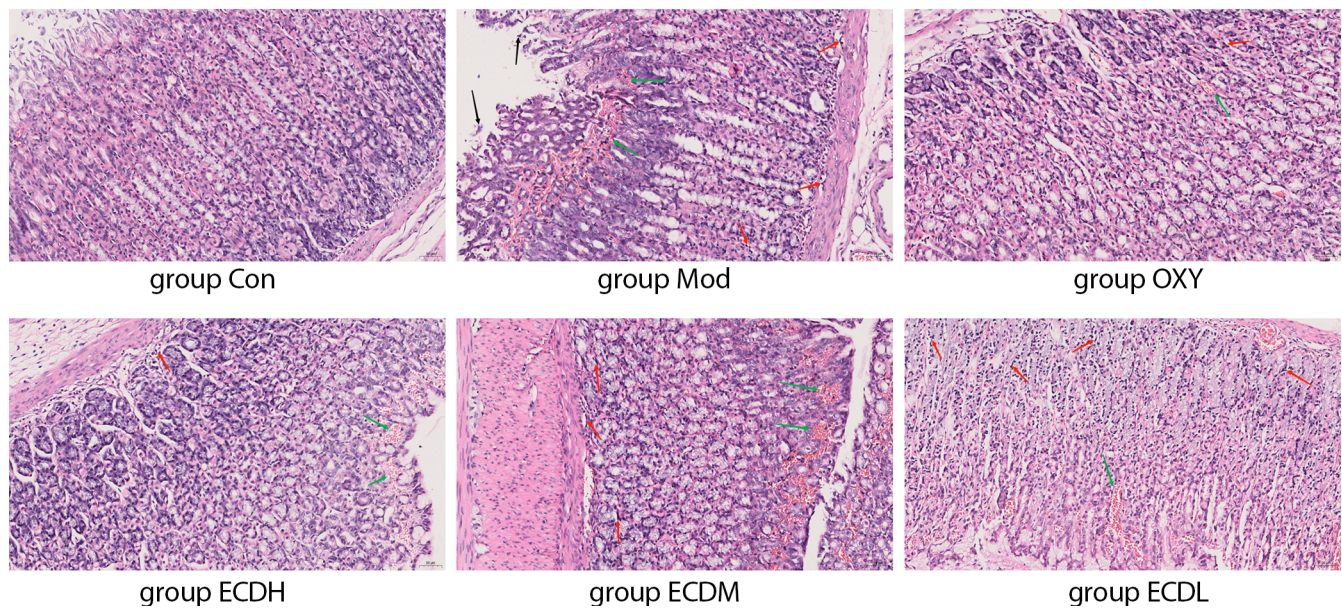


Fig. 2. Hematoxylin and eosin (H&E) staining of rat gastric tissue. Black arrows indicate mucosal epithelial shedding; green arrows indicate inflammatory cell infiltration; red arrows indicate mucosal edema and hemorrhage ($n = 3$)

Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group.

Erchen decoction promotes the proliferation of gastric injured cells

Cells in the Con group exhibited strong PCNA staining, indicating normal proliferative activity. In the Mod group, staining intensity was significantly reduced compared with the Con group (Dunn’s test: $Z_{Con\ vs\ Mod} = 14.667$, adjusted $p_{Con\ vs\ Mod} = 0.005$), suggesting that ethanol-induced gastric injury may suppress cell proliferation. In contrast

to the Mod group, the OXY group demonstrated significantly stronger staining (Dunn’s test: $Z_{OXY\ vs\ Mod} = -12.333$, adjusted $p_{OXY\ vs\ Mod} = 0.025$), indicating that OXY treatment may promote the proliferation of damaged cells. Compared with the Mod group, PCNA staining in the Erchen decoction groups increased in a dose-dependent manner. The ECDH group exhibited the most intense staining and highest proliferative activity, whereas the ECDL group showed the lowest proliferative activity (Fig. 3).

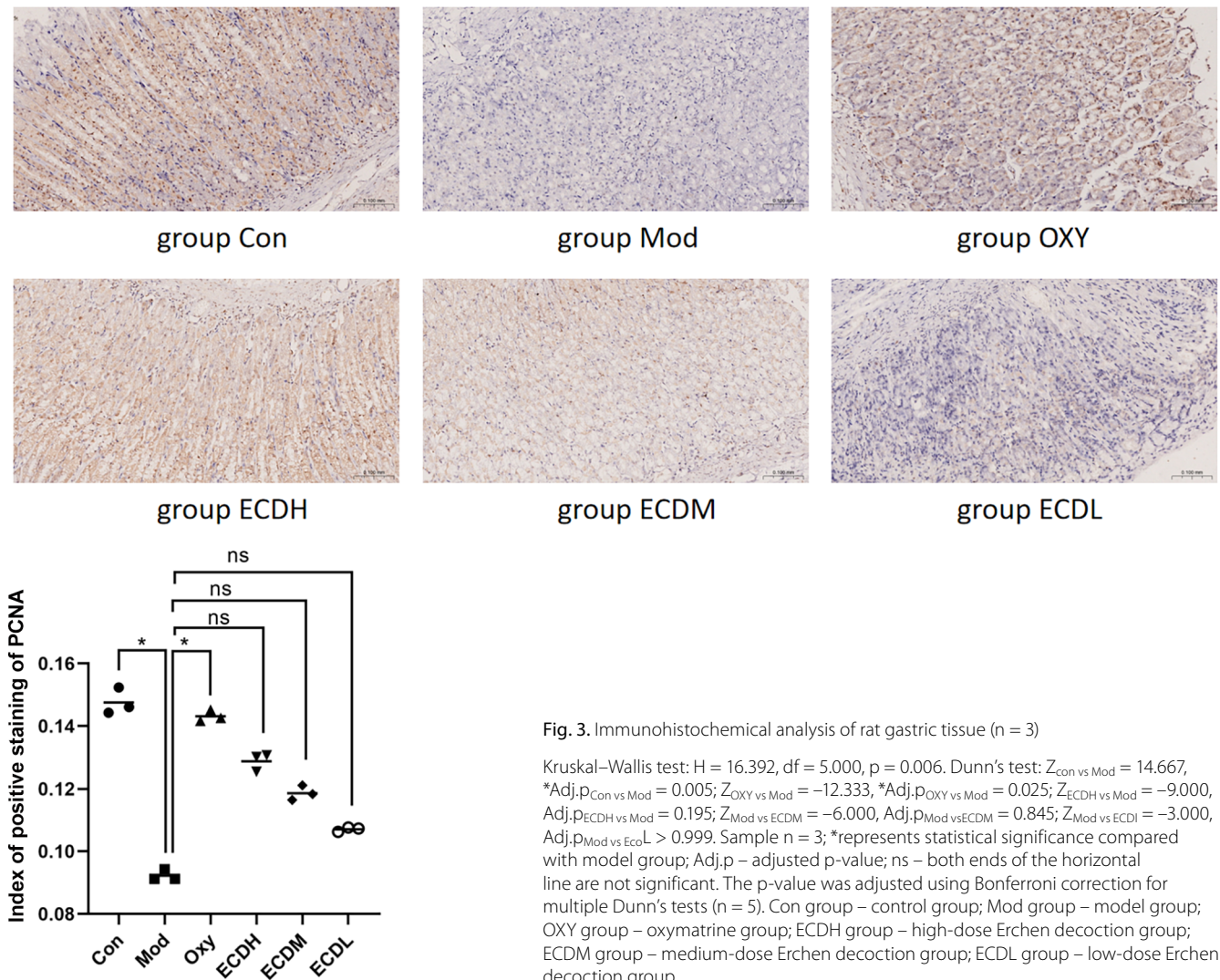


Fig. 3. Immunohistochemical analysis of rat gastric tissue (n = 3)

Kruskal–Wallis test: $H = 16.392$, $df = 5,000$, $p = 0.006$. Dunn's test: $Z_{Con vs Mod} = 14.667$, $*Adj.p_{Con vs Mod} = 0.005$; $Z_{OXY vs Mod} = -12.333$, $*Adj.p_{OXY vs Mod} = 0.025$; $Z_{ECDH vs Mod} = -9.000$, $Adj.p_{ECDH vs Mod} = 0.195$; $Z_{Mod vs ECDM} = -6.000$, $Adj.p_{Mod vs ECDM} = 0.845$; $Z_{Mod vs ECDL} = -3.000$, $Adj.p_{Mod vs EcoL} > 0.999$. Sample $n = 3$; *represents statistical significance compared with model group; Adj.p – adjusted p-value; ns – both ends of the horizontal line are not significant. The p-value was adjusted using Bonferroni correction for multiple Dunn's tests ($n = 5$). Con group – control group; Mod group – model group; OXY group – oxytetracycline group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group.

Erchen decoction improved the structure of gastric damaged cells

The Con group exhibited normal gastric ultrastructure, including well-defined mitochondria and intact cell membranes. Compared with the Con group, the Mod group showed marked mitochondrial swelling and cell membrane damage, indicating inflammation and cellular injury. The OXY and Erchen decoction groups demonstrated attenuation of these pathological changes, particularly in the ECDH group, where cellular architecture was substantially improved compared with the Mod group. Although the ECDM and ECDL groups showed partial structural improvement, these effects were less pronounced than those observed in the ECDH group (Fig. 4).

Erchen decoction inhibited the expression of inflammatory factors

Compared with the Con group, the Mod group exhibited significantly higher levels of tumor necrosis factor

alpha (TNF- α ; Dunn's test: $Z_{Mod vs Con} = -44.500$, adjusted $p_{Mod vs Con} < 0.001$) and IL-1 β (IL-1 β ; Dunn's test: $Z_{Mod vs Con} = -42.778$, adjusted $p_{Mod vs Con} < 0.001$), indicating that ethanol administration induced a pronounced inflammatory response. In the OXY group, the levels of these 2 inflammatory markers were comparable to those in the Con group but significantly lower than those in the Mod group (Dunn's test for TNF- α : $Z_{OXY vs Mod} = 34.500$, adjusted $p_{OXY vs Mod} < 0.001$; Dunn's test for IL-1 β : $Z_{OXY vs Mod} = 34.944$, adjusted $p_{OXY vs Mod} < 0.001$), suggesting a significant anti-inflammatory effect of OXY treatment.

Compared with the Mod group, the ECDH group exhibited significantly reduced levels of TNF- α (Dunn's test: $Z_{ECDH vs Mod} = 28.000$, adjusted $p_{ECDH vs Mod} < 0.001$) and IL-1 β (Dunn's test: $Z_{ECDH vs Mod} = 27.111$, adjusted $p_{ECDH vs Mod} = 0.001$), indicating that Erchen decoction decreased inflammation, with a more pronounced effect at higher doses. Compared with the Mod group, the ECDM group showed significantly lower IL-1 β levels (Dunn's test: $Z_{Mod vs ECDM} = 21.167$, adjusted $p_{Mod vs ECDM} = 0.020$) and intermediate TNF- α levels, whereas the ECDL group

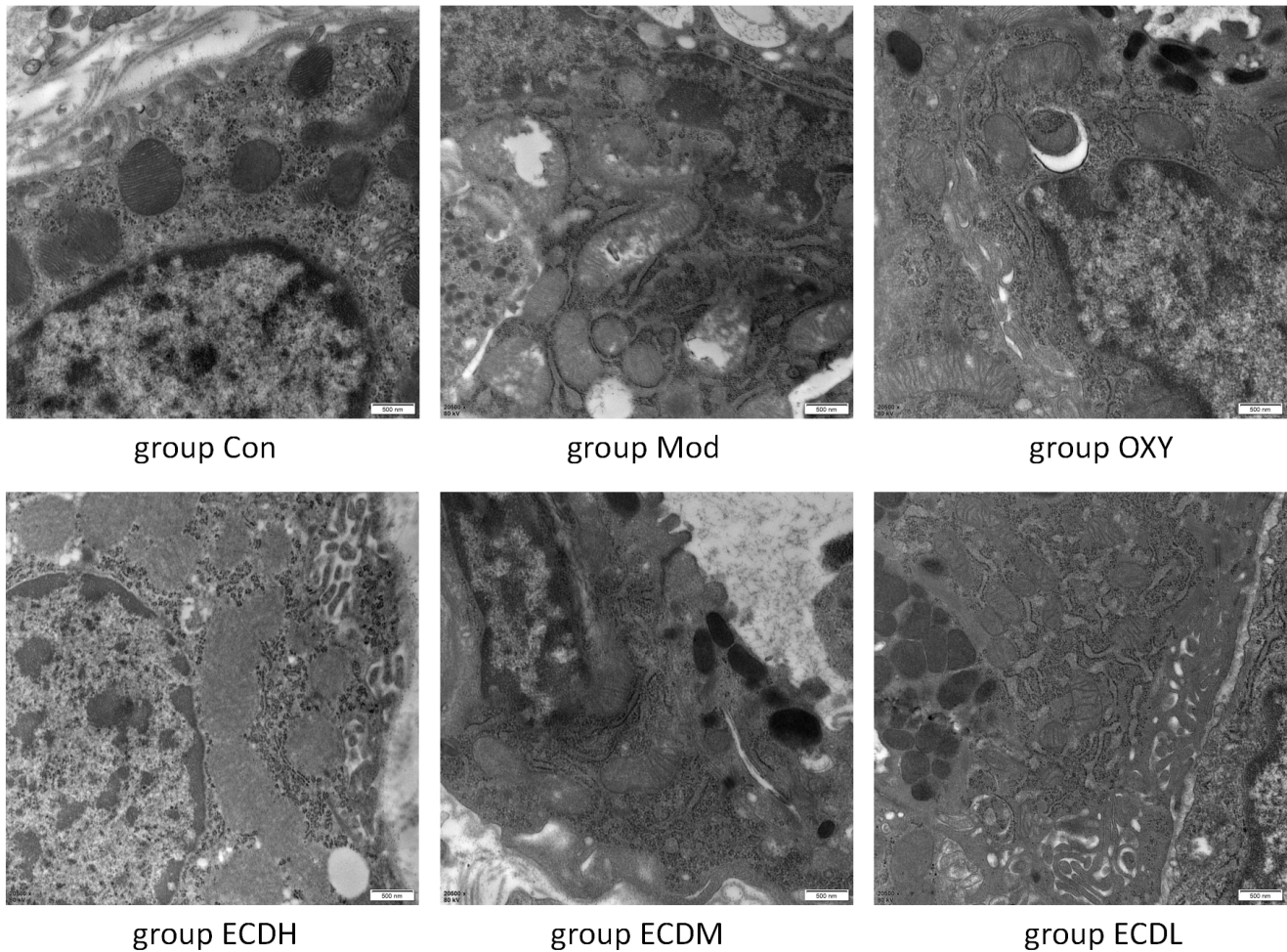


Fig. 4. Ultrastructural morphology of gastric tissue cells in different treatment groups observed by transmission electron microscopy (TEM). The scale bar represents 500 nm (x5,000 magnification; n = 3)

Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group.

Table 4. Expression of TNF-α and IL-1β detected with enzyme-linked immunosorbent assay (ELISA)

Group	TNF-α (pg/mL)	IL-1β (pg/mL)
Con	13.7617 (13.3233, 13.9506)	42.3 (41.44, 43.19)
Mod	91.685 (90.6436, 93.0133)*	168.48 (164.78, 170.61)*
OXY	14.9689 (14.5849, 15.2907) ^Δ	43.56 (42.48, 44.3) ^Δ
ECDH	16.3317 (16.2662, 16.7924) ^Δ	44.49 (44.11, 45.06) ^Δ
ECDM	18.1257 (17.7901, 18.4629)	45.45 (44.68, 45.84) ^Δ
ECDL	44.3981 (43.3273, 46.056)	86.38 (83.57, 89.27)

TNF-α: Kruskal–Wallis test: H = 49.422, df = 5.000, *Adj.p < 0.001. Dunn’s test: Z_{Mod vs Con} = -44.500, Adj.p_{Mod vs Con} < 0.001; Z_{OXY vs Mod} = 34.500, ^ΔAdj.p_{OXY vs Mod} < 0.001; Z_{ECDH vs Mod} = 28.000, ^ΔAdj.p_{ECDH vs Mod} < 0.001; Z_{Mod vs ECDM} = 19.000, Adj.p_{Mod vs ECDM} = 0.050; Z_{Mod vs ECDL} = 9.000, Adj.p_{Mod vs ECDL} > 0.999.
 IL-1β: Kruskal–Wallis test: H = 46.492, df = 5.000, Adj.p < 0.001. Dunn’s test: Z_{Mod vs Con} = -42.778, *Adj.p_{Mod vs Con} < 0.001; Z_{OXY vs Mod} = 34.944, ^ΔAdj.p_{OXY vs Mod} < 0.001; Z_{ECDH vs Mod} = 27.111, ^ΔAdj.p_{ECDH vs Mod} = 0.001; Z_{Mod vs ECDM} = 21.167, ^ΔAdj.p_{Mod vs ECDM} = 0.020; Z_{Mod vs ECDL} = 9.000, Adj.p_{Mod vs ECDL} > 0.999.
 *represents statistical significance compared with Con group; ^Δrepresents statistical significance compared with Mod group. Sample n = 9; p-value was adjusted by Bonferroni correction for multiple Kruskal–Wallis tests (n = 2) and multiple Dunn’s tests (n = 5). Adj.p – adjusted p-value; Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group; TNF-α – tumor necrosis factor alpha; IL-1β – interleukin-1 beta; Z – z-score/standard score; H – H-index; df – degrees of freedom.

demonstrated intermediate levels of inflammatory markers. These findings suggest a mild anti-inflammatory effect

at low and medium doses, which was less pronounced than that observed in the high-dose group (Table 4).

Table 5. SOD activity and MDA detection of serum oxidative stress markers

Group	SOD (U/mL)	MDA (nmol/mL)
Con	17.77 (17.67, 17.93)	2.19 (1.81, 3.23)
Mod	8.91 (8.69, 9.32)*	6.71 (5.81, 7.23)*
OXY	16.54 (15.57, 16.81) ^Δ	3.1 (1.94, 3.36) ^Δ
ECDH	15.85 (15.61, 16.26) ^Δ	2.84 (2.58, 2.84) ^Δ
ECDM	15.61 (15.59, 15.73) ^Δ	2.71 (2.58, 3.23) ^Δ
ECDL	11.88 (11.72, 12.32)	4.52 (4.26, 4.9)

SOD: Kruskal–Wallis test: $H = 46.510$, $df = 5.000$, $Adj.p < 0.001$. Dunn's test: $Z_{Mod vs Con} = 45.000$, * $Adj.p_{Mod vs Con} < 0.001$; $Z_{OXY vs Mod} = -30.111$, $^{\Delta}Adj.p_{OXY vs Mod} < 0.001$; $Z_{ECDH vs Mod} = -27.556$, $^{\Delta}Adj.p_{ECDH vs Mod} = 0.001$; $Z_{Mod vs ECDM} = -23.333$, $^{\Delta}Adj.p_{Mod vs ECDM} = 0.010$; $Z_{Mod vs ECDL} = -9.000$, $Adj.p_{Mod vs ECDL} > 0.999$. MDA: Kruskal–Wallis test: $H = 36.215$, $df = 5.000$, $Adj.p < 0.001$. Dunn's test: $Z_{Mod vs Con} = -34.833$, * $Adj.p_{Mod vs Con} < 0.001$; $Z_{OXY vs Mod} = 31.222$, $^{\Delta}Adj.p_{OXY vs Mod} < 0.001$; $Z_{ECDH vs Mod} = 30.444$, $^{\Delta}Adj.p_{ECDH vs Mod} < 0.001$; $Z_{Mod vs ECDM} = 28.611$, $^{\Delta}Adj.p_{Mod vs ECDM} < 0.001$; $Z_{Mod vs ECDL} = 9.889$, $Adj.p_{Mod vs ECDL} = 0.910$. *represents statistical significance compared with Con group; $^{\Delta}$ represents statistical significance compared with Mod group. Sample $n = 9$; p-value was adjusted using Bonferroni correction for multiple Kruskal–Wallis tests ($n = 2$) and multiple Dunn's tests ($n = 5$). Adj.p – adjusted p-value; Con group – control group; Mod group – model group; OXY group – oxytmatine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group; TNF- α – tumor necrosis factor alpha; IL-1 β – interleukin-1 beta; SOD – superoxide dismutase; MDA – malondialdehyde; Z – z-score/standard score; H – H-index; df – degrees of freedom.

Erchen decoction enhanced the anti-oxidation of cells

Superoxide dismutase activity in the Mod group was significantly lower than that in the Con group (Dunn's test: $Z_{Mod vs Con} = 45.000$, adjusted $p_{Mod vs Con} < 0.001$), whereas MDA levels were significantly higher (Dunn's test: $Z_{Mod vs Con} = -34.833$, adjusted $p_{Mod vs Con} < 0.001$). These findings indicate that ethanol induced pronounced oxidative stress, reduced antioxidant capacity, and increased lipid peroxidation. The OXY, ECDH, and ECDM groups exhibited significantly higher SOD activity than the Mod group (Dunn's test: $Z_{OXY vs Mod} = -30.111$, adjusted $p_{OXY vs Mod} < 0.001$; $Z_{ECDH vs Mod} = -27.556$, adjusted $p_{ECDH vs Mod} = 0.001$; $Z_{Mod vs ECDM} = -23.333$, adjusted $p_{Mod vs ECDM} = 0.010$), along with significantly lower MDA levels (Dunn's test: $Z_{OXY vs Mod} = 31.222$, adjusted $p_{OXY vs Mod} < 0.001$; $Z_{ECDH vs Mod} = 30.444$, adjusted $p_{ECDH vs Mod} < 0.001$; $Z_{Mod vs ECDM} = 28.611$, adjusted $p_{Mod vs ECDM} < 0.001$). These results suggest that these interventions enhanced antioxidant defenses and attenuated oxidative damage. In the Erchen decoction treatment groups, SOD activity in the ECDH and ECDM groups was comparable to that in the Con group, and MDA levels were similarly low, indicating favorable antioxidant effects. Superoxide dismutase activity in the ECDL group also improved; however, the effect was less pronounced than that observed in the ECDH and ECDM groups (Table 5).

Erchen decoction inhibited the expression of inflammation-related proteins

The expression levels of the inflammation- and apoptosis-related proteins TLR4, NF- κ B, NLRP3, and ASC were analyzed with WB. Compared with the Con group,

the Mod group exhibited higher expression levels of these proteins. In contrast, protein expression in the OXY group was reduced relative to the Mod group, indicating that OXY treatment effectively alleviated ethanol-induced inflammatory activation. Similarly, protein levels in the 3 Erchen decoction-treated groups were lower than those in the Mod group, suggesting that Erchen decoction exerts anti-inflammatory effects (Fig. 5).

Erchen decoction inhibited the expression of proteins related to the activation pathway of inflammatory cells

Western blot analysis demonstrated higher expression of proteins involved in inflammatory pathway activation in the Mod group compared with the Con group, including significantly elevated levels of IL-18 (Dunn's test: $Z_{Con vs Mod} = -14.667$, adjusted $p_{Con vs Mod} = 0.005$) and IL-1 β (Dunn's test: $Z_{Con vs Mod} = -14.333$, adjusted $p_{Con vs Mod} = 0.005$). These findings suggest that ethanol exposure induced a pronounced inflammatory response. Compared with the Mod group, the OXY group exhibited reduced levels of cleaved caspase-1, IL-1 β , and IL-18, indicating that OXY treatment suppressed inflammatory activation and associated cell signaling. The levels of cleaved caspase-1, IL-1 β , and IL-18 were lower in the ECDH, ECDM, and ECDL groups than in the Mod group. Notably, the reduction in IL-1 β levels between the ECDH and Mod groups was statistically significant (Dunn's test: $Z_{ECDH vs Mod} = 11.333$, adjusted $p_{ECDH vs Mod} = 0.045$). Although no statistically significant differences were observed among the other Erchen decoction-treated groups, the overall trend was consistent, suggesting that Erchen decoction exerted a dose-dependent suppressive effect on inflammation, with the highest dose (ECDH) showing the most pronounced effect (Fig. 6).

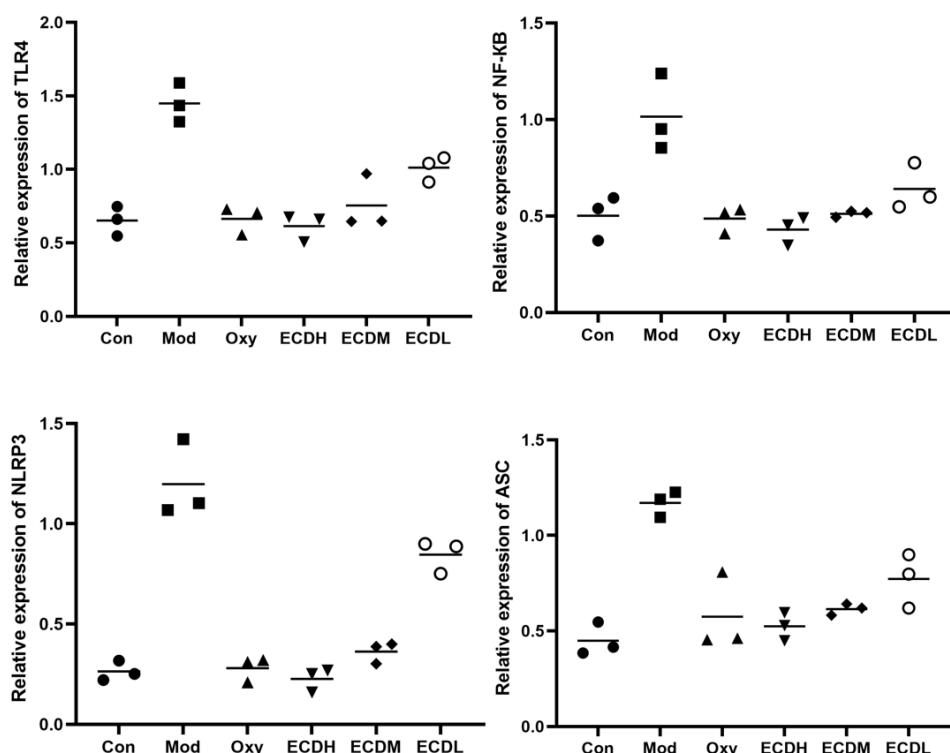
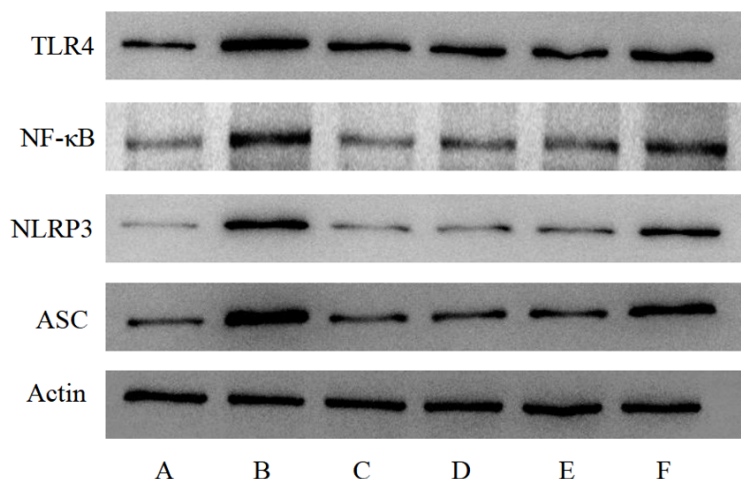


Fig. 5. Western blot analysis of inflammation- and apoptosis-related protein expression. A. Con group; B. Mod group; C. OXY group; D. ECDH group; E. ECDM group; F. ECDL group

Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group; Adj.p – adjusted p-value.
 Toll-like receptor 4 (TLR4): Kruskal–Wallis test: $H = 11.713$, $df = 5.000$, $Adj.p = 0.156$.
 Nuclear factor kappa-B (NF-κB): Kruskal–Wallis test: $H = 13.023$, $df = 5.000$, $Adj.p = 0.092$.
 NLR family pyrin domain containing 3 (NLRP3): Kruskal–Wallis test: $H = 13.936$, $df = 5.000$, $Adj.p = 0.064$.
 Apoptosis-associated speck-like protein (ASC) Kruskal–Wallis test: $H = 12.883$, $df = 5.000$, $Adj.p = 0.096$.
 Sample $n = 3$. The p-value was adjusted using Bonferroni correction for multiple Kruskal–Wallis tests ($n = 4$).

Discussion

Gastric damage caused by excessive alcohol consumption is characterized by bleeding, gastric ulceration, erosion, and related lesions. Injured gastric tissue cells exhibited inflammatory infiltration, accompanied by decreased proliferative activity and increased apoptosis. Under ethanol stimulation, the expression of inflammation-related markers, including $TNF-\alpha$ and $IL-1\beta$, was significantly elevated, along with increased oxidative stress levels.

Erchen decoction was first recorded in the well-known Song Dynasty medical text *Taiping Huimin Heji Ju Fang* and has been widely used in TCM. Modern studies have demonstrated that Erchen decoction can reduce inflammatory mediators in tissues and is therefore frequently

applied in conditions such as pneumonia, bronchitis, and hepatitis.^{27–29} In the present study, we confirmed that Erchen decoction effectively alleviates ethanol-induced gastric injury by regulating inflammatory factors and enhancing antioxidant capacity.

The gastric histopathological findings in the Erchen decoction-treated groups indicated a dose-dependent therapeutic effect, with higher doses producing greater histological improvement. Notably, the high-dose Erchen decoction achieved a therapeutic effect comparable to that of OXY. As a proliferation-associated nuclear antigen, PCNA expression is closely linked to cell proliferation.^{30–32} In the Mod group, ethanol exposure resulted in a significant reduction in PCNA expression, suggesting that ethanol not only disrupted the structural integrity of the gastric mucosa but also impaired cellular

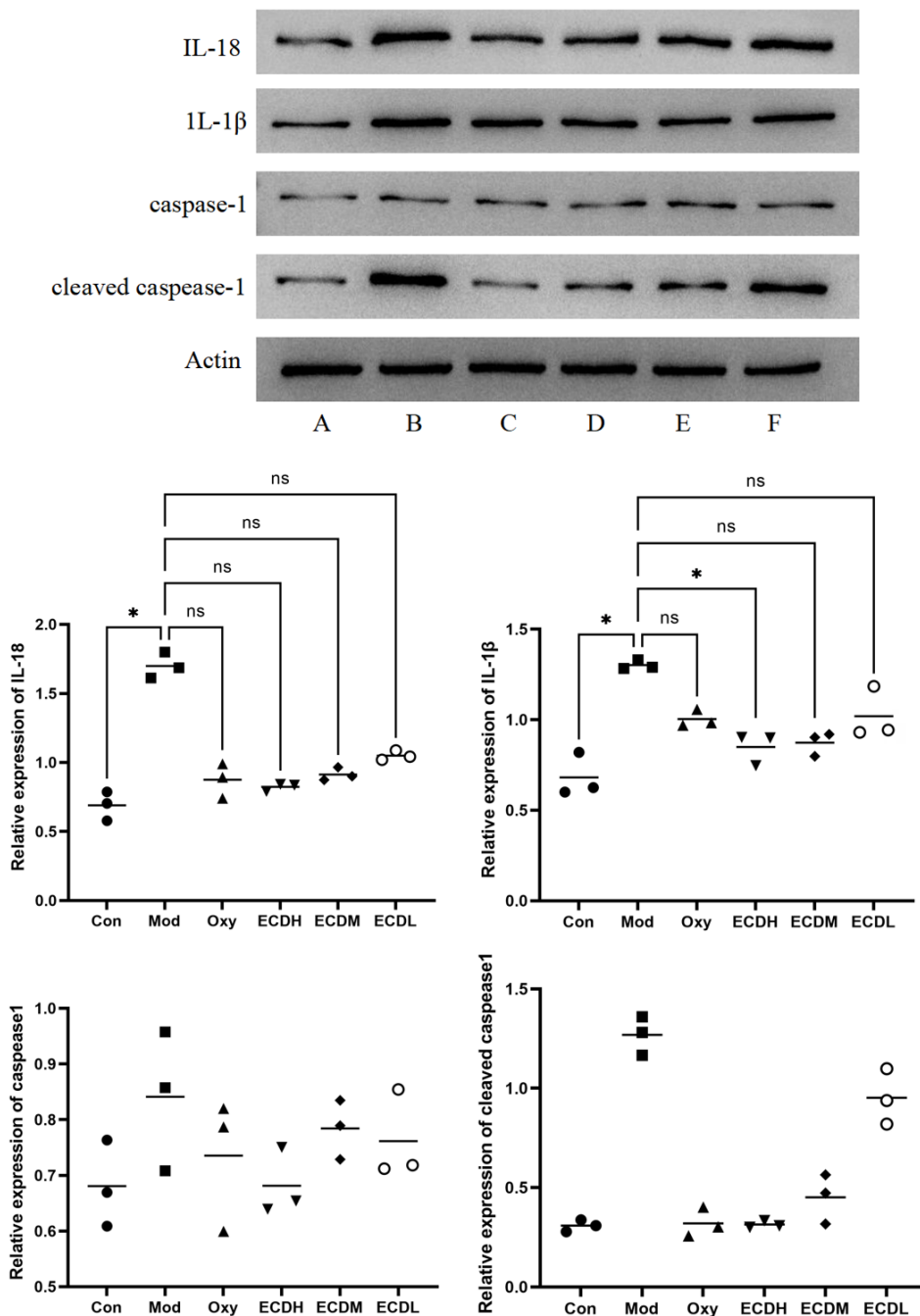


Fig. 6. Western blot analysis of protein expression in the inflammatory signaling pathway. A. Con group; B. Mod group; C. OXY group; D. ECDH group; E. ECDM group; F. ECDL group

Con group – control group; Mod group – model group; OXY group – oxymatrine group; ECDH group – high-dose Erchen decoction group; ECDM group – medium-dose Erchen decoction group; ECDL group – low-dose Erchen decoction group; Adj.p – adjusted p-value. Interleukin-18 (IL-18): Kruskal–Wallis test: $H = 14.988$, $df = 5.000$, $Adj.p = 0.040$. Dunn's test: $Z_{Con vs Mod} = -14.667$, $*Adj.p_{Con vs Mod} = 0.005$, $Z_{OXY vs Mod} = 9.000$, $Adj.p_{OXY vs Mod} = 0.195$; $Z_{ECDH vs Mod} = 11.000$, $Adj.p_{ECDH vs Mod} = 0.060$; $Z_{Mod vs ECDM} = 7.333$, $Adj.p_{Mod vs ECDM} = 0.460$; $Z_{Mod vs ECDL} = 3.000$, $Adj.p_{Mod vs ECDL} > 0.999$. Interleukin-1 beta (IL-1β): Kruskal–Wallis test: $H = 15.175$, $df = 5.000$, $Adj.p = 0.040$. Dunn's test: $Z_{Con vs Mod} = -14.333$, $*Adj.p_{Con vs Mod} = 0.005$; $Z_{OXY vs Mod} = 4.000$, $Adj.p_{OXY vs Mod} > 0.999$; $Z_{ECDH vs Mod} = 11.333$, $*Adj.p_{ECDH vs Mod} = 0.045$; $Z_{Mod vs ECDM} = 10.333$, $Adj.p_{Mod vs ECDM} = 0.090$; $Z_{Mod vs ECDL} = 5.000$, $Adj.p_{Mod vs ECDL} > 0.999$. Caspase 1: Kruskal–Wallis test: $H = 5.608$, $df = 5.000$, $Adj.p > 0.999$. Cleaved caspase 1: Kruskal–Wallis test: $H = 13.585$, $df = 5.000$, $Adj.p = 0.072$. Sample $n = 3$; *represents statistical significance compared with model group; ns, both ends of the horizontal line are not significant. The p-value was adjusted using Bonferroni correction for multiple Kruskal–Wallis tests ($n = 4$) and multiple Dunn's post hoc tests ($n = 5$).

proliferative capacity. Compared with the Mod group, PCNA staining was more intense in the OXY group, indicating that OXY enhanced the proliferation of damaged cells and promoted gastric mucosal repair and regeneration. Similarly, PCNA staining intensity in the Erchen decoction groups increased in a dose-dependent manner.

Among the treatment groups, proliferative activity was greatest in the ECDH group, whereas the ECDL group also showed enhanced proliferation, albeit to a lesser extent. These findings indicate that Erchen decoction not only attenuates ethanol-induced gastric mucosal injury but also promotes the growth and repair of damaged gastric mucosal cells. Erchen decoction treatment reduced

mitochondrial swelling and cell membrane disruption, thereby inhibiting cellular injury and the potential subsequent dysfunction or death.³³ Following OXY treatment, the levels of inflammatory markers TNF- α and IL-1 β were comparable to those in the Con group and significantly lower than those in the Mod group. Although the high-dose Erchen decoction did not achieve the same magnitude of effect as OXY, it still significantly reduced ethanol-induced inflammation, suggesting that high-dose Erchen decoction exerts a substantial anti-inflammatory effect.

Superoxide dismutase is a key antioxidant enzyme responsible for eliminating harmful superoxide radicals, whereas MDA is a byproduct of lipid peroxidation in cell

membranes and is commonly used as a marker of oxidative damage.³⁴ Compared with the Con group, the Mod group showed significantly reduced SOD activity and significantly elevated MDA levels. These changes indicate that ethanol exposure markedly impaired antioxidant defense capacity.^{11,13,14} Both the OXY group and the ECDH/ECDM/ECDL groups demonstrated varying degrees of improvement. Superoxide dismutase activity increased and MDA levels decreased in all treatment groups compared with the Mod group, suggesting that these interventions enhanced antioxidant defenses and attenuated oxidative damage. Overall, these findings indicate that Erchen decoction alleviates ethanol-induced gastric mucosal injury, at least in part, by increasing SOD activity and reducing MDA levels. Activation of the NF- κ B pathway represents a central mechanism in ethanol-induced gastric injury. As a key regulator of the inflammatory response, the NF- κ B pathway plays a pivotal role in promoting the expression of pro-inflammatory cytokines.^{35,36} Alcohol exposure activates the NF- κ B pathway, leading to elevated levels of inflammatory mediators such as TNF- α and IL-1 β , thereby exacerbating gastric mucosal inflammation and injury. In the present study, ethanol exposure was associated with increased expression of NF- κ B and related inflammatory signaling proteins, including TLR4, NLRP3, and ASC, further indicating activation of the NF- κ B pathway in ethanol-induced gastric injury. Notably, this study further explored the regulatory effects of Erchen decoction on this pathway. Erchen decoction effectively suppressed NF- κ B activation and the expression of downstream inflammatory mediators, particularly in the ECDH group. In addition, Erchen decoction reduced the levels of cleaved caspase-1, caspase-1, IL-1 β , and IL-18, further suggesting that it alleviates ethanol-induced gastric mucosal injury, at least in part, through inhibition of the NF- κ B pathway. These findings are important for understanding the mechanisms by which Erchen decoction mitigates chronic ethanol-induced gastric injury and suggest that its protective effects may be mediated by suppression of NF- κ B signaling, thereby reducing inflammatory mediator release and inflammatory cell activation.

Limitations of the study

However, we acknowledge that our study has several limitations. Erchen decoction is a complex TCM formulation, and the specific active components and key target proteins responsible for regulating chronic ethanol-induced gastric injury remain unclear. We intend to address this important issue in future investigations.

Conclusions

This study confirmed that Erchen decoction alleviates ethanol-induced gastric injury by reducing inflammation,

attenuating oxidative stress, preserving cellular structural integrity, and regulating the NF- κ B signaling pathway. These findings not only suggest a potential TCM-based therapeutic approach for ethanol-induced gastric injury but also provide a scientific basis for further investigation of Erchen decoction in the treatment of other inflammation- and oxidative stress-related diseases.

Data Availability Statement

The datasets supporting the findings of the current study are openly available in the Zenodo repository at <https://doi.org/10.5281/zenodo.16417628>.


Consent for publication


Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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