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Anti-ulcer Potential of Myco-fabricated Copper Oxide and Ferric Oxide Nanoparticles Against Ethanol Induced Gastric Ulcers in Male Albino Rats

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Abstract

Gastric ulcers are a prevalent stomach disease that requires timely medical attention and appropriate treatment to prevent different complications. In this study, the protective effects of myco-fabricated copper oxide nanoparticles (CuO-NPs) and Iron Oxide nanoparticles (Fe_3O_4-NPs) against ethanol-induced acute gastric ulcers in rats were assessed. The rats were administered ethanol *via* gavage to create an acute gastric ulcer model, after which serum and stomach tissue samples were collected for biochemical analysis. The results indicated that pretreatment with CuO-NPs and Fe_3O_4-NPs significantly reduced the gastric ulcer index and increased the ulcer prevention rate. These findings suggest that both types of nanoparticles offer protection against gastric ulcers. Moreover, CuO-NPs and Fe_3O_4-NPs exhibited antioxidant properties by decreasing malondialdehyde levels and increasing the activity of superoxide dismutase and nitric oxide, as well as anti-inflammatory effects by reducing tumor necrosis factor-α, interleukin-6, interleukin-1β, myeloperoxidase levels, and increasing prostaglandin E2 levels in serum and gastric tissue, as confirmed by histopathological studies. Both CuO-NPs and Fe3O4-NPs improved ethanol-induced acute gastric ulcers in rats, primarily through their antioxidant and anti-inflammatory activities. These findings suggest the protecting and therapeutic roles of the myco-fabricated CuO-NPs and Fe_3O_4-NPs from natural and green approaches.

Keywords: Gastric Ulcer; Metal Oxide; Nanoparticles; Myco-fabricated; Albino Rats.

1. Introduction

Gastric ulcer (GU) is a prevalent gastrointestinal disorder that affects people globally. It is estimated that approximately 8–10% of the world's population suffers from peptic ulcers, with gastric ulcers accounting for 5% of these cases [1]. GU has diverse causes, primarily stemming from an imbalance between protective factors and aggressive gastric elements, including physical, chemical, or physiological stressors such as alcohol overconsumption, tobacco use, or excessive NSAID intake for conditions like fever and migraine, leading to oxidative stress and subsequent ulceration in gastrointestinal tissues [2, 3]. Other significant factors include alcohol consumption and *Helicobacter pylori* infection, with *H. pylori* cytotoxin contributing substantially to cellular vacuolization and mucosal injury through the release of lipopolysaccharides and chemotactic proteins [4]. Copper (Cu) is one of the essential trace elements found in the biological system mostly in the cupric form (Cu^{++}) with maximum concentrations in the liver and brain. It is essential for many important biological functions such as myelin production and maintenance, melanin production, synthesis of collagen and elastin, and more [5]. Additionally, copper has been shown to have medicinal effects for cardiovascular diseases and bone healing, in addition to its antitumor and antibacterial activity [6]. Copper also acts as an antioxidant and pro-oxidant; It scavenges free radicals as an antioxidant and promotes free radical stress in Alzheimer's disease as a prooxidant [7]. Recent advancements in nanotechnology have facilitated the production of copper nanoparticles (CuO-NPs) at the nanoscale. Nanoparticles possess the ability to accumulate in damaged or inflamed tissues due to their size and the permeability of injured vasculature, thereby exerting therapeutic effects through various mechanisms [8-12]. Environmentally friendly methods for nanoparticle synthesis, particularly using fungi have gained tremendous attraction [13-20]. Biosynthesis of CuO-NPs *via* natural microorganisms such as microbial metabolites, notably endophytes, is considered sustainable and eco-friendly compared to conventional chemical methods. This approach has sparked interest in exploring CuO-NPs for their potential antimicrobial, antioxidant, and anticancer properties, aiming to develop novel therapeutic strategies for various diseases, including gastric ulcers induced by ethanol [21].

Several fungal strains, including *Stereum hirsutum*, *Trichoderma asperellum*, *Aspergillus fumigatus*, and *Aspergillus terreus*, have been utilized for the synthesis of CuO-NPs [22-25]. Iron, another essential element crucial for hemoglobin, myoglobin, and enzymatic functions, plays a vital role in maintaining health. Iron oxide nanoparticles, particularly Fe_3O_4, have been extensively used in biomedical applications due to their antibacterial, antifungal, antiviral, and anti-inflammatory

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properties [26]. The use of biological methods, such as enzymes, microorganisms, and plant extracts, for synthesizing iron oxide nanoparticles (Fe_3O_4) has further enhanced their appeal for biomedical uses, improving drug stability and tissue permeability and thereby reducing required dosages [27]. Iron oxide nanoparticles have shown promise in treating diseases associated with *H. pylori* [28]. In this study, our objective was to evaluate the efficacy ofmyco-fabricated CuO-NPs and Fe_3O_4-NPs as green agents in protecting against ethanol-induced gastric ulcers in rat models.

1. Experimental

2.1. Animals

The experiments were carried out on a total of 60 male albino rats weighing 180–200 g and aged 9–10 weeks. Rats were kept in our animal house for one week before the experimental study and were fed on a normal rat diet with water available ad libitum. After one week of acclimation, rats were divided into six groups. The care and treatment of the animals were carried out under the supervision of the biology department, and nuclear research center guide for animals.

The research protocol with serial number F/85A/23 to oversee and monitor experimental animals was approved by the National Centre for Radiation Research and Technology's Research Ethics Committee.

2.2. Copper oxide and Ferric oxide nanoparticles

Synthesis of CuO-NPs and Fe_3O_4-NPs was carried out according to a previous study using gamma irradiated *Aspergillus terreus* ORG-1 fungus [25, 29]. The synthesized NPs were characterized using several techniques including UV-Vis spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction, and transmission electron microscopy according to the previous study [25]. CuO-NPs and Fe_3O_4-NPs mean sizes of 18.95 and 32.41 nm, respectively with a hexagonal crystal structure [25].

2.3. Experimental Groups

Rats were equally divided into six groups.

1st group: The control group included rats administered 1 ml distilled water orally daily throughout the experiment.

2nd group: The induction group included rats that fasted for 48 hours before administration of ethanol (5ml/kg) orally on the last day of the experiment to induce experimentally acute stomach ulcer.

3rd group: CuO-NPs group included rats that received CuO-NPs (10 mg/kg /day) orally for three weeks.

4th group: Fe_3O_4-NPs group included rats that received Fe_3O_4NPs (0.4 mg/kg /day) orally for three weeks.

5th group: included rats that received CuO-NPs (10mg/kg /day) orally for three weeks then gastric ulceration was induced on the last day of the experiment by intubating 80% ethanol (5ml/kg).

6th group: included rats that received Fe_3O_4-NPs (0.4 mg/kg /day) orally for three weeks then gastric ulceration was induced on the last day of the experiment by intubating 80% ethanol (5ml/kg).

2.4. Establishment of ethanol-induced gastric ulcer model

Rats in groups other than the control, CuO-NPs, and Fe_3O_4-NPs groups were administered 5 ml/kg ethanol (Sigma-Aldrich, USA) gavage to induce acute gastric ulcers. Two hours later, the rats were sacrificed by cervical dislocation. Blood and gastric tissues were collected for analysis. Tissue samples were centrifuged to obtain serum for biochemical evaluation. Stomach tissues were homogenized and centrifuged, with the supernatant used for biochemical analysis. Additional samples were fixed in formal saline, processed, embedded, and sectioned for histological examination.

2.5. Histopathological examination

Gastric tissues were fixed in 10% formalin for over 48 hours, dehydrated in alcohol, embedded in paraffin, and sliced into 4μm thick sections. Hematoxylin and eosin (H&E) staining was performed, and pathological changes were observed under an optical microscope.

2.6. Gross observation of gastric mucosa and assessment of gastric tissue injury

The stomachs were opened, washed with cold saline, dried, and photographed. The gastric ulcer index (GUI) was used to assess ulcer size, with scores ranging from 0 (no injury) to 5 (ulcer >4mm). The inhibition rate of gastric ulcers was calculated as [(GUI of ethanol group − GUI of the treated group) / GUI of ethanol group] × 100.

2.7. Identification of biochemical indexes associated with oxidative stress

Gastric homogenate was prepared by mixing rat gastric tissue with cold PBS at a ratio of 1 to 9.

The homogenate was centrifuged for 15 min at 5000 rpm and 4°C. The supernatant was collected and frozen at −80°C. SOD and MDA activities were determined according to the kit manufacturer's method.

2.8. Identification of Biochemical Indexes Associated with Inflammatory Reaction

Two hours after the sample was fixed with ethanol, rats' blood was collected from the orbit and centrifuged at 4°C 3500rpm/min for 15min, then the supernatant was collected and frozen at −80°C. Levels of TNF-α were resolute using ELISA kit (Cusabio Biotech Co., Ltd), IL-6, IL-1β, IL-2, IL-4, IL-10, were detected in both serum and stomach contents by using enzyme-linked assay (ELISA), according to equipment instructions (Bio-Tehne Ltd, R&D systems, Inc., USA). MPO and PGE2 were assessed using enzyme-linked assay (ELISA), according to equipment instructions (Cusabio Biotech Co., Ltd) and NO were determined.

2.9. Statistical analysis

Statistical investigation of the obtained results was performed using ONE-WAY ANOVA ($P<0.05$) and arranged as Duncan's multiple sequences (Brown et al. 2005) using SPSS software (version 15, IBM, USA).

3. Results

3.1. Impact of CuO-NPs and Fe_3O_4-NPs on alteration of pro-inflammatory cytokines

We examined the activity of myeloperoxidase, a biochemical marker of neutrophil infiltration, after ethanol induction. As shown in Table 1, the MPO activity in the stomach increased significantly after ethanol administration; this confirmed that ethanol induces neutrophil activation and infiltration. Rats treated with CUONPs and Fe_3O_4-NPs significantly reduced MPO activity compared with the control group. To better assess this, for example, we measured the levels of key inflammatory factors e.g., tumor necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1β (IL-1β) in gastric homogenates to evaluate anti-inflammatory activity of CUO-NPs and Fe_3O_4-NPs. Cytokine levels in gastric tissues increased significantly in rats with severe ethanol-induced gastric damage. Interestingly, the pretreatment with CuO-NPs and Fe_3O_4-NPs effectively reduced TNF, IL-6, and IL-1β in the gastric tissue. This study shows that CuO-NPs and Fe_3O_4-NPs have physiological gastro protective effects that help reduce neutrophil infiltration and inflammation.

Table 1: Effect of CuO-NPs and Fe_3O_4-NPs on alteration of pro-inflammatory cytokines and in stomach rats induced by ethanol

Groups	IL-1B (pg/ml)	MPO (ng/ml)	IL-6 (pg/ml)
G1	204.00 ± 8.15^{d}	0.45 ± 0.08^{b}	251.00 ± 3.29^{b}
G2	929.35 ± 85.88^{a}	16.50 ± 3.04^{a}	1001.50 ± 3.08^{a}
G3	265.33 ± 14.84^{bc}	0.59 ± 0.19^{b}	224.67 ± 6.92^{d}
G4	272.33 ± 8.41^{b}	0.65 ± 0.16^{b}	234.83 ± 3.97^{c}
G5	236.83 ± 5.12^{bcd}	1.01 ± 0.13^{b}	251.67 ± 6.09^{b}
G6	229.00 ± 5.55^{cd}	1.23 ± 0.36^{b}	248.50 ± 6.28^{b}

The calculated mean is for triplicate measurements from two independent experiments ± SD, means with different superscripts in the same column for each nanoparticle are considered statistically different (LSD test, $P \leq 0.05$).

3.2. Impact of CuO-NPs and Fe_3O_4-NPs on gastric PGE-2 level and INOS contents.

Ethanol administration showed a significant reduction in the gastroprotective PGE-2 and INOS compared to the control group (Table 2). However, pretreatment with CuO-NPs and Fe_3O_4-NPs significantly increased the levels of PGE-2 and INOS compared with the induction group. Also, the results of the present study revealed a significant increase ($P < 0.05$) in TNF level (G2), compared to the other groups (Table 2). These alternations were almost returned to normalcy in rats supplemented with CuO-NPs and Fe3O4-NPs.

Table 2: Effect of myco-fabricated CuO-NPs and Fe_3O_4-NPs on gastric PGE-2 level and INOS content.

Groups	PGE-2 (pg/ml)	TNF (pg/ml)	INOS (ng/ml)
G1	53.08 ± 5.73^{a}	257.83 ± 5.64^{d}	7.66 ± 0.79^{a}
G2	10.32 ± 1.45^{d}	906.50 ± 13.94^{a}	0.53 ± 0.13^{c}
G3	43.33 ± 5.51^{b}	304.67 ± 10.50^{c}	8.02 ± 1.05^{a}
G4	37.53 ± 5.66^{c}	307.00 ± 12.93^{c}	6.24 ± 1.32^{b}
G5	46.44 ± 3.93^{b}	296.17 ± 8.45^{c}	5.33 ± 1.45^{b}
G6	43.42 ± 3.24^{b}	327.33 ± 7.69^{b}	5.51 ± 1.21^{b}

The calculated mean is for triplicate measurements from two independent experiments ± SD, means with different superscripts in the same column for each nanoparticle are considered statistically different (LSD test, $P \leq 0.05$).

3.3. Impact of CuO-NPs and Fe_3O_4-NPs on oxidative stress and antioxidant indicators.

Ethanol-induced gastric damage is often associated with oxidative stress in the gastrointestinal tract. Therefore, we evaluated the levels of oxidative biomarkers NO, MDA, and SOD in gastric homogenates (Table 3). Ethanol administration induced oxidative stress, as evidenced by a significant increase in MDA levels ($p>0.05$) and an accompanying significant decrease in SOD and NO activity compared to control rats ($p>0.05$). While pretreatment with CuO-NPs and Fe_3O_4-NPs significantly reduced the length of MDA in gastric cells.

Table 3: Effect of myco-fabricated CuO-NPs and Fe3O4-NPs on oxidative stress and antioxidant indicators in stomach rats administrated with ethanol

Groups	MDA (nmol/ml)	NO (umol/ml)	SOD (u/ml)
G1	0.45 ± 0.18^{d}	92.07 ± 7.40^{a}	218.58 ± 4.86^{a}
G2	17.88 ± 0.71^{a}	10.01 ± 1.70^{d}	20.45 ± 1.57^{f}
G3	0.98 ± 0.13^{c}	73.45 ± 5.52^{b}	177.14 ± 7.65^{b}
G4	0.91 ± 0.13^{c}	89.06 ± 5.15^{a}	156.05 ± 5.58^{c}
G5	4.09 ± 0.33^{b}	42.92 ± 3.79^{c}	132.29 ± 10.84^{d}
G6	4.24 ± 0.30^{b}	70.34 ± 5.86^{b}	106.38 ± 11.06^{e}

The calculated mean is for triplicate measurements from two independent experiments ± SD, means with different superscripts in the same column for each nanoparticle are considered statistically different (LSD test, $P \leq 0.05$).
Means with different superscripts in the same column are considered statistically different (LSD test, $P \leq 0.05$).

3.4. Impact of CuO-NPs and Fe_3O_4-NPs on pH and total acidity of the stomach.

As shown in Table 4, rats with gastric lesions showed a significant decrease in pH (Figures 1, 2 and 3) compared to the normal control group. However, pretreatment with CuO-NPs and Fe_3O_4-NPs was able to increase the pH value (Table 4) in the gastric lesions of rats compared with the control group. The results showed that CuO-NPs and Fe_3O_4-NPs ameliorated ethanol-induced elevation in gastric pH. Moreover, both CuO-NPs at (10 mg/kg) and Fe_3O_4-NPs at (0.4 mg/kg) dose did not show any reduction in total acidity compared to the negative control.

Table 4: Effect of myco-fabricated CuO-NPs and Fe_3O_4-NPs on pH and total acidity in the stomach of rats administrated with ethanol.

Groups	pH	Total acidity
G1	2.73 ± 0.25^{ab}	292.45 ± 6.64^{bc}
G2	1.24 ± 0.23^{d}	358.69 ± 38.72^{a}
G3	2.66 ± 0.38^{ab}	275.33 ± 21.67^{c}
G4	2.93 ± 0.58^{a}	323.00 ± 55.97^{ab}
G5	2.09 ± 0.07^{c}	310.17 ± 14.15^{bc}
G6	2.49 ± 0.23^{b}	304.00 ± 9.70^{bc}

The calculated mean is for triplicate measurements from two independent experiments ± SD, means with different superscripts in the same column for each nanoparticle are considered statistically different (LSD test, $P \leq 0.05$).

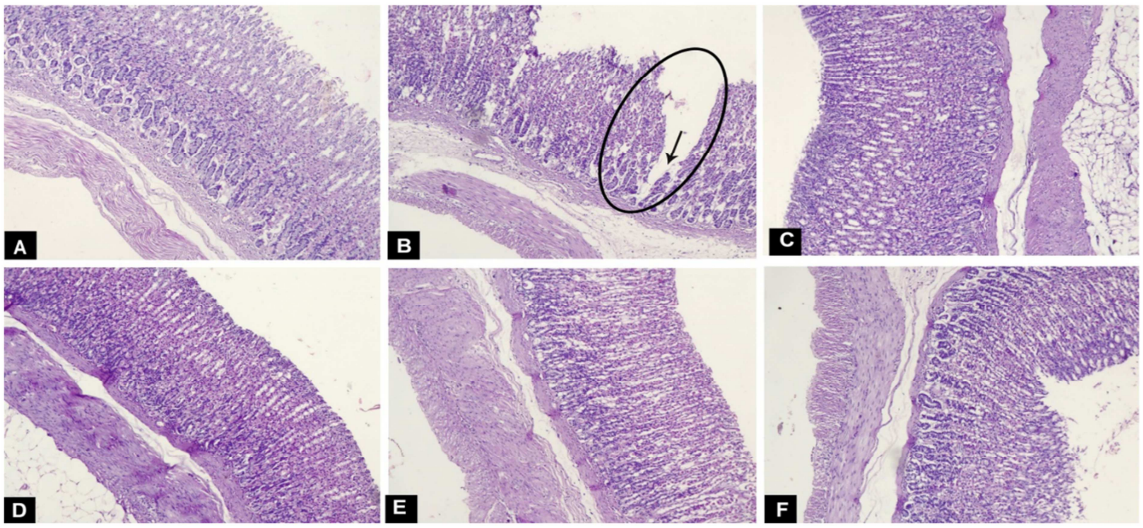

Figure 1: Representative H&E photomicrograph of the gastric sections from different studied treated groups. (A) the stomach of the control group showed normal mucosal lining; and tightly long-packed tubular fundic gastric glands (Gg) that were composed of isthmus (I), middle neck (N), and deep base(B). Muscularis mucosa (Mm) is observed. Gastric glands open into the lumen through gastric pits (curved arrow) (B) The stomach of an ethanol-treated animal revealed ulcer formation (oval shape) extending up to the base associated with necrosis (arrow). (C) the stomach of animals treated with CuO-NPs group showed normal mucosal lining with intact isthmus (I), neck (N), and base (B) of the gastric gland. (D) the stomach of animals treated with Fe_3O_4-NPs showed normal mucosal lining. (E) stomach of animals treated with ethanol + CuO-NPs revealed the disappearance of degeneration of the gastric glands and mostly were near to normal architecture of the histological gastric structures. (F) stomach of an animal treated with ethanol + Fe_3O_4-NPs revealed a marked decrease in degeneration; however, the gastric mucosa still has degeneration (arrow).

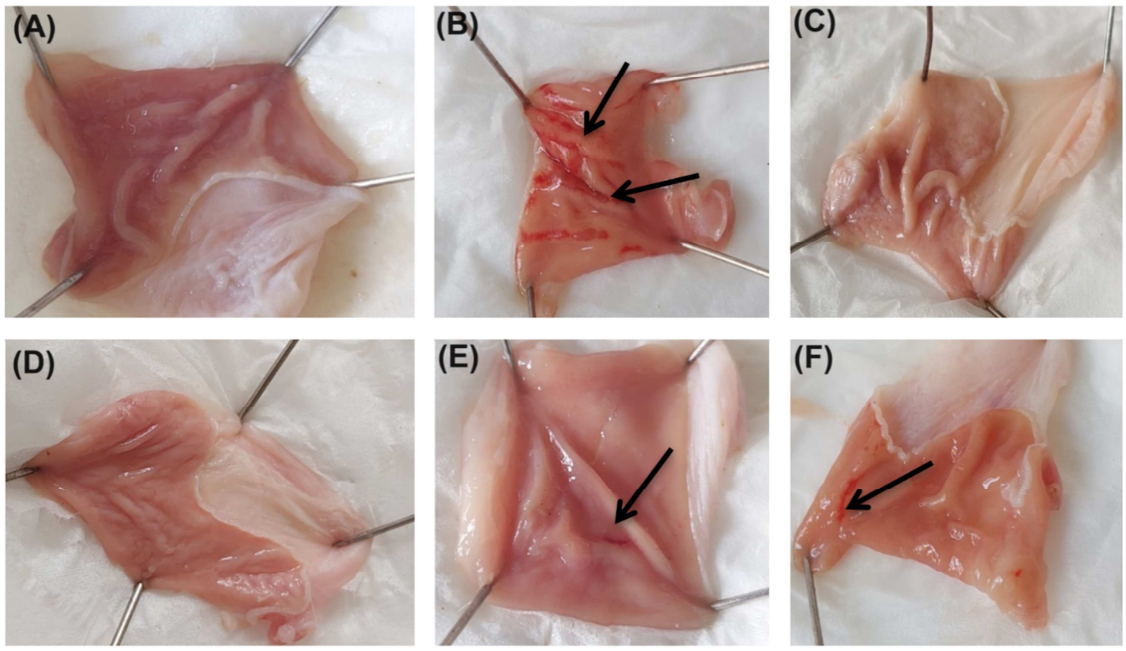

Figure 2: Protective effects of CuO-NPs and Fe_3O_4-NPs on ethanol-induced gastric mucosal lesions in rats. **(A–F)** Representative stomach images from each group. Rats were pretreated orally with distilled water **(A)**, rats administered ethanol 5ml/kg **(B),** rats received CuO-NPs 10 mg/kg **(C),** rats received Fe3O4-NPs 0.4 mg/kg /day **(D),** rats received CuO-NPs (10mg/kg /day) for three weeks then gastric ulceration was induced **(E),** rats received Fe_3O_4-NPs (0.4 mg/kg /day) orally for three weeks then gastric ulceration was induced **(F)**.

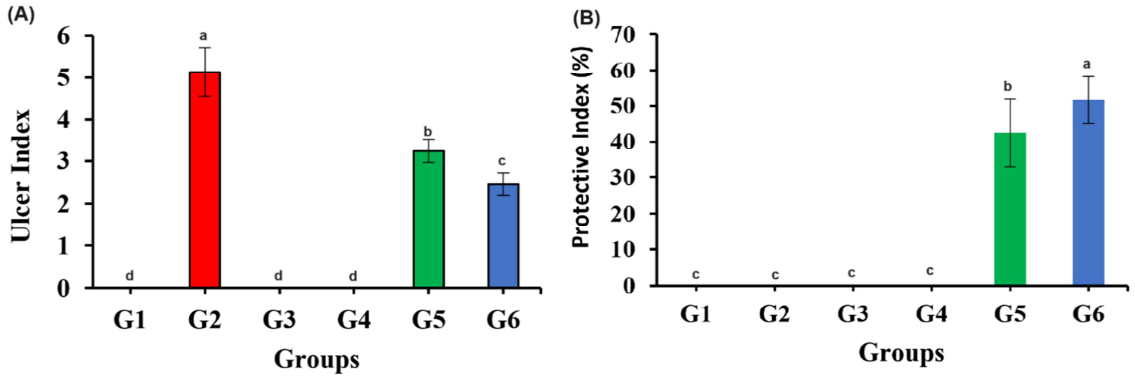

Figure 3: Ulcer index (A), and Protective index (B) of CuO-NPs and Fe3O4-NPs on ethanol-induced gastric mucosal lesions in rats.

4. Discussion

Finding medications and health supplements for treating stomach ulcers remains a significant and challenging area of medical research. Hence, this study focused on evaluating the potential of CuO-NPs and Fe_3O_4-NPs as therapeutic candidates to mitigate gastric ulcers. We employed an experimental model induced by 80% ethanol (5ml/kg) to simulate gastric ulcer conditions, which closely mimics human gastric ulcer pathophysiology and aids in assessing the anti-ulcer properties and underlying mechanisms of potential drugs [30-32]. Ethanol is a prominent cause of gastric ulcers, primarily due to its dehydration effects on mucosal cell barriers and its cytotoxicity, which triggers ROS release from leukocytes and inflammatory cytokines [33, 34].

The study revealed that CuO-NPs and Fe_3O_4-NPs effectively reduced ethanol-induced gastric lesions in rats. Specifically, these nanoparticles decreased the incidence of gastric ulcers, enhanced their prevention, and ameliorated gastric mucosal damage. Beyond clinical observations, the study also investigated the impact of CuO-NPs and Fe_3O_4-NPs on oxidative and inflammatory markers. In normal physiological conditions, the balance between oxidants and antioxidants is maintained by various enzymatic systems [35]. Imbalances, characterized by decreased antioxidant enzyme activities like SOD and increased reactive oxygen species such as MDA, are associated with gastric ulcer development [36]. SOD, a potent antioxidant enzyme, converts harmful superoxide into less reactive substances, whereas excessive ROS can lead to lipid peroxidation and cellular damage, notably high MDA levels, which severely affect gastric mucosa integrity [37-41]. The study found that pretreatment with CuO-NPs and Fe_3O_4-NPs significantly increased SOD activity and reduced MDA content, highlighting their antioxidant potential [42, 43].

Inflammation represents another critical mechanism in ethanol-induced gastric injury in rats [44, 45]. Oxidative stress in gastric tissues triggers neutrophil infiltration and upregulates inflammatory cytokines like TNF-α, IL-6, and IL-1β [46]. TNF-α, in particular, exacerbates gastric ulceration by inducing neutrophil accumulation and disrupting gastric microcirculation, while elevated TNF-α levels promote further cytokine release, intensifying inflammation [47]. MPO serves as a marker for neutrophil infiltration, and decreased MPO activity suggests anti-inflammatory effects in experimental models [48]. The study demonstrated that CuO-NPs and Fe_3O_4-NPs pretreatment significantly reduced TNF-α, IL-6, IL-1β, and MPO levels in serum and gastric tissues, consistent with previous findings [49].

Protective factors like PGE2 and NO are crucial for gastric mucosa integrity, promoting mucus secretion, blood flow maintenance, and reducing gastric acid secretion [50]. PGE2 also aids in gastric cell proliferation and mucosal repair [51]. CuO-NPs and Fe_3O_4-NPs were found to increase NO and PGE2 levels in rat gastric mucosa, thereby protecting against mucosal damage induced by ethanol [52]. Despite NO's role in ulcer formation, it plays a pivotal role in ulcer healing by eliminating damaged cells from regenerating mucosa [53]. Thus, CuO-NPs and Fe_3O_4-NPs exhibit gastroprotective effects through antioxidant and anti-inflammatory mechanisms, modulating the iNOS pathway.

The study also assessed gastric pH levels across experimental groups, noting significant increases in acidity compared to controls. Ethanol administration induced gastric mucosal damage, contributing to elevated acidity and lower pH levels. However, groups treated with CuO-NPs or Fe_3O_4-NPs showed acidity increases but maintained pH levels closer to normal, indicating protective effects against ethanol-induced ulceration. The nanoparticles likely interacted with gastric HCl to form $CuCl_2$ or $FeCl_3$, which have acidic pH values close to gastric pH. This interaction could explain the observed histopathological improvements in gastric mucosa integrity in treated groups [54].

5. Conclusions

Oral administration of CuO-NPs and Fe3O4-NPs at specific doses effectively protected against ethanol-induced gastric ulcers by maintaining pH levels close to normal and exerting antioxidant and anti-inflammatory effects. Overall, myco-fabricated CuO-NPs and Fe3O4-NPs demonstrated promising therapeutic potential in healing acute gastric ulcers in experimental rat models. The results of this study support the use of a dose of CuO-NPs at (10 mg/kg) and Fe3O4-NPs at (0.4 mg/kg) in the prevention of gastric ulcers. Therefore, more research is needed. Consequently, these findings may pave the way for their possible applications in healing acute gastric ulcers.

6. Conflicts of interest
There are no conflicts to declare".

7. Author contribution
Doaa S. Mansour, Reham M. Morsi, Hoda A. Mansour, Salwa N.A. Mater, H. M. Essam, and Shaimaa A. Mousa experimental methodology design, experimental, data analysis, original draft writing, manuscript revision, and proofreading. El-Sayed R. El-Sayed research topic suggestion, experimental methodology design, experimental, data analysis, original draft writing, manuscript revision, and proofreading. All authors read and approved the manuscript.

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