Novel role of Zn(II)–curcumin in enhancing cell proliferation and adjusting proinflammatory cytokine-mediated oxidative damage of ethanol-induced acute gastric ulcers

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ABSTRACT

Alcohol consumption can induce gastric ulcers and zinc deficiency. Zinc complexes were reported to have anti-ulcer activity as it acts as an anti-inflammatory and antioxidant. Zn(II)–curcumin complex and its solid dispersions (SDs) were synthesized and evaluated for its gastroprotective activity and mechanism against ethanol-induced ulcer. The Swiss murine fibroblast cell line (3T3) was used as an alternative in vitro model to evaluate the effects of Zn(II)–curcumin on cell proliferation. Zn(II)–curcumin were administered orally for seven consecutive days prior to induction of ulcers using ethanol. Gross and microscopic lesions, immunological and biochemical parameters were taken into consideration. The results showed that solid dispersions (SDs) of Zn(II)–curcumin (2.5–20 μM) enhanced the proliferation of 3T3 cells more significantly than curcumin at the same concentrations (P < 0.01). Oral administration of Zn(II)–curcumin (12, 24 and 48 mg/kg) SDs dose-dependently prevented formation of ulcer lesions induced by ethanol. The levels of proinflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and oxidative stress superoxide dismutase (SOD), glutathione peroxidase (GPX-Px), malonaldehyde (MDA) and H^+–K^+–ATPase were in the rats exposed to ethanol in ulceration have been altered. Zn(II)–curcumin prevented formation of ulcer lesions, significantly inhibited TNF-α and IL-6 mRNA expression, increased the activity of SOD and GSH-Px, reduced MDA levels and H^+–K^+–ATPase in mucosa of rats compared to controls (P < 0.05). These findings suggest that the gastroprotective activity of Zn(II)–curcumin complex might contribute in stimulating cell proliferation and adjusting the proinflammatory cytokine-mediated oxidative damage to the gastric mucosa.

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1. Introduction

Alcohol consumption is an independent risk factor for ulcers and increases the risk of ulcers in patients taking non-steroidal anti-inflammatory drug (NSAID) therapy [1]. Proinflammatory cytokines and oxidative damage are two major etiological factors that are suggested to play important roles in the development of ulcer [2]. It was suggested that proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) play key roles in the acute phase inflammation as well as in maintenance and regulation of the severity of gastric ulcers [3]. Inflammatory responses are accompanied by increased production of reactive oxygen species (ROS). ROS generation in mitochondria is believed to play a important role in TNF-induced cytotoxic activity [4].

Alcohol abuse is frequently associated with zinc deficiency, impaired zinc absorption is another likely cause of zinc deficiency in alcoholics [5]. Zinc is essential for cell proliferation, differentiation, and viability. Zinc deficiency can cause poor wound healing, elevation in ROS stress, induces oxidative damage and alterations in the antioxidant defense system [6]. Zinc complexes were reported to have anti-ulcer activity as it acts as an anti-inflammatory and antioxidant [7,8]. One type of protein/energy supplement containing zinc (30 mg) significantly improved the rate of ulcer healing in patients with stage 2, 3 or 4 pressure ulcers [9]. Polaprezinc (zinc l-carnosine), a novel anti-ulcer drug, protected against colonic mucosal injury through induction of heat shock protein 72 and suppression of nuclear factor-kappa B (NF-kB) activation, and is commonly used for the treatment of gastric ulcers in Japan [10].

In traditional medicine, perennial herb Curcuma longa, a condiment which is also known as turmeric that gives a distinctive flavor to curry, has been used to treat gastrointestinal disorders [11]. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hept-
significantly reduced the gastric volume, free acidity and total acid-dose-dependently inhibited the formation of gastric lesions, and surface area). Compared to curcumin at 24 mg/kg, Zn(II)–curcumin and 68 mg zinc, respectively, in adult humans based on the body treated to rats in a pylorus-ligature induced gastric ulcer model SDs of Zn(II)–curcumin (12, 24 and 48 mg/kg, p.o.) were adminis-<sup>1</sup> We also synthesized Zn(II)–curcumin, a mononuclear (1:1) zinc complex of curcumin, and prepared SDs of Zn(II)–curcumin and PVP in a ratio of 1:6, to improve the dissolution of Zn(II)–curcumin. SDs of Zn(II)–curcumin (12, 24 and 48 mg/kg, p.o.) were adminis-<sup>2</sup>serum albumin (BSA) as a standard. One milliliter of Bradford re-<sup>3</sup>bovine serum (FBS), 50 g/mL streptomycin and 50 U/mL penicillin<br>at 4 °C for 20 min. The supernatants were then used after the rats were sacrificed, the stomachs were rapidly re-<sup>4</sup>Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the major active constituent of Curcuma loga. Administration curcumin (20, 40 and 80 mg/kg) exerts its antiulcer activity not only by affecting oxidative stress and total antioxidant capacity but also by inhibiting IL-6 secretion and preventing apoptosis on experimental gastric ulcer in rats [12]. Curcumin protects ethanol ulcer by its potent antioxidant activity through scavenging of ROS and inhibition of TNF-α expression [13].

The maximum solubility of curcumin is reported to be 11 ng/mL in plain aqueous solutions; therefore, curcumin is only slightly absorbed in the gastrointestinal tract [14]. The oral bioavailability of curcumin is very low, and is approximately only 1% in rats [15]. In our previous work, SDs of curcumin were prepared by drying a co-sprayed mixture of curcumin and polyvinylpyrrolidone (PVP) to improve the dissolution and absorption in vivo [16]. SDs of curcumin (equivalent to curcumin 10, 30 and 90 mg/kg, p.o.) have exhibited potent anti-gastric ulcer effects in different rat ulcer models [17]. We also synthesized Zn(II)–curcumin, a mononuclear (1:1) zinc complex of curcumin, and prepared SDs of Zn(II)–curcumin and PVP in a ratio of 1:6, to improve the dissolution of Zn(II)–curcumin. SDs of Zn(II)–curcumin (12, 24 and 48 mg/kg, p.o.) were adminis-trated to rats in a pylorus-ligature induced gastric ulcer model (equivalent to 135, 270 and 340 mg Zn(II)–curcumin and 17, 34 and 68 mg zinc, respectively, in adult humans based on the body surface area). Compared to curcumin at 24 mg/kg, Zn(II)–curcumin dose-dependently inhibited the formation of gastric lesions, and significantly reduced the gastric volume, free acidity and total acidity (P < 0.05) [18]. The aim of this study was to investigate the effect-iveness of Zn(II)–curcumin in the treatment of ethanol-induced acute gastric ulcers, and determine the effect of Zn(II)–curcumin on cell proliferation, regulation the expression of proinflammatory cytokines TNF-α and IL-6 and oxidative damage.

2. Materials and methods

2.1. Experimental animals

Male adult Sprague–Dawley rats (6–7 weeks old, 200–250 g) were housed in a facility at the Laboratory of Traditional Chinese Medicine and Marine Drugs, School of Life Sciences, Sun Yat-sen University which is approved by the Guangdong Experimental Ani-mals Association (Guangzhou, China). Animals were housed in a storage room under conditions of constant temperature (23 ± 1 °C), relative humidity of 50 ± 5% and under a 12:12 h light–dark cycle (lights on at 07:00 h) until the start of the experiment. The animals were maintained on a standard pellet diet and water ad libitum. All procedures regarding animal care and use were car-ried out based on the guidelines of the animal ethics committee of Sun Yat-sen University (Guangzhou, China). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Chemicals

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] and Zn(II)–curcumin (greater than 99% purity) were manufactured by the Guangdong Zhongda Greenfield Bio-tech Co., (Guangzhou, China). PVP was purchased from BASF Chemical Ltd., (New Jersey, USA). Lansoprazole tablets were obtained from Shengfan Pharmaceutical Company Limited (Henan, China). Peni-cillin and streptomycin were purchased from Haibei Pharmaceuti-cal Co., (Shijiazhuang, China).

2.3. 3T3 cell culture and proliferation assays

3T3 Swiss murine fibroblasts (CCL-92; ATCC) were obtained from the School of Life Sciences, Jinan University (Guangzhou, China), and cultured in Dulbecco’s Modified Eagle Medium (DMEM; Genview, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), 50 μg/mL streptomycin and 50 U/mL penicillin at 37 °C in an atmosphere containing 5% CO<sub>2</sub>. To assess the effect of Zn–curcumin, curcumin and zinc acetate on cell growth, 3T3 cells were incubated for 24 h with Zn–curcumin SDs, curcumin SDs or zinc acetate (equivalent to 5, 10, 20 and 40 μmol/L Zn–curcumin (curcumin or zinc acetate) for 24 h. DMEM media containing FBS with or without 125 mg/L PVP were used as the vehicle control and normal control, respectively. The following day, the media was replaced with fresh DMEM, and the cells were incubated with 0.5 mg/mL 3-[4,5-dimethyl-thiazol-yl-2]-2,5-diphenyl tetrazolium bromide (MTT) solution for 4 h at 37 °C. The formation of the violet formazan precipitate was measured at a wavelength of 492 nm using a microplate reader (Thermo Coulter, Lafayette, CO, USA).

2.4. Induction of gastric ulcers and evaluation of the gastric ulcer area

Rats were randomly divided into seven experimental groups, each containing ten animals. The normal and control groups received PVP vehicle (300 mg/kg) throughout the course of the experiments. The treatment groups received different doses of Zn(II)–curcumin SDs (equivalent to Zn(II)–curcumin 12, 24 and 48 mg/kg, p.o.). The curcumin group received curcumin SDs (equiv-alent to curcumin 24 mg/kg, p.o.), and lansoprazole (7.8 mg/kg, p.o.) was used as the positive control for a period of 7 days. Oral lansoprazole (30 mg/day) provided effective relief of symptoms and healing of duodenal ulcer in 75 ± 100% of patients after 4 weeks [19]. In this model, lansoprazole (7.8 mg/kg) was used as the positive control for a period of 7 days, equivalent to 90 mg/ day for adult humans calculated by body surface area.

After fasting for 24 h prior to the experiment, rats were fed orally with 75% ethanol (0.5 mL/100 g body weight) to induce the maximum level of acute ulcers, while the normal group received sterile water only. Four hours after induction, the rats were killed by cervical dislocation.

After the rats were sacrificed, the stomachs were rapidly removed, opened along the greater curvature and rinsed with normal saline to remove the gastric contents and blood clots in order to assess the extent of gastric damage. The degree of gastric mucosal damage was evaluated from digital pictures, and rated for gross damage was evaluated from digital pictures, and rated for gross pathology according to the ulcer score scale described by Dekanski et al. as follows [20]: 0 = no damage; 1 = blood at the lumen; 2 = pin-point erosions; 3 = one to five small erosions < 2 mm; 4 = more than five small erosions < 2 mm; 5 = one to three large erosions > 2 mm; 6 = more than three large erosions > 2 mm. The inhibition percentage was calculated using the formula: [(U<sub>untreated</sub>− U<sub>treated</sub>) U<sub>untreated</sub>] × 100.

2.5. Measurement of antioxidant enzymes, H<sup>+</sup>–K<sup>+</sup>-ATPase activity and malonaldehyde (MDA) level

After the macroscopic analyses, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and H<sup>+</sup>–K<sup>+</sup>-ATPase enzyme activities and the MDA levels were determined in rat stomach tissues. To prepare the tissue homogenates, stomach tissues were homog-enized with liquid nitrogen using a pestle and mortar. The homog-enized tissues (0.5 g each) were mixed with 4.5 mL of homogenization Tris-buffer (10 mM, pH 7.4), homogenized on ice using an Ultra-Turrraks homogenizer for 15 min, filtered and centri-fuged at 1000g at 4 °C for 20 min. The supernatants were then used to determine the enzymatic activities.

The concentration of protein in the homogenized mucosa solution was determined by the Bradford method [21] using bovine serum albumin (BSA) as a standard. One milliliter of Bradford re-agent was added to 100 μL of the diluted sample and absorbance
was measured at 595 nm. Protein concentrations were expressed in mg/L.

SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium (NBT) to form a formazan dye [22]. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction. Enzyme activity leading to 50% inhibition was considered as 1 U/mg protein. Decomposition of H$_2$O$_2$ in the presence of this reaction. Enzyme activity leading to 50% inhibition was considered as 1 U/mg protein. Decomposition of H$_2$O$_2$ in the presence of reduced glutathione (10 mM), NADPH (4 mM), and 1 U enzymatic activity of glutathione reductase in PBS buffer, pH 7.8. Absorbance was read at 3 min and 10 min and the change was recorded. Results were expressed as units per mg protein.

Glutathione peroxidase (GSH-Px) activity was quantified by the method described by Yoshikawa et al. [24]. GSH-Px activity was evaluated by following the decrease in absorbance at 365 nm induced by 0.25 mM H$_2$O$_2$ in the presence of reduced glutathione (10 mM), NADPH (4 mM), and 1 U enzymatic activity of glutathione reductase in PBS buffer, pH 7.8. Absorbance was read at 3 min and 10 min and the change was recorded. Results were expressed as units per mg protein.

The H$^+$–K$^+$-ATPase activity was assayed following the description by Reyes-Chilpa et al. [25]. The reaction mixture (1 mL) contained enzyme in 20 mM Tris–HCl, pH 7.4, 2 mM MgCl$_2$ and 2 mM KCl. The reaction was started by the addition of 2 mM ATP and incubated for 30 min at 37 °C. The reaction was terminated by the addition of ammonium molybdate and trichloroacetic acid mixture followed by centrifugation at 2000g. The amount of inorganic phosphate released from ATP was determined spectrophotometrically at 400 nm. The H$^+$–K$^+$-ATPase activities were expressed as micromoles of P$_i$ released per hour per mg of protein.

Tissue MDA levels were determined by the method described by Ohkawa et al. [26]. The corpus mucosa was homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added to a solution containing 0.2 mL of 80 g/L sodium lauryl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate and 0.3 mL of distilled water. This mixture was heated at 98 °C for 1 h, and 5 mL of n-butanol:pyridine (15:1) was added when the mixture had cooled. The mixture was vortexed for 1 min, centrifuged for 30 min at 4000 rpm and the absorbance of the supernatant was measured at 532 nm. A standard curve was obtained using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. The results were expressed as nmol/min per mg protein.

2.6. Quantification of TNF-α and IL-6 mRNA by reverse transcription-PCR (RT-PCR)

Full-thickness specimens from the stomach were obtained to quantify TNF-α mRNA and IL-6 mRNA using RT-PCR. The samples were immersed in RNA Stabilization Reagent and stored at −70 °C. Total RNA was extracted using RNA Stabilization Reagent, according to the protocol provided by the manufacturer, and quantified by measuring the absorbance at 260 nm. Complementary DNA was synthesized from 1 μg total RNA from each sample in 20 μL of reaction buffer using SuperScript II reverse transcriptase. The cDNA (1 μL) was then amplified in 20 μL of reaction buffer for 30 cycles of denaturation (94 °C for 30 s), annealing (50 °C for 30 s), and extension (72 °C for 30 s) using the following primers: for TNF-α: sense, 5′-CAACGCTTTCTGTCAGT-3′ and antisense, 5′-GCTACGGCCTGTGACTC-3′ (expected product 145 bp); IL-6: sense, 5′-CCTCTTGAGAGTGTGAG-3′ and antisense, 5′-CTCTGCTGTTGTCTTC-3′ (expected product 384 bp), and β-actin: sense, 5′-TCACCACTCCTGCAGAC-3′, and antisense 5′-AGGATACAGGGA CAACA-3′ (expected product 207 bp). The PCR products were analyzed by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. The PCR product sizes were estimated using a 100 bp marker.

2.7. Histological analysis

After the stomach specimens for RT-PCR were obtained, the remaining tissues were immediately immersed and fixed in 4% paraformaldehyde in phosphate buffer (pH 7.6) for 48 h at 4 °C. Paraffin sections (5 μm thick) were prepared and stained with Mayer’s hematoxylin and eosin according to standard procedures. Tissue preparations were observed and microphotographed under a light microscope.

2.8. Statistical analysis

The values are expressed as the mean ± S.D. for 10 animals in each group. The data were analyzed using SPSS/13 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Dunnett’s T3 multiple comparisons test. The significance levels were analyzed at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

3. Results

3.1. Zn(II)–curcumin stimulates the proliferation of 3T3 cells

Zinc supplementation using extracellular cation chelators increases the proliferation of numerous cell types in vitro [27]. The 3T3 cell line is the most frequently used model to investigate the influence of zinc on cell growth. As shown in Fig. 1, PVP supplementation (125 mg/L) did not affect the proliferation of 3T3 cells, compared to the normal control ($P > 0.05$). Supplementation with 5–40 μmol/L Zn(II)–curcumin increased cell proliferation by approximately 13–60%, with a maximum effect at 20 μmol/L. Amongst the curcumin supplemented cells, increasing concentrations of curcumin from 5 to 10 μmol/L stimulated cell proliferation by 8–17%, compared to normal control cells ($P < 0.01$). Higher concentrations of curcumin (20–40 μmol/L) inhibited cell proliferation by between 0% and 35%. Amongst the zinc acetate supplemented cells, increasing the concentration of zinc acetate from 5 to 40 μmol/L increased cell proliferation by 14–47%, and had a maximal effect at 20 μmol/L. The MIT proliferation assay also showed that Zn(II)–curcumin stimulated the proliferation of 3T3 cells more significantly than curcumin or zinc acetate at the same concentrations ($P < 0.01$), indicating that curcumin and zinc ions have a synergistic effect on cell proliferation.
3.2. Anti-gastric effects of Zn(II)-curcumin SDs on ethanol-induced gastric ulcers

As shown in Fig. 2A, no macroscopic or microscopic lesions were observed in the normal control group. Compared to normal controls, intragastric administration of 75% ethanol induced macroscopic morphological changes, such as linear hemorrhages, mucosal erythema in the mucosal layer and edema in the submucosal layer in control group rats (Fig. 2B). Surface mucous cells were pyknotic and desquamated from mucosa. Photomicrographs of ethanol-induced gastric ulcers in the control group showed ulcers with a loss of mucosa exposing the muscularis mucosa.

Fig. 2. Effect of Zn(II)-curcumin on macroscopic and microscopic changes in the gastric mucosa induced by ethanol in rats. Ulcers were induced in the (A) normal group; (B) control group damaged by ethanol after pretreatment with PVP 300 mg/kg; (C) lansoprazole group treated with ethanol after pretreatment with lansoprazole 7.8 mg/kg. (D–F) Zn(II)-curcumin groups treated with ethanol after pretreatment with Zn(II)-curcumin 12 mg/kg, 24 mg/kg or 48 mg/kg. (G) Curcumin group treated with ethanol after pretreatment with curcumin 24 mg/kg. The left panel represents macroscopic morphological changes (gross observation) and the right panel illustrates microscopic histopathological observation (magnification: ×100). (H) Changes in the mean gross lesion index and inhibition rate. Each value represents the mean ± S.D. value of ten animals. Significance is represented as **P < 0.001 compared to the control group and *P < 0.05 compared to the curcumin group.
However, rats pretreated with Zn(II)-curcumin (12, 24 and 48 mg/kg) displayed a marked attenuation of histopathological changes induced by ethanol.

The gastric mucosa of rats treated with Zn(II)-curcumin were almost normal in appearance (Figs. 2D–F). Zn(II)-curcumin treated rats showed increased epithelial regeneration. Necrosis of the surface mucous cells was rarely observed, and if noted was not severe. Zn(II)-curcumin treatment significantly reduced the ulcer index (P < 0.001). When the total sum of gross mucosal changes was measured and the ulcer index was determined, the ulcer index of the ethanol-administered groups was 73.7 ± 14.4; however, the ulcer index of rats pretreated with 12, 24 and 48 mg/kg Zn(II)-curcumin were 39.9 ± 8.6, 26.5 ± 7.2 and 18.4 ± 6.8 (45.9%, 64.0% and 75.0% protection), respectively (Fig. 2H). However, the ulcer index was greater in the curcumin-treated group receiving 24 mg/kg than in Zn(II)-curcumin group receiving the same dose (P < 0.01).

The curcumin-treated group prevented 53.7% of the gastric ulcer, compared to the 64.0% inhibition rate by Zn(II)-curcumin (24 mg/kg), which revealed a synergistic effect between curcumin and zinc ion. Lansoprazole (7.8 mg/kg) showed a less potent
reduction in the ulcer index (72.5%), which was slightly lower than Zn(II)–curcumin at a dose of 48 mg/kg (75.0%; P > 0.05).

3.3. The effect of Zn(II)–curcumin SDs on antioxidant enzymes of gastric ulcer rats

According to Fig. 3, intragastric administration of 75% ethanol significantly reduced SOD and GSH-Px activities, compared with the normal group (P < 0.001). Administration of Zn(II)–curcumin (equivalent to Zn(II)–curcumin 12, 24 and 48 mg/kg, p.o.) alleviated the antioxidant status by significantly increasing the activities of SOD and GSH-Px in a dose-related manner. Zn(II)–curcumin at 12–48 mg/kg increased the GSH-Px level to 87.3 ± 13.8, 101.2 ± 13.4 and 112.7 ± 22.2 U/mg protein, in comparison to controls (48.7 ± 17.5 U/mg protein; P < 0.001 for all parameters). Furthermore, the group receiving Zn(II)–curcumin displayed higher GSH-Px levels than the group administered with curcumin at the same dose (P < 0.05), which demonstrates the synergistic effect of curcumin and zinc ion.

3.4. Effects of Zn(II)–curcumin SDs on H+–K+–ATPase and MDA level

According to Fig. 4, H+–K+–ATPase activity and MDA levels were significantly accelerated in stomach tissue after ethanol-induced acute gastric ulcer formation. Pretreatment with Zn(II)–curcumin at doses of 12–48 mg/kg remarkably attenuated H+–K+–ATPase activity and MDA levels in stomach tissue in a dose-related manner. Compared with controls, H+–K+–ATPase activity reduced to 5.31 ± 0.99, 4.65 ± 0.69 and 4.31 ± 0.58 μmol Pi/mg protein hour in the group receiving Zn(II)–curcumin (P > 0.05, P < 0.001 and P < 0.001 respectively). Zn(II)–curcumin significantly reduced MDA content to 1.42 ± 0.20, 1.24 ± 0.13 and 1.16 ± 0.16 nmol/mg protein when treated with 12, 24 and 48 mg/kg respectively (P < 0.01, P < 0.001 and P < 0.001), H+–K+–ATPase activity in the curcumin group (24 mg/kg) was lower than in the control group, but this difference was not statistically significant (P > 0.05), which indicated that Zn(II)–curcumin more potently reduced H+–K+–ATPase activity than curcumin alone. Zn(II)–curcumin not only suppressed H+–K+–ATPase activity, but also totally prevented an increase in MDA levels.

3.5. Effect of Zn(II)–curcumin SDs on TNF-α and IL-6

Compared with the normal group, severe gastric mucosal damage was induced after oral administration of 75% ethanol, which contributed to increased expression of the proinflammatory factors TNF-α and IL-6 mRNA (P < 0.001). In the case of Zn(II)–curcumin, 12, 24 and 48 mg/kg Zn(II)–curcumin attenuated the expression of TNF-α mRNA in a dose-related manner. The expression of TNF-α mRNA in the group treated with curcumin (equivalent to 24 mg/kg curcumin) was higher than the group administered 24 mg/kg Zn(II)–curcumin SDs (P < 0.05), which indicated Zn(II)–curcumin had a stronger ability to suppress TNF-α mRNA expression due to the synergistic effects of curcumin and zinc ion. The expression of IL-6 mRNA was lower in the groups receiving 24 and 48 mg/kg Zn(II)–curcumin than the positive control group administered lansoprazole (7.8 mg/kg).

4. Discussion

It is well known that venous ulcers have a slow healing rate. Fibroblasts are a dermal element which is critical for ulcer healing. Fibroblasts isolated from venous ulcers display slow growth and...
enhances the proliferation of fibroblasts due to the presence of zinc [31]. Polaprezinc (zinc-carnosine) prevented by addition of zinc sulfate [31]. Curcumin (20 and 30 μM) lead to a dose-dependent inhibition of cell proliferation [P < 0.05, P < 0.001] [33]. Curcumin can dose-dependently inhibit cell proliferation and DNA synthesis at equimolar concentrations ranging from 25 to 50 μM curcumin for 24 h, 3T3-L1 cell proliferation [P < 0.05, P < 0.01] and excessive lipid peroxidation can cause increased GSH consumption. In contrast, an increase in gastric non-protein sulphydryl content limits the production of oxygen-derived free radicals, and could be related to gastric protection in different experimental models such as the ethanol-induced ulcer model [43]. Lipid peroxidation is an important mechanism of cellular injury, and MDA is one of the end products. Hence, determination of MDA levels can be used to access lipid peroxidation [44]. Increases in H+-K+-ATPase and MDA and decreases in SOD and CAT levels in the gastric tissue were observed in ethanol-induced and CRS-induced gastric ulceration [45].

Curcumin can attenuate increased lipid peroxidative damage, and prevent depletion of GSH, GSH-Px and CAT activities, which are markers of renal injury and urinary excretory indices during gentamicin-induced oxidative stress [46]. Curcumin can effectively accelerate ulcer healing in a rat chronic acetic acid-induced gastric ulcer model via a mechanism involving inhibition of gastric acid secretion and anti-inflammatory activity preventing inducible nitric oxide synthase (iNOS) and TNF-α production [47]. Curcumin exerted an anti-ulcer activity by affecting oxidative stress and total antioxidant capacity, as well as inhibiting IL-6 secretion and preventing apoptosis in a pylorus-ligated model [48].

Zinc has an important role in cell-mediated immune functions and also functions as antioxidant and anti-inflammatory agent. Zinc deficiency has been shown to significantly increase the gastric secretory volume, acid and pepsin in the rat stomach by retarding growth of the animals, producing ulceration and aggravating the formation of glandular ulceration [49]. Administration of zinc was found to preserve the integrity of the intestinal glycoalyx against indomethacin-induced small intestinal damage in the rat, and inhibited the growth of enterobacteria and their adherence to the mucosa [50]. The preventive effects of zinc supplementation in a group receiving zinc gluconate were linked to a significantly decreased incidence of infections and ex vivo generation of TNF-α and plasma oxidative stress markers compared with a placebo group [8]. A zinc compound [dichlorido-zinc(II)-4-(2-(5-methoxy-benzylidene amino)ethyl) piperazin-1-iumphenolate] showed the gastroprotective activity and block oxidative damage during ethanol-induced ulcer in rats through reducing the production of TNF-α.
and IL-6 [35]. Our earlier studies suggested that Zn(II)–curcumin (12, 24 and 48 mg/kg) prevented pylorus ligation-induced lesions in rats by inhibiting NF-κB, TGF-β1, and IL-8 mRNA expression [18]. The anti-ulcer effects of Zn(II)–curcumin may be attributable to its capacity to reduce gastric acid secretion and enhance the mucosal defense mechanisms through the suppression of NF-κB-mediated inflammation. Our previous studies also demonstrated that the Zn(II)–curcumin complex showed significant gastroprotective and antidepressant effects by participating in free radical scavenging and increasing expression of HSP70, thereby attenuating the increase in iNOS expression during cold-restraint stress (CRS)-induced gastric ulcers in rats [51].

In this study, the mRNA expression of TNF-α and IL-6 increased in the gastric tissue of ethanol induced ulcer, accompanying with lower activities of SOD and GPX-Px, higher level of MDA, indicating that ethanol induces inflammation and ROS generation. Zn(II)–curcumin at 12, 24 and 48 mg/kg protected against ethanol-induced ulcer lesions by up to 75%, similar to the effect of lansoprazole, a known anti-ulcer drug, at 7.8 mg/kg b.w. (72.5%). Accordingly, the activity of gastric H−K−ATPase was measured in the stomach homogenate, which showed a twofold upregulation of the enzyme in the ulcer condition, which was normalized by treatment with Zn(II)–curcumin. Administration of Zn(II)–curcumin resulted in a significant increase in SOD and GSH-Px, and downregulating MDA levels and expression of TNF-α and IL-6 mRNA, suggesting that Zn(II)–curcumin effectively enhance the mucosal defense mechanism through the preventing of proinflammatory cytokine-induced oxidative damage.

In conclusion, Zn(II)–curcumin complex has notably and dose-dependently protect the gastric mucosa against ethanol-induced injury. The compound activity is considered as a synergistic effect between curcumin and zinc. These results also show that antioxidant activity of Zn(II)–curcumin via enhance cell proliferation and adjusting proflammaotry cytokine-induced oxidative damage during ulcer.

Conflicts of interest statement
The authors declare that there are no conflicts of interest.

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