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Multi-trace elements-enriched functional drink accelerates gastric ulcer repair *via* the HGF/c-Met/STAT3 pathway

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ABSTRACT

Heavy alcohol consumption can lead to various gastrointestinal disorders, with gastric ulcers being the most prevalent. In this study, we explored the reparative effects of a functional drink (FD) enriched with trace elements (TEs) on ethanol-induced gastric ulcers in rats and elucidated its underlying mechanism. Rats were administered anhydrous ethanol (5 mL/kg) orally to induce gastric ulcers and were then randomly assigned to a model, an omeprazole (OME, 20 mg/kg), FD with low-dose (FD-L), medium-dose (FD-M), high-dose (FD-H), FD without added TEs (FD without TEs), and an omeprazole+low-dose group (OME + L). FD increased the activities of antioxidant factors (T-SOD, GSH, CAT, and IL-10), and decreased the inflammatory factors levels (TNF-α, IL-6, MDA, COX-2, and iNOS) by promoting the HGF/c-Met/STAT3 pathway. It also effectively regulate the abundance and diversity of intestinal flora. Finally, FD reduced the severity of gastric mucosal injury caused by invasive factors, improved the morphological structure of gastric mucosal cells, enhanced the ability of oxidative stress, and inhibited excessive inflammatory response. In addition, the combination of OME and FD-L did not significantly affect the therapeutic efficacy of OME, but reduced its adverse effects. Ultimately, FD presentation had a pro-healing effect on the ethanol-induced gastric ulcer model. In conclusion, FD has a wide range of developmental prospects and offers an attractive approach and protocol for the treatment of clinical gastric ulcers.

1. Introduction

Gastric ulcers are a prevalent digestive disorder, affecting up to 10 % of the global population (Kuna et al., 2019). They are characterized by ulceration of the gastric mucosa, bleeding, perforation, and an increased risk of other serious complications, leading to a high mortality rate (Sung et al., 2009). The primary cause of gastric ulcers include *Helicobacter pylori* infection (Simões et al., 2024), prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) (D. Zhou et al., 2020), and excessive alcohol consumption (Aziz et al., 2019). Among these, alcohol consumption is a significant contributor to the development of stomach ulcers. It is detrimental to the gastric mucosa due to impaired blood microcirculation in the gastric lining, tissue necrosis, and reduced blood flow and mucus secretion in the gastric mucosa (Ma & Liu, 2014). Studies have demonstrated that ethanol induces gastric mucosal damage by promoting mucosal epithelial cell apoptosis, inflammatory response, and oxidative stress in gastric tissues (Xie et al., 2017). In particular, inflammatory cell infiltration and oxidative stress play crucial roles in

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the pathogenesis of ethanol-induced gastric mucosal injury (Brito et al., 2018). The drugs currently used for treating gastric ulcers mainly consist of acid inhibitors and gastric mucosal protectors. Among them, proton pump inhibitors (PPIs) are the most widely utilized class of medications (Selim et al., 2023). However, recent studies indicates that prolonged use of PPIs increases the risk of gastric adenocarcinoma and can lead to various serious side effects, including hepatotoxicity, headaches, and, in rare cases, Cushing's syndrome (Fagundes et al., 2021). Therefore, it is of significant importance to identify novel treatment approaches for gastric ulcers that have minimal toxic side effects and substantial therapeutic efficacy.

Micronutrients, as essential trace minerals, are indispensable for maintaining optimal human health. They play a crucial role in various physiological processes, including body growth, development, and functional metabolism (Ruan et al., 2023). Elements such as iron, zinc, selenium, and copper are vital components of the immune and antioxidant defence systems (Torres-Vega et al., 2012). Additionally, metallic elements possess significant antioxidant properties (Sabina Janciauskiene, 2024). Trace elements (TEs) are intricately linked to the bodily functions and are involved in normal physiological activities, encompassing metabolic regulation, tissue repair, growth, and development (Islam et al., 2023).

While numerous studies have explored the relationship between individual elements and gastric ulcers, it is important to recognize that the human body is a complex organism. The physiological and biochemical functions of TEs are multifaceted, and the effects of a single element may not fully represent real-world scenarios (Olechno et al., 2021). The pharmacological interactions between multiple TEs and disease are often underappreciated. For instance, zinc has been widely utilized in the treatment of peptic ulcers due to its mucosal cytoprotective and anti-inflammatory effects, which are mediated through antioxidant, cytokine regulatory and membrane stabilising properties (Efthymakis & Neri, 2022). In our previous research, we developed a TEs solution to treat rheumatoid arthritis. This solution induced the transition of macrophages from M1-type to M2-type, enhanced antioxidant enzymes, scavenged reactive oxygen species at the site of the lesion, inhibited chondrocyte apoptosis, and reduced inflammatory responses (Wang, Yin, et al., 2024). The supplementation of multiple micronutrients can more comprehensively address the nutritional needs of the body, improving the absorption and utilization of micronutrients. Moreover, it can prevent and alleviate nutritional deficiency symptoms caused by individual micronutrients, thereby synergistically optimizing the overall dietary effect.

On this basis, we prepared a functional drink (FD) enriched with a variety of TEs. This FD is a type of soft drink with health benefits, designed to meet the nutritional needs of certain special populations. FD, as a novel beverage, not only offers high functional perception and good taste but also aligns with the current health demands of consumers. Furthermore, considering the toxic side effects of omeprazole (OME) and the modulating effect of micronutrients on the immune system, the combination of OME with this FD holds significant research value. Therefore, the present study not only provides a FD containing multiple TEs for repairing gastric ulcers but also evaluates its reparative effects on ethanol-induced gastric ulcers in rats and its antioxidant mechanism. Additionally, it offers a new clinical perspective to reduce treatment cost and alleviate the side effects in patients.

2. Material and methods

2.1. Materials

Trace element solution was provided from Beijing Jinshan Ecology company (Beijing, China). Omeprazole enteric-coated capsules (15240309) purchased from Hebei Mushi Drug Co Ltd. (Hebei, China). Anhydrous ethanol (TG10013) purchased from Beijing Tong Guang Fine Chemicals Company (Beijing, China). TNF-α (Lot 240,524,516), IL-6 (Lot 240,524,485), IL-10 (Lot 240,524,490) were purchased from CloudClone Biotechnology Co. (Beijing, China); T-SOD (MPC2401003), MDA (MPC2401003), GSH (MPC2401015) and CAT (MPC2401004) were purchased from Wuhan Xavier Biological Co. (Wuhan, China) ; COX-2 antibody (90494), iNOS (141279) antibody, HGF antibody (143087), c-Met antibody (134618) were purchased from Wuhan Three Eagles Biotechnology Co. (Wuhan, China).

2.2. Experimental animals

Male SD rats (SPF, 6-week-old, 180–200 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Animal Licence No.: SYXK (Beijing) 2021-0050. The animals were housed and experimented in the SPF Grade Animal Experimentation Centre of the Institute of Pharmaceutical Research, Beijing Medical College, Beijing, China, under the following conditions: temperature (25 ± 2) °C, relative humidity less than 70 %, day and night alternation for 12 h, good ventilation, free feeding and watering of the animals, and the experiments were initiated after 1 week of acclimatization. All animal experiments were approved by the Laboratory Animal Ethics Committee (00004407) of the Institute of Pharmaceutical Sciences, Peking Union Medical College.

2.3. FD preparation and composition

The team previously developed a TEs solution (Wang, Yin, et al., 2024). Considering the taste and utilization, a FD was prepared based on this solution. The specific formulations are shown in Table S1.

With a volumetric quantity of 500 mL, we prepared three doses of FDs: low (adding 0.5 mL TEs), medium (adding 1 mL TEs), and high (adding 1.66 mL TEs). This approach allows for the evaluation of the effects of varying concentrations of TEs in the FD on health benefits and consumer acceptance.

2.4. Long-term safety of FD

Healthy SD rats ($n = 6$) were allowed to drink the FD-H without restriction every day for 7 days (FD group). Simultaneously, a control group (n = 6) was established, and the rats in the control group drank pure water daily. A total of 12 rats were utilized. 1 mL of blood was collected from the fundus venous plexus of rats at 8 days. Then, all rats were euthanized. Histopathologic analyses of the main organs of the rats were conducted to assess the impact of the FD on their health.

2.5. Establishment of gastric ulcer model

SD rats were acclimatized and fed for 1 week to establish an ethanol-induced gastric ulcer model. Briefly, the rats were fasted for 24 h, but allowed water. Gastric ulcers were induced by slowly administration of anhydrous ethanol (5 mL/kg) using a gavage needle probed through the mouth to the cardia. They were randomly divided into Model group (Model), omeprazole group (OME, 20 mg/kg, Positive), functional drink with low dose trace elements group (FD-L), functional drink with medium dose trace elements group (FD-M), functional drink with high dose trace elements group (FD-H), omeprazole and functional drink with low dose trace elements group (OME + L), Functional drink without added trace elements groud (FD without TEs). Six rats were used in each group. In addition, a Control group of 6 healthy SD rats was established. All rats in the intervention groups were gavaged with 1 mL of the respective solution once a day for 7 consecutive days. The Control and Model groups were gavaged with an equal amount of saline.

2.6. Collection tissues

After the final administration, 1 mL of blood was collected from the fundus venous plexus of rats. The blood samples were centrifuged (3000

rpm, 10 min, 4 °C). The supernatant was aspirated and transferred to −80 °C refrigerator for storage and reserve. Concurrently, the rats were euthanised by inhalation of excess carbon monoxide 1 h. The stomachs were rapidly removed and incised along the greater curvature. The gastric contents were rinsed with pre-cooled saline and the surface was blotted dry with filter paper to record gastric mucosal damage under a fixed-range camera. A portion of the gastric tissue was fixed in 10 % formalin solution, while the remaining tissue was quickly stored in a −80 °C refrigerator for further analysis.

2.7. Determination of ulcer index

The rat gastric ulcer injury score was adjusted according to the guth scoring criteria as shown in Table S2. Ulcer index = sum of ulcer index of all animals in each group / number of animals.

2.8. Histopathological evaluation

Fixed rat stomach tissues were dehydrated using a fully automated dehydrator, embedded and sectioned. Sections from each group were taken for hematoxylin-eosin staining (H&E staining) and sealed for histopathological examination.

2.9. Serum cytokine assay

Serum levels of TNF-α, IL-6 and IL-10 were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

2.10. Detection of T-SOD, MDA, GSH, CAT, HGF, and c-Met levels in rat gastric

Frozen stomach tissue was thawed and then 4-fold pre-cooled saline was added. After homogenization, supernatant was centrifuged and protein concentration in supernatant was determined by BCA protein concentration assay kit. The supernatant was taken to measure the levels of T-SOD, MDA, GSH, CAT, HGF, and c-Met.

2.11. Apoptosis rate in rat gastric

Fixed rat stomach were dehydrated using a fully automatic dehydrator, embedded, sectioned. The TUNEL apoptosis detection kit was used, and the operation was strictly in accordance with the instructions of the manual for TUNEL staining. Apoptotic cells were observed under fluorescence microscope and apoptotic cell analysis was performed.

2.12. Expression of COX-2, iNOS, HGF and c-Met proteins

Fixed rat stomach were dehydrated using an automatic dehydrator, embedded, sectioned, and then routinely deparaffinised to water and placed in a staining vat containing 3 % hydrogen peroxide for 10 min at room temperature, and rinsed three times with PBS. After high-temperature repair of the antigen, the slices were closed at room temperature for 20 min, and the slices were titrated with diluted primary antibodies against COX-2 (1:100), (1:100), iNOS (1:100), HGF (1:100), c-Met (1:100), and STAT3 (1:500), respectively. Incubate overnight at 4 °C, dropwise add the secondary rabbit antibody, incubate at a constant temperature of 37 °C for 30 min, and rinse 3 times with PBS. The optical density and area of all the captured images were determined using the Image J analysis system.

2.13. Changes in abundance and diversity of rat intestinal flora detected by 16S RNA sequencing

Gastric ulcer model rats were divided into two groups. One group was gavaged with FD for 5 consecutive days, while the other group was given free access to water. Fecal samples were randomly collected from naturally excreted feces. Immediately after collection, the feces were stored at −80 °C to prevent degradation of microbial DNA. Subsequently, total microbial DNA was extracted from the samples. Species abundance and diversity were assessed for each sample using alpha diversity analysis, and differences in microbial community structure between samples were compared using phylum analysis and genus analysis. By combining the results of the analyses with the phenotypic data of the experimental groups, the changes in the rat gut microbial community under specific conditions and their correlations were revealed.

2.14. Statistical analysis

All data were presented as mean ± standard deviation and each experiments was performed at least three times. Statistical analysis was tested with the Prism 9.5 software (GraphPad Software) by Tukey's multiple comparison tests and one-way analysis of variance. The differences were considered significant, when * P values <0.05, ** $P < 0.01$, and *** $P < 0.001$.

3. Results

3.1. Evaluation of FD

This FD was clarified, with no visible foreign matter, and exhibited a slightly yellow colour. It had a moderately sweet and sour in taste. The types and concentrations of TEs in the three different FDs are shown in Table S3.

3.2. Long-term safety of FD

After 7 d of continuous consumption of the FD without restriction, the rats in the FD group continued to exhibit normal appearance and behavior. The results of pathological examination revealed normal morphology of the main organs (heart, liver, spleen, lung, kidney, stomach, and intestines) (Fig. 1 A). There was no evidence of inflammation, mucosal erosion, or other abnormal phenomena, indicating that the integrity and health of the stomach and intestines were fully maintained. As depicted in Fig. 1 B, compared with the Control group, the rats of FD group showed no significant changes in the levels of ALT, AST, CREA, and BUN. Therefore, we conclude that the FD is non-toxic.

3.3. Effect of FD on rats gastric

After gastric ulcer model was established, the weight of rats in Model and FD without TEs group decreased rapidly (Fig. 2 A and S1). Some rats died in the Model group. The weight of intervention group recovered gradually. The gastric mucosa of the Control group was structurally intact, light pink in colour, with more folds, and no haemorrhages or oedema (Fig. 2 B). Compared with the Control group, the gastric mucosa of the Model and FD without TEs group showed a severe bleeding band with a width of about 2 mm, longitudinally penetrating through the entire gastric mucosa portion, with severe bruising of the submucosal layer, extensive oedema, gastric mucosal relaxation, thinning, and severe damage, with almost complete loss of the gastric mucosal surface structure in the focal area, and a significant increase in the ulcer index ($P < 0.01$) (Fig. 2 C). Compared with the Model and FD without TEs group, the gastric mucosal damage of rats in each intervention group showed different degrees of improvement. The ulcer index was significantly reduced in the FD-M group ($P < 0.05$). Among them, the OME + L group and the OME and FD-H groups had less gastric mucosal damage, significantly less haemorrhagic damage, and only a few blood spots and mild oedema. The gastric ulcer index was significantly lower ($P < 0.01$). The results suggested that FD improved gastric mucosal damage caused by ethanol, and the efficacy showed a dose-dependent pattern.

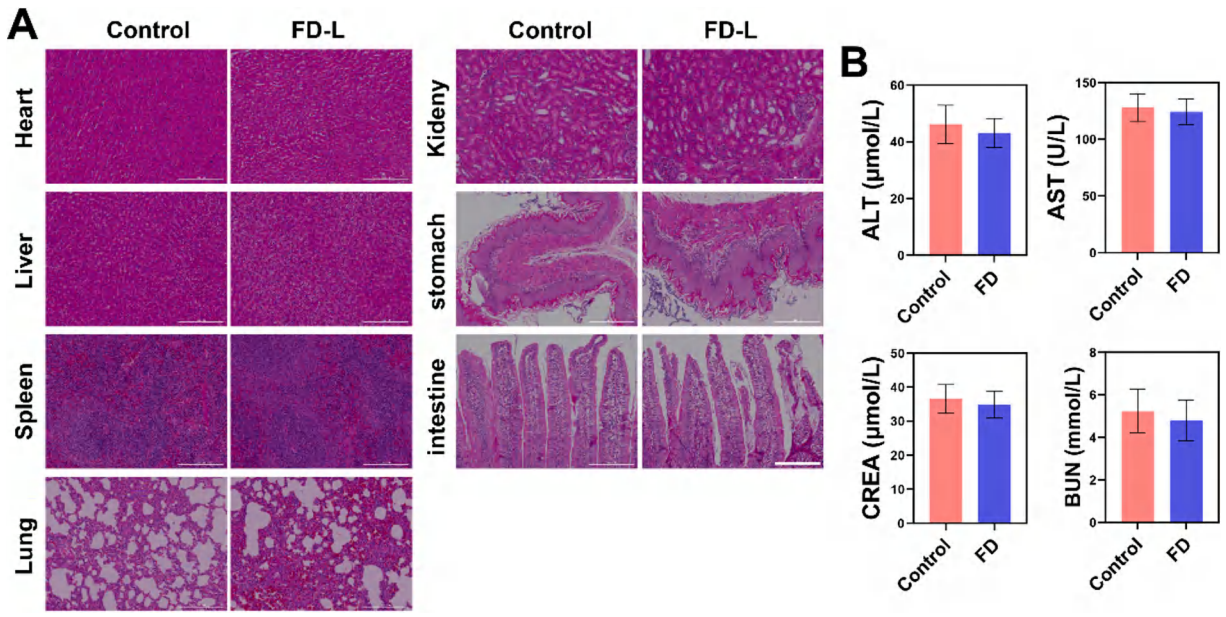

Fig. 1. Safety. (A) H&E staining of the main organs (heart, liver, spleen, lung, kidney, stomach and intestine) harvested from different groups, scale = 200 μm. (B) Biomarkers of ALT, AST, CREA, and BUN from rats ($n = 6$).

3.4. Histopathological examination of rat stomachs

The results of H&E staining were shown in Fig. 2 D, the gastric mucosa structure of Control group was intact. The mucosal epithelial cells were not detached. The gastric glands were abundant, arranged in a regular and close manner and clearly visible. The epithelial cells were in normal morphology without necrosis. The mucosal layer was not obviously damaged, and the submucosa was not oedematous with no infiltration of inflammatory cells. The Model and FD without TEs group were structural damage to the mucosal, submucosal, muscular and plasma layers of some of the gastric tissues. The mucosal epithelium and lamina propria were necrotic. The structure of the gastric gland in the necrotic area was blurred, with a large number of mural and principal cells undergoing necrotic and cell lysis. Compared with the Model and FD without TEs group, there were different degrees of improvement in the gastric mucosal damage of rats in each intervention group. The FD-L and FD-M groups was slight damage to the gastric tissue structure, superficial degeneration and necrosis of the mucosal layer, blurring of the morphology and structure of the mural cells. Congestion and dilatation of blood vessels were observed in the local areas. The lesions in the FD-H and OME groups and the OME + L group were significantly reduced, and the gastric tissue was structurally intact, clear, and clearly stratified, with the submucosa, muscularis mucosae, and epimysium closely connected. The results suggested that FD possesses a superior gastric mucosal protective effect, and the effect is a dose-dependence.

3.5. TNF-α, IL-6, and IL-10 levels

TNF-α is a central cytokine in the inflammatory response, driving the inflammatory both directly by inducing the expression of inflammatory genes and indirectly by inducing cell death, stimulating inflammatory immune responses, and disease development (van Loo & Bertrand, 2023). IL-6 is a classic pro-inflammatory cytokine that maintains homeostasis in the body. Excessive and persistent dysregulation of IL-6 synthesis has pathological effects on acute systemic inflammatory response syndromes and chronic immune-mediated diseases, respectively. IL-10 is an anti-inflammatory cytokine that limits immune activation of innate and adaptive immune cells and also inhibits the expression of inflammatory cytokines such as TNF-α and IL-6 through activation of macrophages (York et al., 2024). The ELISA results showed that TNF-α and IL-6 levels were significantly higher and IL-10 levels were significantly lower in Model and FD without TEs group compared to Control group (Fig. 3). Compared with Model and FD without TEs group, TNF-α and IL-6 levels were differently improved in the intervention group and were significantly reduced in the FD-M, FD-H, OME, and OME + L groups ($P < 0.001$). IL-10 expression levels were significantly higher in the FD-L, FD-M, FD-H, OME, and OME + L groups ($P < 0.001$). These results suggest that FD could significantly reduce the production of pro-inflammatory cytokines and significantly increase the expression of anti-inflammatory cytokines in the rats gastric, thus effectively inhibiting the occurrence and development of gastric ulcers.

3.6. The expression of T-SOD, MDA, GSH and CAT in rats gastric

T-SOD is the only known enzyme that directly scavenges superoxide radicals. T-SOD activity reflects the degree of intracellular oxidative stress, and cells become susceptible to oxidative damage when their levels are reduced (Borgstahl & Oberley-Deegan, 2018). MDA is one of the most commonly used biomarkers of lipid peroxidation. It reflects the rate and intensity of lipid peroxidation in the body, and also indirectly reflects the degree of tissue peroxidative damage (Ito et al., 2019). GSH possesses antioxidant and integrative detoxification effects, aiding in the maintenance of normal immune system function (Georgiou-Siafis & Tsiftsoglou, 2023). CAT is a core antioxidant enzyme in most organisms that catalyses the decomposition of hydrogen peroxide, thereby protecting cells from oxidative damage (Baker et al., 2023). In summary, SOD, MDA, GSH, and CAT play significant biological roles in living organisms, protecting cells from oxidative damage by synergistically converting reactive oxygen species into harmless substances. At the same time, they can also be used as an indicator of the degree of oxidative stress in an organism, reflecting the health status of the organism. Compared to the Control group, T-SOD, GSH, and CAT activities were significantly lower and MDA levels were significantly higher in Model and FD without TEs group (Fig. 4 A, B, and C). Compared with Model and FD without TEs group, T-SOD, GSH, and CAT levels improved to different degrees in each intervention group, and were significantly higher in the FD-H group, and significantly higher in the FD-L, FD-M, OME, and OME + L groups. Compared with Model and FD without TEs group, MDA expression levels were significantly lower in the FD-M, FD-H, OME, and OME + L groups, and significantly lower in the FD-L group

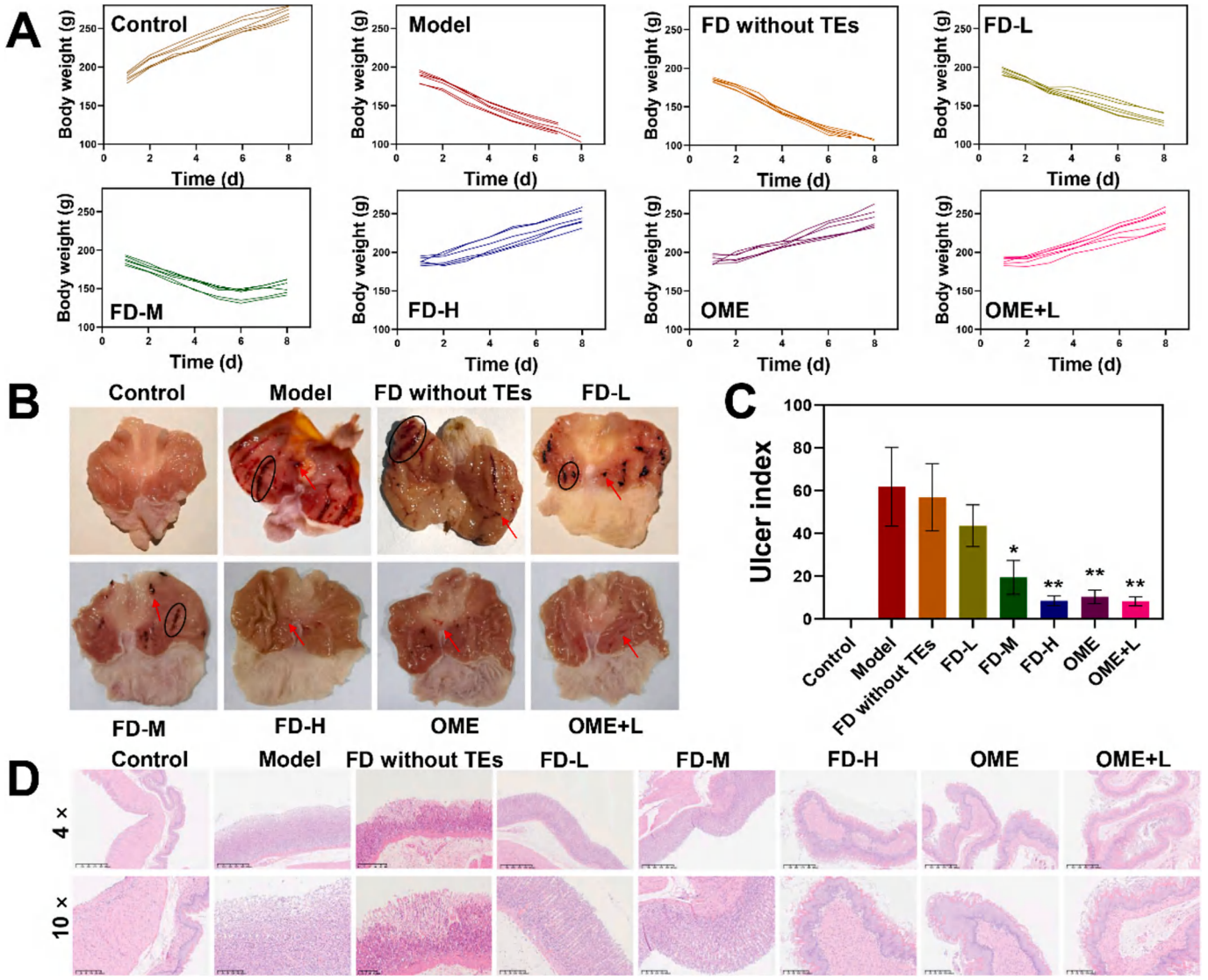

Fig. 2. Gastric mucosal damage in each group. (A) Body weight. (B) Photos of rats stomach tissue from different groups. The dark red and black parts of the figure are ulcers (red arrows indicate punctate ulcers, black arrows indicate strip ulcers. (C) Ulcer index ($n = 6$). Compared with Model group * $P < 0.05$, ** $P < 0.01$. (D) Histopathological and morphological changes of the large stomach in each group (scale bar = 200 μm). TEs: trace elements; FD: functional drink; FD without TEs: Functional drink without added trace elements; FD-L: functional drink with low dose trace elements; FD-M: functional drink with medium dose trace elements; FD-L: functional drink with high dose trace elements; OME: omeprazole; OME + L: omeprazole and functional drink with low dose trace elements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

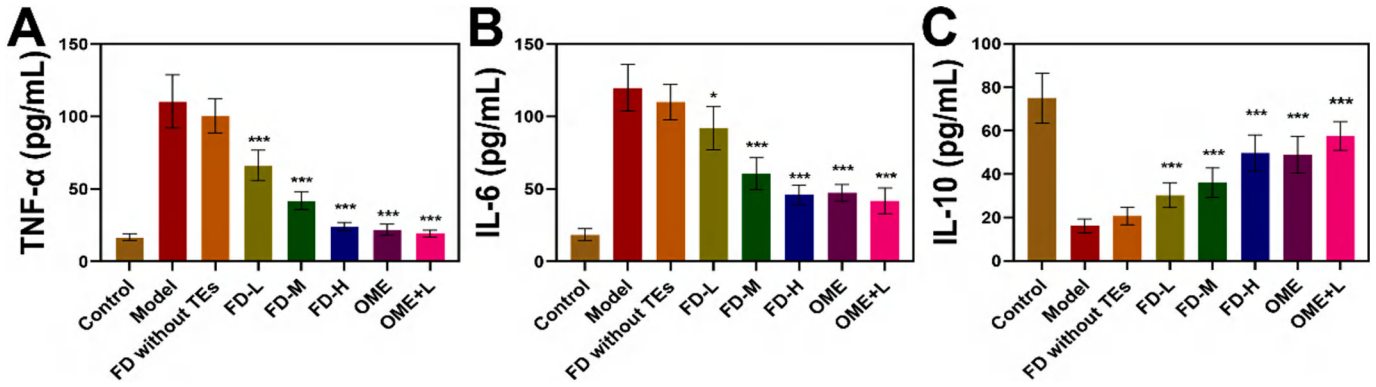

Fig. 3. Serum levels of (A) TNF-α, (B) IL-6 and (C) IL-10 ($n = 6$). Compared with Model group, * $P < 0.05$, *** $P < 0.001$. **TNF-α: Tumor necrosis factor α; IL-6: Interleukin-6; IL-10: Interleukin-10.**

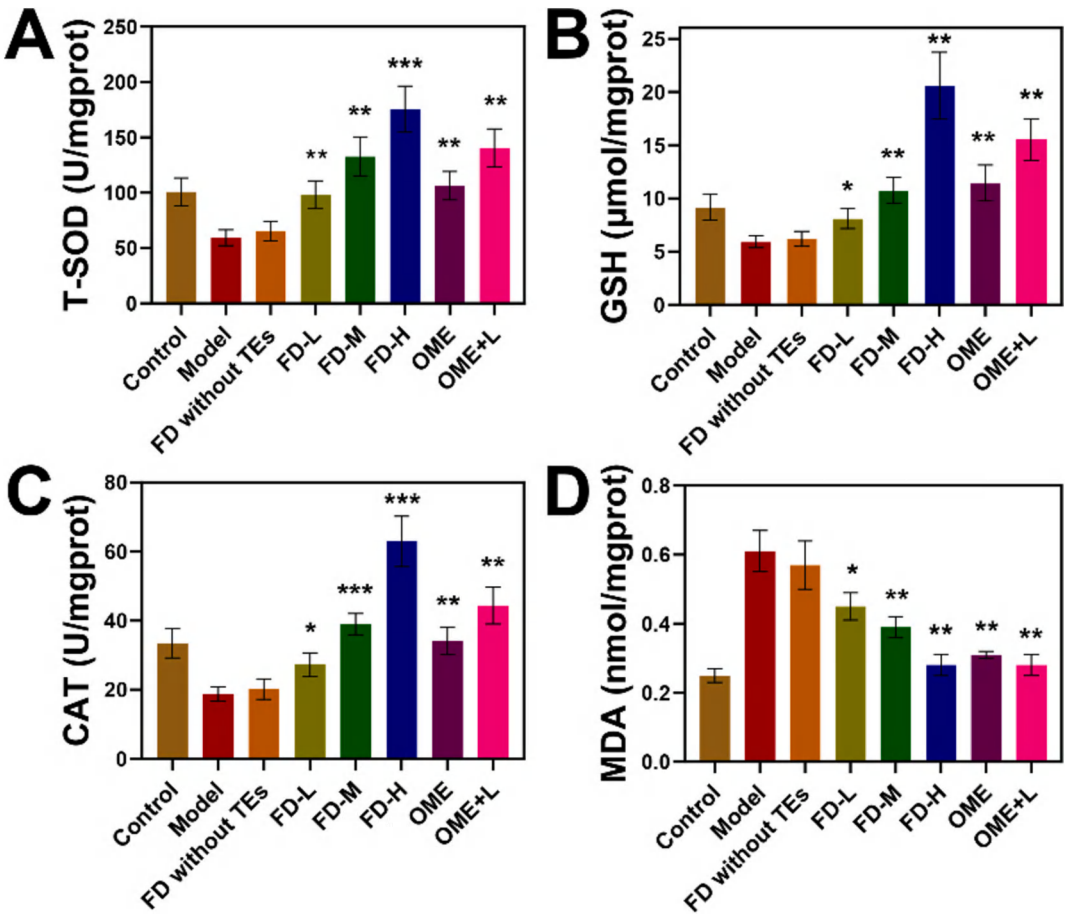

Fig. 4. Oxidase levels of (A) T-SOD, (B)GSH, (C) CAT, and (D) MDA in the rats gastric tissues of difference groups ($n = 6$). Compared with Model group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. T-SOD: Total Superoxide Dismutase; GSH: Glutathione; CAT: Catalase; MDA: Malondialdehyde.

(Fig. 4 D). The results suggest that FD possesses superior antioxidant effects.

3.7. Apoptosis of rats gastric cells

The results of histopathological were shown in Fig. 5, where normal cell nuclei exhibit blue fluorescence and apoptotic cell nuclei display green fluorescence. The percentage of apoptosis was very low in Control group and significantly higher in the gastric tissue of rats in Model group ($P < 0.001$) (Fig. 5 A and B). Compared with Model and FD without TEs group, the percentage of apoptosis in the gastric tissue of all intervention groups was reduced to varying degrees. The FD-H, OME, and OME + L groups had low percentage of apoptosis ($P < 0.001$) (Fig. 5B). While it was significantly lower in the FD-L group ($P < 0.01$). FD was able to alleviate apoptosis caused by oxidative stress.

3.8. Histopathological tests

3.8.1. COX-2

The COX-2 activity is extremely low in normal tissue cells. When cells are stimulated by inflammation and other stimuli, its expression level in inflammatory cells can increase up to 80 times the normal level, leading to inflammatory responses and tissue damage (Wang, Chen, & Zheng, 2023). The results are shown in Fig. 6 A and C. Compared with Control group, the expression of COX-2 in the rats gastric of Model and FD without TEs group was significantly elevated ($P < 0.001$). Compared with Model and FD without TEs group, the FD-H group significantly reduced the expression level of COX-2 in gastric tissues ($P < 0.001$). The FD-M, OME, and OME + L groups were able to significantly reduce the expression level of COX-2 in gastric tissues ($P < 0.01$).

3.8.2. iNOS

iNOS is a key mediator of immune activation and inflammation. Its overexpression can lead to damage to the body from the immune-inflammatory response (Cinelli et al., 2020). The results are shown in Fig. 6 B and D. Compared with Control group, the expression levels of iNOS in the gastric tissue of Model and FD without TEs group were significantly higher ($P < 0.01$). Compared with Model and FD without TEs group, the FD-H, OME, and OME-L groups were able to significantly reduce the expression levels of iNOS in gastric tissues ($P < 0.01$).

3.8.3. HGF

HGF is a multifunctional factor that regulates the growth, motility and morphogenesis of a wide range of cells. It plays an important role in embryogenesis, wound healing, angiogenesis, tissue and organ regeneration, and morphogenetic effects (Nakamura et al., 2011). The results are shown in Fig. 7 A, D, and F. Compared with Control group, the expression levels of HGF in the gastric tissue in the Model and FD without TEs group were decreased. Compared with the Model and FD without TEs group, the FD-H group and the OME + L group were able to significantly increase the expression level of HGF in gastric tissues ($P < 0.001$). The FD-M and OME groups were able to significantly increase the expression level of HGF in gastric tissues ($P < 0.01$).

3.8.4. c-Met

c-Met is a receptor tyrosine kinase that plays a crucial role in various physiological responses, including embryogenesis, tissue regeneration, cellular homeostasis, and wound healing (Viticchiè & Muller, 2015).

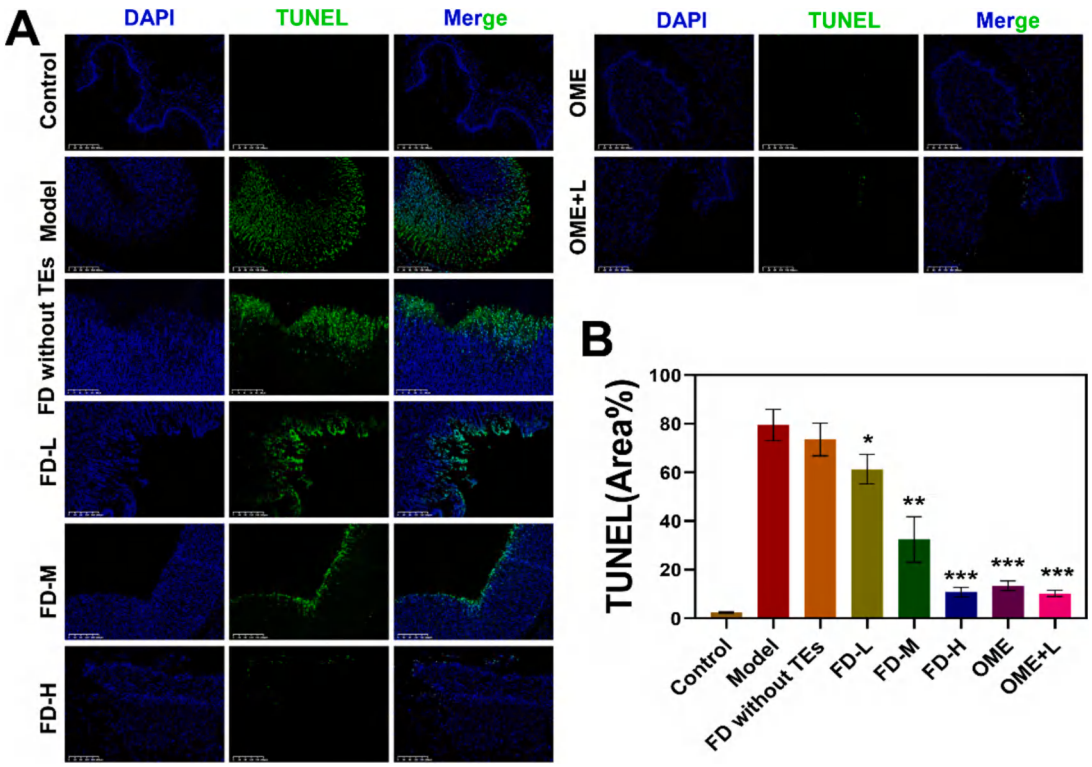

Fig. 5. Detection of apoptosis in rat stomach tissue. (A) TUNEL staining (scale bar = 200 μm). (B) Apoptosis rate ($n = 3$). Compared with Model group, ** $P < 0.01$, *** $P < 0.001$.

The results are shown in Fig. 7 B, E, and G. c-Met expression level was decreased in the rats gastric of Model and FD without TEs group compared with Control group. Compared with Model and FD without TEs group, the FD-L, FD-M, FD-H, OME, and OME + L groups were able to significantly increase the expression level of c-Met in gastric tissues ($P < 0.01$).

3.8.5. STAT3

The morphogenetic effects of the HGF/c-Met pathway are mediated by signal transducers and activators of transcription (STAT)3. STAT3 activates several target genes involved in diverse cellular processes such as cytokine signaling, cell proliferation and development, and immune response in microenvironment. The results are shown in Fig. 7 C and E. STAT3 expression levels were decreased in the gastric tissue in Model and FD without TEs group compared with Control group. Compared with Model and FD without TEs group, the FD-L, FD-M, FD-H, OME, and OME + L groups were able to increase the expression level of c-Met in gastric tissues.

Histopathological results showed that FD exerted antioxidant effects by promoting the expression of HGF/c-Met/STAT3 pathway and decreasing the expression of COX-2 and iNOS.

3.9. sRNA sequencing results

The results showed that the length of the clean tags of the samples ranged from 400 to 440 bp, which falls within the normal range (300–500 bp), and the sequencing quality was qualified (Fig. 8 A). Species richness and sample diversity were assessed using alpha diversity indices (alpha diversity index), including Chao-1, Observed-species, PD-whole-tree, and Shannon indices. It was observed that bacterial diversity and species abundance were significantly upregulated in the gut flora of rats receiving FD. This is due to the fact that a variety of micronutrient ingredients can promote the production of SCFAs in the gut, thereby regulating the composition of gut microorganisms (Scarpellini et al., 2022). Based on the results of the analysed phyla analysis (Fig. 8 B), the most significant changes were a decrease in the abundance of the thick-walled phyla and the increase in the abundance of the anamorphic phyla. According to the results of the genera of the bacterial group (Fig. 8 C and D), there was a significant decrease in the content of Lachnospiraceae, Helicobacter, and Narenga. And a significant increase in the proportion of genera, such as Clostridiales and Azospirillum. In summary, FD can effectively regulate the abundance and diversity of intestinal flora. However, the inhibitory effect on specific pathogenic bacterium and the potential mechanism related to the treatment of gastric ulcers equire further exploration.

4. Discussion

Gastric ulcers are deep defects in the wall of the stomach throughout the mucosa and the muscular layer (Tarnawski & Ahluwalia, 2021). They occur when endogenous and exogenous factors severely damage the barrier and repair capacity of gastric mucosa. Tissue necrosis is caused by vascular and microvascular damage, leading to mucosal ischaemia, hypoxia, cessation of nutrient and oxygen delivery, and free radical formation. TEs, both essential and non-essential, play an important role in various biological processes in living systems. Organic TEs are typically formed by binding to amino acids, proteins, or organic acids to create organic chelates or complexes. This structure enhances the stability of trace minerals in the digestive tract and increases their bioavailable, reducing the risk of toxicity and strengthening the immune system (Cui et al., 2024). These essential micronutrients are directly related to the metabolic and physiological processes of living organisms, and their excesses or deficiencies can lead to serious bodily dysfunction or, in the worst-case scenario death. TEs are present in the body in extremely small amounts but have powerful biological effects (Islam et al., 2023).

Studies have demonstrated that ROS are released from neutrophils during ethanol metabolism. This process causes endothelial damage and microcirculatory disturbances, thereby inhibiting the healing of gastric injury. It leads to apoptosis and depletion of the protective portion of the

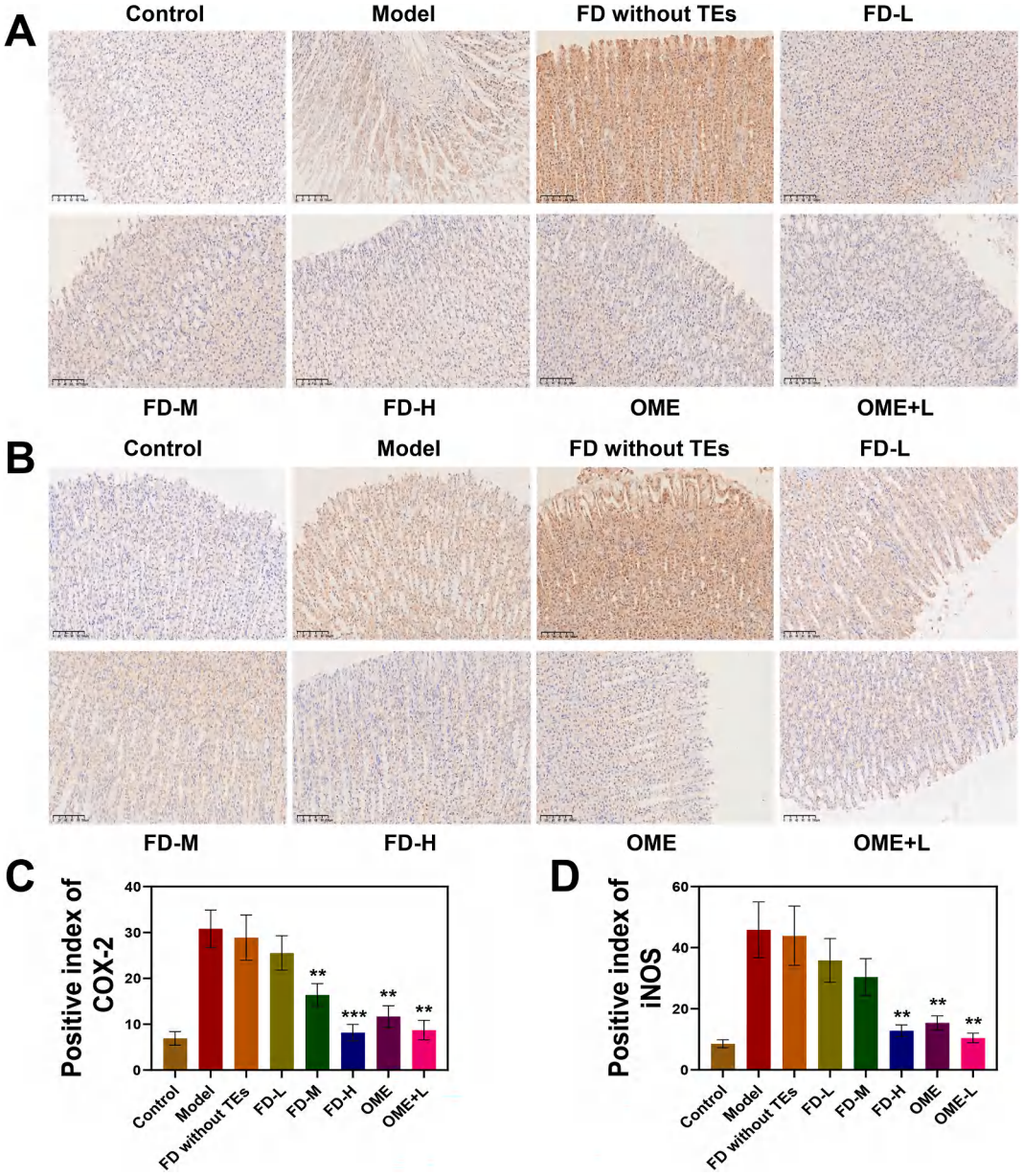

Fig. 6. Histopathological testing. (A and C) COX-2 ($n = 3$). (B and D) iNOS (n = 3) (scale bar = 100 μm). Compared with model group, ** P < 0.01, *** $P < 0.001$. COX-2: cyclooxygenase-2; iNOS: inducible Nitric oxide synthase.

mucosal cells. The metabolic reaction of ethanol, catalysed by alcohol dehydrogenase and xanthan oxidase, generates oxidative free radicals in gastric tissues, thereby enhancing lipid peroxidation (Vijayan et al., 2022). Additionally, numerous studies have shown that HGF, c-Met, and STAT3 receptors play crucial roles in embryonic development, organ morphogenesis, wound healing, and tissue repair. These roles are mediated through the activation of various signaling pathways involved in cell proliferation, motility, survival, differentiation, scattering and morphogenesis (Noriega-Guerra & Freitas, 2018).

Based on the antioxidant effect of metal elements, we have prepared FD containing a variety of TEs. This drink is characterized by its good taste, stable properties, strong antioxidant effect, and easy to consumption, using natural minerals as raw materials. The biosafety evaluation of FD is an essential part of beverage. Our team has previously demonstrated that TEs do not adversely affect liver and kidney function in rats, and they exhibit good biocompatibility and safety (Wang, Yin, et al., 2024). The present study further investigated the pro-healing effect of FD on ethanol-induced gastric ulcers in rats. The results from the rats experiments that FD increased the activities of antioxidant enzymes such as T-SOD, GSH and CAT, as well as the content of anti-inflammatory factor IL-10 in serum. It also decreased the levels of inflammatory factors TNF-α and IL-6 in serum, and MDA, COX-2, and iNOS in gastric tissues by promoting the expression of the HGF/c-Met/STAT3 pathway. Meanwhile, FD can effectively regulate the abundance and diversity of intestinal flora. Ultimately, FD reduced the severity of gastric mucosal injury caused by invasive factors, improved the morphological structure of gastric mucosal cells, enhanced the ability of oxidative stress, inhibited excessive inflammatory response, and thus achieved the effect of repairing gastric ulcer. In addition, the combination of OME and FD-L did not significantly affect the therapeutic efficacy of OME, but reduced its adverse effects. In conclusion, FD demonstrated a pro-healing effect on the ethanol-induced gastric ulcer model.

The fundamental beverages (FD without TEs) are rich in numerous beneficial components, including Taurine, Choline, Lysine, Sucralose, Pantothenic acid, Vitamin B6, and Vitamin B12. While some of these

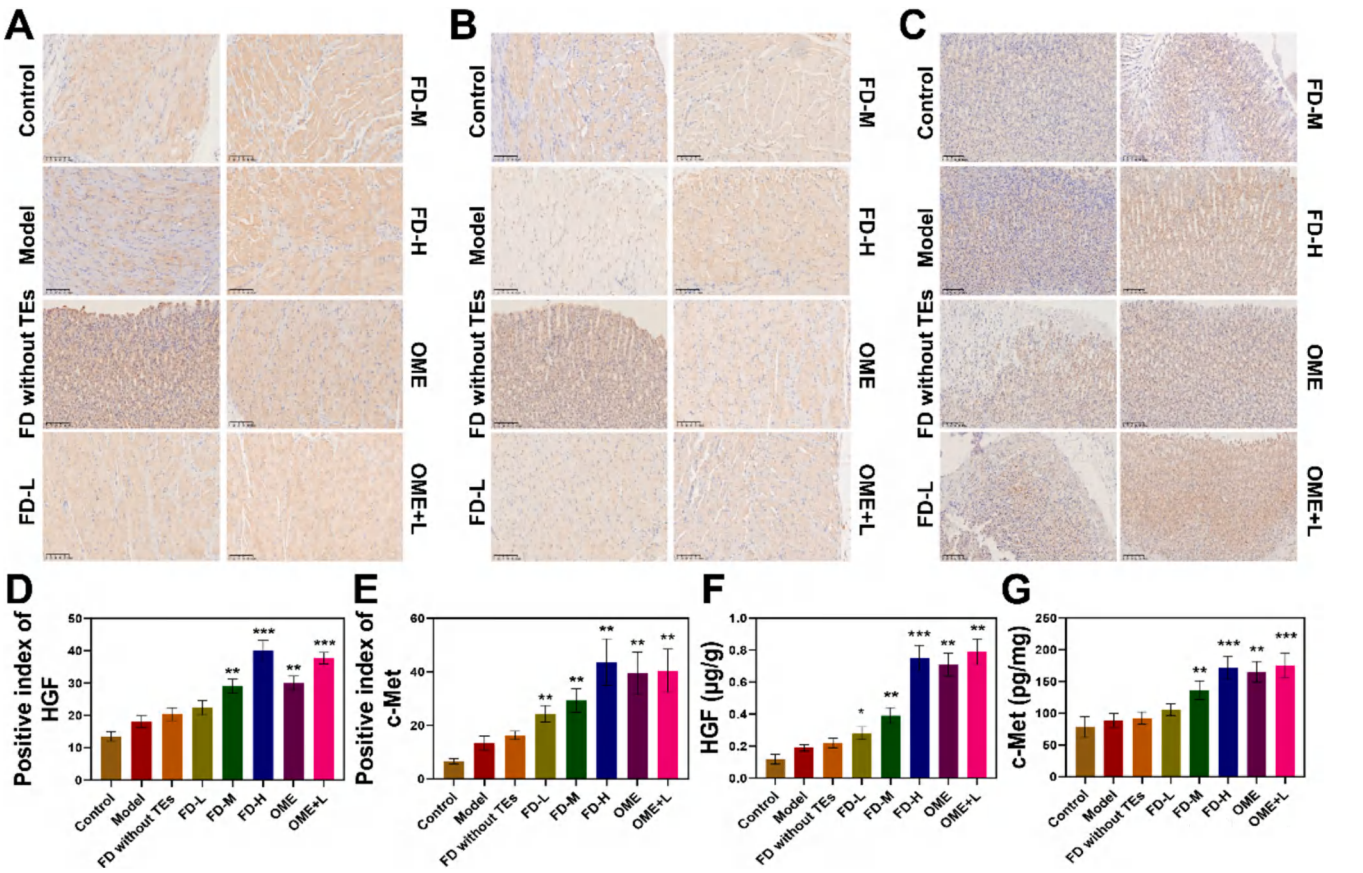

Fig. 7. Histopathological testing. (A, D, and F) HGF ($n = 3$). (B, E, and G) c-Met (n = 3). (C) STAT3 (scale bar = 100 μm). Compared with Model group, ** P < 0.01, *** $P < 0.001$. HGF: Hepatocyte growth factor; c-Met: c-Mesenchymal-epithelial transition factor; STAT3: Signal transducer and activator of transcription 3.

substances might theoretically facilitate the healing of gastric ulcers, they do not demonstrably exert a significant impact in practice. It is plausible that prolonged consumption is required to observe substantial benefits. We posit that the pro-restorative influence of FD is predominantly attributed to the inclusion of TEs. Although the other constituents within FD do not individually yield significant effects, the TEs interact synergistically with them to enhance the repair of the gastric mucosa.

However, this study also has some limitations. Although this FD demonstrates a favorable pro-healing effect, we did not conduct a trial-by-trial experiment with individual element. Of course, we have found some reports on the effectiveness of individual elements in the treatment of gastric ulcers. Zinc is an element that performs several functions in the body. It plays an important role in the activation of more than 300 enzymes and acts as an antioxidant in counteracting and inhibiting oxidative stress (Lee, 2018) (Wang, Liu, et al., 2024). Zinc L- carnosine has potent antioxidant, anti-inflammatory, and genome stability-enhancing effects and is used as a gastric ulcer treatment in Japanese clinics (Ooi et al., 2017). Currently, selenium is recognized as an essential TEs of importance to human health (Kieliszek, 2019) (Wang, Liu, et al., 2023). The antioxidant and anti-inflammatory activities of selenium and lecithin combination on gastric ulcers in ethanolic mice are mediated by modulating IGF-1/PTEN/Akt/FoxO3a signaling pathway (Youssef et al., 2024). Elemental selenium is a cofactor for many enzymes, is important in preventing oxidative stress, and exhibits the highest activity as a free radical scavenger and anti-cancer agent. Iron is a TEs with one of the most complete biological functions. The biological role of iron is characterized by the fact that it is indispensable in cellular respiration and various biochemical processes, providing normal functioning of the cells and organs. Iron also plays an important role in the production of free radicals (Milto et al., 2016). Iron therapy has a significant therapeutic effect on inflammatory diseases caused by oxidative stress (Loveikyte et al., 2023). Magnesium is a cofactor in more than 300 enzyme systems that regulate various biochemical reactions in the body. Magnesium is also essential for the structural function of proteins, nucleic acids, or mitochondria (Gröber et al., 2015). Clinical observation of magnesium aluminum carbonate combined with rabeprazole-based triple therapy in the treatment of gastric ulcer-related bleeding (P.-Z. Zhou et al., 2022). These studies have examined the effect of one or two TEs on the organism. But we have always considered life as a unified organism. Individual elements do not reflect real life situations. Multi-elements are still widely used as mandatory supplements. Secondly, this study explored the mechanism of FD antioxidant and anti-inflammatory damage only from an animal model, and its role and mechanism should be confirmed at the cellular level. In light of the above findings and limitations, we still need to carry out relevant experiments to study and explore the antiulcer mechanism of FD at a deeper level.

5. Conclusion

In this study, we developed a FD enriched with various TEs, characterized by its pleasant taste, stable properties, potent antioxidant capacity, and ease of consumption. Our rat experiments demonstrated that FD increased the activities of antioxidant factors (T-SOD, GSH, CAT, and IL-10), and decreased the levels of inflammatory factors (TNF-α, IL-6, MDA, COX-2, and iNOS) through the promotion the HGF/c-Met/STAT3 pathway expression. Concurrently, FD effectively regulate the abundance and diversity of intestinal flora. Ultimately, FD mitigated the severity of gastric mucosal injury caused by invasive factors, improved the morphological structure of gastric mucosal cells, enhanced the ability of oxidative stress, and bolstered excessive inflammatory response. Furthermore, the combination of OME and FD-L did not

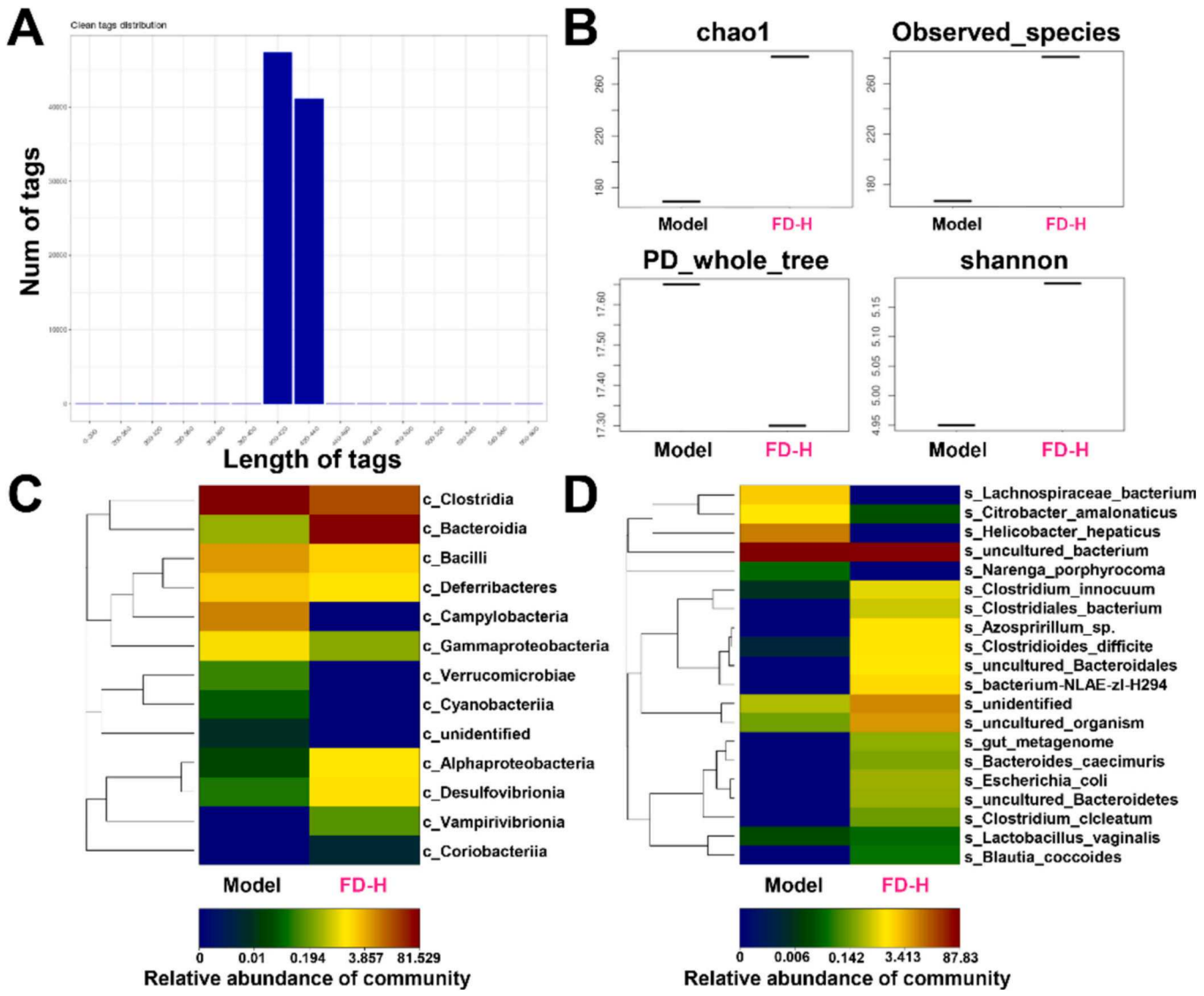

Fig. 8. Gut microbiota. (A) Sample cleanliness. (B) Microbiota diversity. (C) Heat map of phylum analysis. (D) Heat map of genus analysis.

significantly alter the therapeutic efficacy of OME, but reduced its adverse effects. Ultimately, FD showed a pro-healing effect on the ethanol-induced gastric ulcer model. In the future, we will investigate the therapeutic effects of individual elements like zinc, iron, and magnesium on gastric ulcers and reveal their therapeutic mechanisms.

Ethics statement

Male wistar rats (SPF, 6-week-old, 160–180 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Animal Licence No.: SYXK (Beijing) 2021-0050. The animals were housed and experimented in the SPF Grade Animal Experimentation Centre of the Institute of Pharmaceutical Research, Beijing Medical College, Beijing, China, under the following conditions: temperature (25 ± 2) °C, relative humidity less than 70 %, day and night alternation for 12 h, good ventilation, free feeding and watering of the animals, and the experiments were initiated after 1 week of acclimatization. All animal experiments were approved by the Laboratory Animal Ethics Committee (**00004407**) of the Institute of Pharmaceutical Sciences, Peking Union Medical College.

CRediT authorship contribution statement

Yongnan Piao: Visualization, Methodology, Investigation, Formal analysis, Data curation. **Nuoya Wang:** Methodology, Formal analysis. **Mingji Jin:** Formal analysis. **Jianyu Piao:** Formal analysis. **Mingfeng Han:** Formal analysis. **Zifei Wang:** Formal analysis. **Chunhua Quan:** Formal analysis. **Jishan Yin:** Resources, Methodology. **Zhonggao Gao:** Resources, Project administration, Funding acquisition. **Wenxiang Cui:** Validation, Supervision. **Shuangqing Wang:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Xiuquan Quan:** Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2025.106674.

Data availability

Data will be made available on request.

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