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ORIGINAL ARTICLE

Protective Effect of *Schisandra chinensis* Extract Against Ethanol-Induced Gastric Ulcers in Mice by Promoting Anti-inflammatory and Mucosal Defense Mechanisms

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Abstract

Schisandra chinensis (Turcz.) Baill., Schisandraceae, is a common Chinese traditional medicine for the treatment of nervous system disorders and cardiovascular diseases. However, little is known about its efficacy in treating gastrointestinal diseases. In the present study, the protective effect of the ethanol extract of *S. chinensis* against ethanol-induced acute gastric lesions was investigated in mice. Furthermore, we developed a method based on high-performance liquid chromatography for the quantification of bioactive lignans with a dibenzocyclooctadiene skeleton. The ethanol extract of *S. chinensis* significantly decreased the ulcer index, sheltered mucosa from lesions, increased levels of superoxide dismutase, decreased malondialdehyde levels, and downregulated plasma levels of tumor necrosis factor-α and interleukin-1β. Our findings demonstrated the gastroprotective properties of the ethanol extract of *S. chinensis*.

Keywords Acute alcohol intake · Cytokines · Gastric mucosa damage · Oxidative stress · Pathological change

Introduction

Gastric ulcers are a common type of gastrointestinal disease and exhibit a high prevalence rate. Studies have shown that the etiology of ulcers is associated with cigarette smoking, stress, infections, nutritional deficiencies, frequent ingestion of non-steroidal anti-inflammatory drugs, and alcohol consumption (Arab et al. 2015; Sidahmed et al. 2019). Among these factors, excessive alcohol consumption has attracted the most attention, as it has been demonstrated to considerably increase the incidence of gastric ulcers (Yamada et al. 2005). However, the pathogenic mechanisms underlying ethanol-induced gastric ulcers have not been fully clarified.

Previous studies have shown that ethanol is a known cause of gastric damage by reducing mucosal protein secretion and prostaglandin E_2 (PGE_2) production (Sidahmed et al. 2015; Sidahmed et al. 2019), as well as by increasing disruptive factors such as oxidative stress and pro-inflammatory cytokines (Li et al. 2018; Sidahmed et al. 2019). Alcohol disrupts the expression of cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), in the pathogenesis of gastric ulcers (Chen et al. 2016; Liu et al. 2016). Thus, controlling the formation of reactive oxygen species (ROS) and inflammatory reactions are essential for the treatment of this pathology. Another key defensive factor against gastric ulcers is the production of gastric mucus. A sufficient amount of gastric mucus can potentiate the action of a protective agent on the gastric mucosa. Therefore, it is an effective protection

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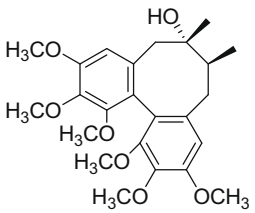
and a method of treatment to directly or indirectly balance the damage and defense factors of gastric ulcers.

Current treatments for gastric ulcers mainly include the administration of proton-pump inhibitors, H2-receptor antagonists, gastric mucosa protective agents, and antibacterial drugs that kill *Helicobacter pylori* (Kuna et al. 2019). However, these treatments are ultimately not effective in preventing ethanol-induced gastric ulcers and have considerable severe side effects during long-term use (Kuna et al. 2019). Thus, there is an urgent need to identify and develop more effective treatments for ameliorating gastric ulcers.

Schisandra chinensis (Turcz.) Baill., Schisandraceae, also known as Chinese magnolia vine ("wuweizi," or five-flavor-fruit), is a medicinal plant that has been extensively used in traditional and modern medicine in China, Japan, and Russia (Hernandez et al. 1988; Panossian and Wikman 2008; Szopa et al. 2017). The fruit of *S. chinensis* (magnolia berry) is used as a tonic, sedative, antitussive, and anti-aging remedy (Dilshara et al. 2013). The components of *S. chinensis* that have exhibited pharmacological activities are lignans with a dibenzocyclooctadiene skeleton, such as schisandrin (**1**), schisantherin A (**2**), deoxyschisandrin (**3**), and γ-schisandrin (**4**). These pharmacological activities included antioxidative, anti-hepatotoxic, anti-ulcerogenic, and anti-inflammatory properties (Hernandez et al. 1988; Huyke et al. 2007; Kang et al. 2014; Szopa et al. 2017). Further studies have shown that the components of magnolia berries exhibit inhibitory effects on the expression levels of TNF-α and IL-1β by deactivation of the mitogen-activated protein kinase pathway (Giridharan et al. 2015), which subsequently prevents the production of pro-inflammatory mediators and cytokines. Furthermore, several previous studies have reported efficacies of this medicinal plant in mitigating central nervous system disorders (*e.g.*, depression, neurosis, and alcoholism), hepatotoxic disease, and peptic ulcers (Panossian and Wikman 2008; Szopa et al. 2017). In addition, other studies have shown that *S. chinensis* extracts, particularly through component lignans or formulations containing deoxyschizandrin, exhibit inhibitory effects on stress-induced gastric ulcers (Hernandez et al. 1988). However, the protective effects and mechanisms of *S. chinensis* extracts on ethanol-induced gastric ulcers have remained unclear, although such extracts have been shown to be effective in the treatment of inflammation and oxidative stress in many other diseases.

In animal experiments, an ethanol-induced gastric ulcer model is often used to screen for compounds that possess anti-ulcer activities (Yang et al. 2017; Li et al. 2018; Sidahmed et al. 2019). Therefore, the aim of the present study was to investigate the effects and possible use of magnolia berry alcohol extracts from *S. chinensis* against ethanol-induced gastric ulcers in mice by measuring the activities of antioxidative substances, levels of cytokines, and pathological changes in gastric mucosa.



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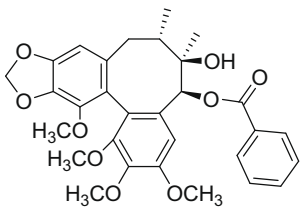

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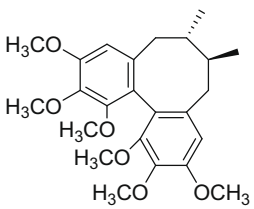

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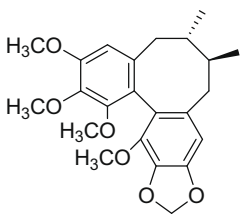

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Materials and Methods

Preparation of Extracts

Berries of *Schisandra chinensis* (Turcz.) Baill., Schisandraceae, were purchased from Zhejiang Zhenyuan Pharmaceutical Co., Ltd. (Shaoxing, China) and identified by Professor Xiuzhen Wang, Faculty of Medicine and Health, Yuanpei College, Shaoxing University, as the dried and mature fruit. In this experiment, the botanical material was stored in a dry, dark, and low-temperature place and a sample was kept in our laboratory for future reference. The purchased samples were dried and homogenized to a fine powder using a grinder before extraction. The powder (30 g) was placed into a round bottom flask and was reflux extracted with 80% ethanol at a material-to-liquid ratio of 1 g per 10 ml at 90 °C for 1 h. This procedure was repeated three times, and the resultant solution was then filtered and concentrated using a rotary vacuum evaporator. The solution was dried in a drying cabinet, resulting in a crude extract (7.29 g) that was stored at 4 °C and diluted with medium to the desired concentration before use.

Preparation of Solutions

For the preparation of the corresponding solutions for each reference compound, 10.3 mg of schisandrin (**1**), 4 mg of schisantherin A (**2**), 6.5 mg of deoxyschisandrin (**3**), and 10.4 mg of γ-schisandrin (**4**) were individually dissolved in 25 ml of methanol. Additionally, 559.5 mg of crude extract was dissolved in 100 ml of methanol.

Quantification of Chemical Marker

In the present study, we used an Agilent 1200 HPLC instrument. Chromatographic conditions were as follows: C_{18} column, 4.6 × 250 mm, 5 μm; mobile phase, A, H_2O; mobile phase B, MeOH; gradient elution was with 60–100% mobile phase B from 0 to 15 min, 100% mobile phase B from 15 to 20 min, and 100–60% mobile phase B from 20 to 25 min; flow rate was 0.5 ml/min and the oven temperature was set at 45 °C; and detection was at 280 nm and the injection volumes of standards and extracts were 5 μl each (He et al. 1997; Lee and Kim 2010). Aqueous methanolic stock solutions containing the four analytes were prepared by mixing compounds **1**–**4** in a 1:1:1:2 volumetric ratio and diluting them to the appropriate concentrations (5%, 10%, 20%, 50%, 80%, and 100%) for the establishment of calibration curves. Six different concentrations of the mixed analyte solutions were injected in triplicate. Variations were expressed as relative standard deviations (RSDs).

Induction of Gastric Ulcers and Treatments

After 1-week adaptation to the environment, mice were randomly divided into 6 groups with 10 mice in each group. In group 1 (control), mice received vehicle (0.9% physical saline). In group 2 (ethanol), mice received absolute ethanol (10 ml/kg) (Li et al. 2018). In group 3 (omeprazole), mice received omeprazole before the induction of ulcers at a dose of 20 mg/kg. Finally, mice in groups 4–6 ($SC_{80–320}$) received the analyzed extracts before the induction of ulcers at doses of 80, 160, and 320 mg/kg for groups 4, 5, and 6, respectively. Mice were fasted for 24 h before the experiment, during which they were provided free access to drinking water. The induction of ulcers was achieved by oral administration (*p.o.*) of absolute ethanol at a dose of 10 ml/kg body weight, while the control group received normal saline instead. One hour later, blood was collected by removal of the eyes. Then, the collected blood was centrifuged for 10 min at 2110×*g* to obtain clean plasma that was stored at − 20 °C until further use. Finally, mice were sacrificed by dislocating their necks. A diagram of the study design is shown in Fig. S1 (Supplementary Material).

Determination of Gastric Ulcer Index

After the mice were euthanized, their stomachs were removed and examined for the observation of mucosal hemorrhagic lesions macroscopically. Each lesion's length, in millimeters, was carefully measured to determine the mean ulcer index (UI). Mucosal lesions were evaluated as follows: no ulcer (0), 1–5 petechiae (< 1 mm) (1), 6–10 petechiae (< 1 mm) (2), > 10 petechiae (< 1 mm) (3), small ulcer (< 2 mm) (2), medium ulcer (2–4 mm) (3), and large ulcer (> 4 mm) (4). If the width was > 1 mm, the points were multiplied by a factor of 2. The UI was equal to the sum of all scores dividing by the number of mice (Li et al. 2018).

Determination of Plasma Malondialdehyde Levels and Superoxide Dismutase Activities

The plasma was centrifuged at 2110×*g* for 10 min at 4 °C, after which plasma malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities were assayed by immunoassay kits according the manufacturer's instructions (Mohan et al. 2020).

Immunoassay Analysis

Plasma TNF-α and IL-1β levels were assayed using monoclonal antibodies for TNF-α and IL-1β, in accordance with the instructions of ELISA kits (Li et al. 2018).

Statistical Analysis

All values are expressed as the mean ± standard error of the mean (SEM). The SPSS statistical software (SPSS 18.0) was used for all statistical analysis. The data were statistically analyzed by one-way analyses of variance (ANOVAs) followed by Newman-Keuls tests or Dunnett's multiple comparison tests for *post hoc* pairwise comparisons. A $p < 0.05$ was considered statistically significant.

Results

HPLC Analysis

Four reference compounds, schisandrin (**1**), schisantherin A (**2**), deoxyschisandrin (**3**), and γ-schisandrin (**4**), were subjected to HPLC analysis to determine their retention times. Figure 1 shows the HLPC-UV chromatograms of mixed standards and the crude extracts from magnolia vine berries; the results showed that the total extract contained the four chemical markers used to obtain the chemical profile. To determine the concentrations of these main components, HPLC was used to establish a standard curve, verify linear ranges, and compute correlation coefficients (R^2) (Table 1). The concentrations of compounds **1**–**4** were 3.57 ± 0.353, 0.94 ± 0.123, 0.63 ± 0.017, and 2.61 ± 0.064 mg/g, respectively; these results are consistent with previously reported findings (Lee and Kim 2010).

Table 1 Linear regression data and precision of investigated compounds from *Schisandra chinensis* extracts

Compounds	Linear regression data			Precision, RSD (%)
	Regressive equation	r^2	Linear range (μg/ml)	
Schisandrin	$y = 19.418x + 11.005$	0.9993	8.24–164.8	3.59
Schisantherin A	$y = 14.180x + 2.9783$	0.9992	2.96–59.2	3.70
Deoxyschisandrin	$y = 20.647x + 13.396$	0.9993	2.60–52.0	4.17
γ–Schisandrin	$y = 16.793x + 1.8455$	0.9998	4.16–83.2	2.81

Evaluation of Gastric Lesions

Gross examinations of the stomachs of mice among the groups are illustrated in Fig. S2. As shown in Fig. S2A, no macroscopic or microscopic lesions were observed in the control group. Compared with the features of the control group, mice treated with absolute ethanol (10 ml/kg) induced macroscopic morphological changes, such as linear hemorrhagic lesions and multifocal mucosal erythema (Fig. S2B). Compared with features in the ethanol-induced ulcer group, mice pretreated with omeprazole (Fig. S2C) and crude extract (Fig. S2D–F) had significantly diminished areas of gastric damage formation and suppression of the formation of hemorrhagic mucosal lesions. The most prominent protection of the gastric mucosa was observed at the middle dose of 160 mg/kg of the crude extract (Fig. S2E). Compared with that of the vehicle group, the group treated with absolute ethanol (10 ml/kg) displayed a marked increase in the UI ($p < 0.001$) (Fig. 2). Pretreatment with omeprazole (20 mg/kg, *i.g.*) or the tested extract (80, 160, or 320 mg/kg, *i.g.*) significantly decreased the UI in the ethanol-induced gastric ulcer groups in

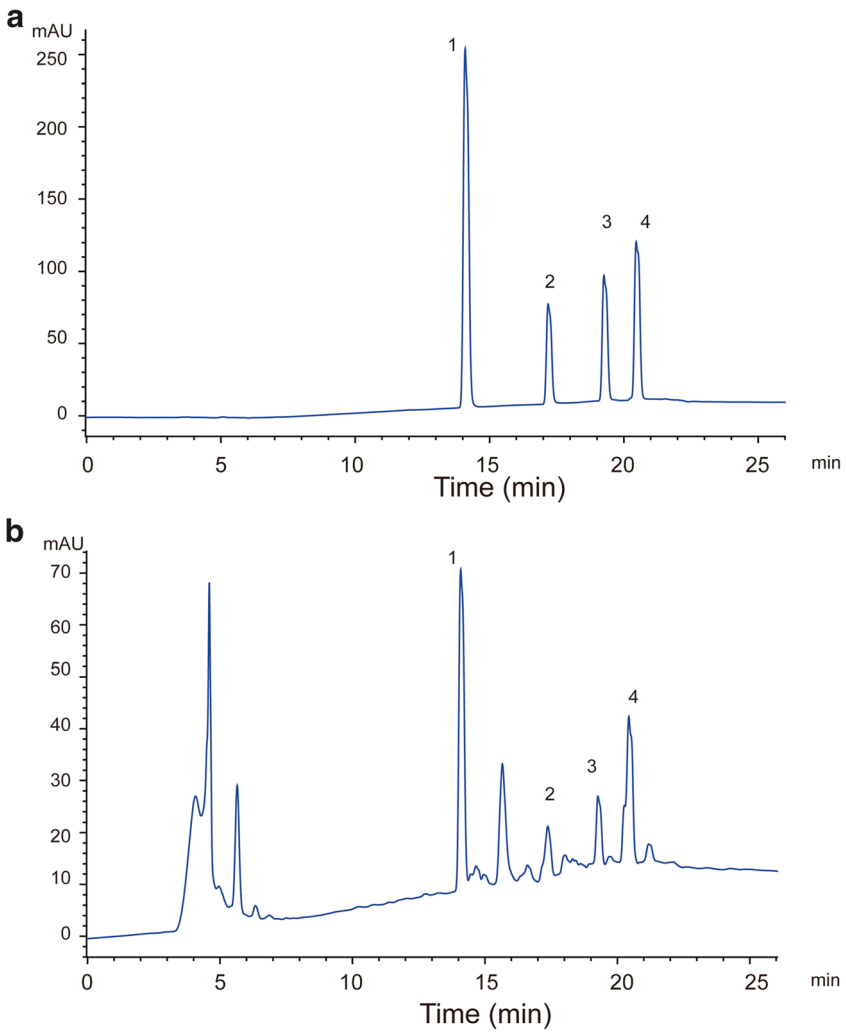

Fig. 1 HPLC-UV chromatograms of mixed chemical markers used as standards (**a**) and crude extracts from berries of *Schisandra chinensis* (**b**). Reference compounds: 1 schisandrin; 2 schisantherin A; 3 deoxyschisandrin; and 4 γ-schisandrin

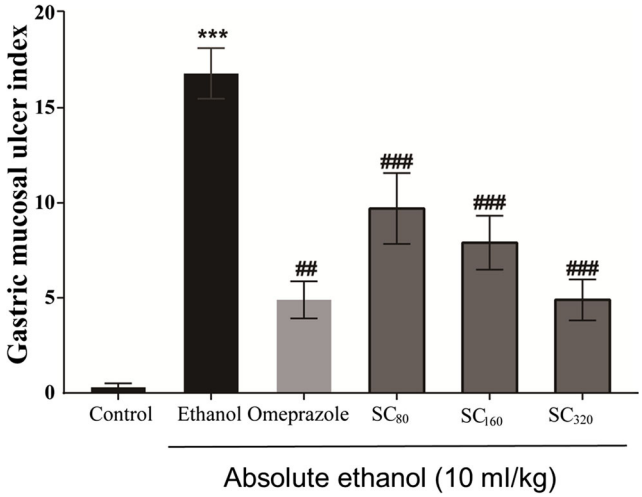

Fig. 2 Effects of the crude extract of *Schisandra chinensis* on the gastric UI in the following groups: the saline-treated group (control); ethanol group (10 mg/kg); omeprazole (20 mg/kg) group; *S. chinensis* (80 mg/kg) group (SC80); *S. chinensis* (160 mg/kg) group (SC160); and *S. chinensis* (320 mg/kg) group (SC320). Each value represents the mean ± SEM from 10 mice. Significance is represented as ***$p < 0.001$ compared with the control group, and ##$p < 0.01$ and ###$p < 0.001$ compared with the ulcer control group

comparison with that of the ethanol group. Lesions in the gastric mucosa were significantly reduced in mice pretreated with the extract of *S. chinensis* at doses of 320 mg/kg (4.9 ± 1.069, $p < 0.001$), 160 mg/kg (7.9 ± 1.418, $p < 0.001$), and 80 mg/kg (9.7 ± 1.856, $p < 0.01$) (Fig. S2D–F). Omeprazole (20 mg/kg), used as the reference drug, also significantly reduced gastric lesions (4.9 ± 0.971, $p < 0.001$) when compared with that of the ulcer control group (16.8 ± 1.323, $p < 0.001$).

Mice treated with absolute ethanol exhibit a significant reduction of thickness in mucosa, a damage of the surface epithelium with epithelial cell deficiency, intense edema, and leucocyte infiltration in the submucosal layer (Fig. 3). As the positive control, omeprazole exhibited relative protection against the effects of ethanol, as observed by an improvement in the superficial region, decreased edema in the submucosa, and only moderate infiltration of neutrophilic inflammatory cells (Fig. 3c). In contrast, pretreatment with *S. chinensis* (Figs. 3d–f) yielded protection against ethanol-induced histopathological disruption and nearly normal mucosal morphological characteristics.

Mucosal Defense Mechanisms

Sections of gastric mucosa from control mice revealed periodic acid-Schiff (PAS)–positive reactions (Fig. S3A). In contrast, in the alcohol-treated group, there was a PAS-negative reaction in

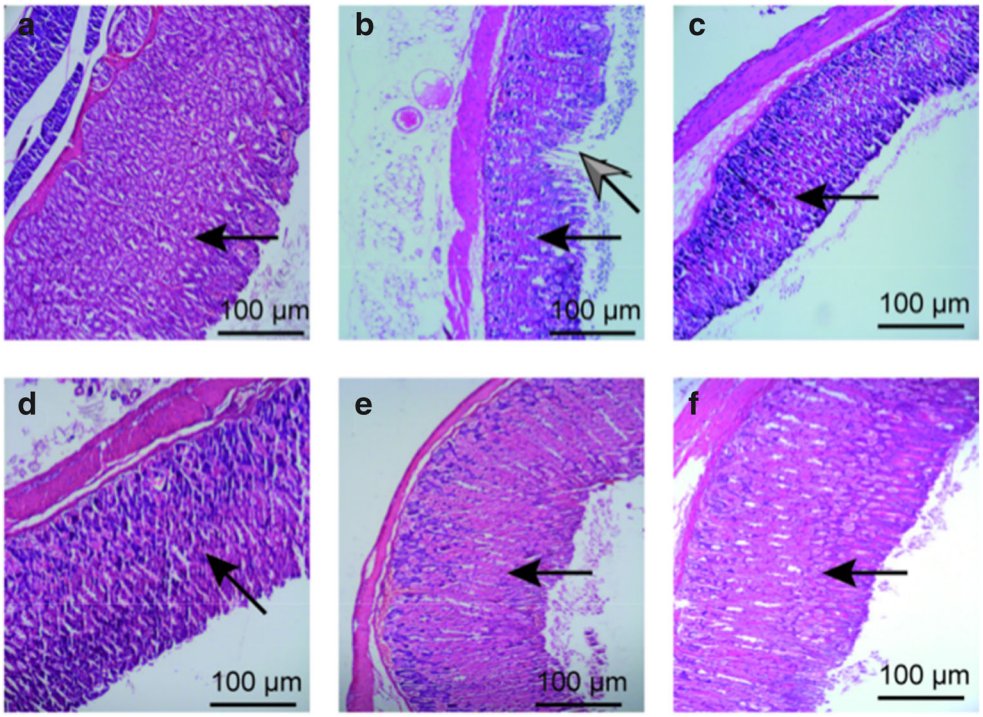

Fig. 3 HE staining of gastric mucosa in mice (× 100). **a** The control group is shown. **b** The absolute ethanol group (10 ml/kg) is shown. Broad erosion (gray arrow) in the upper half of mucosa, extensive edema (black arrow) in the submucosal layer, and encircling by the leukocyte inflammatory zone (black arrow) were observed. **c** The omeprazole group (20 mg/kg) is shown. Mild disruption of the surface epithelial mucosa was present, but deep mucosal damage was absent. **d** The *Schisandra chinensis* (80 mg/kg) group is shown. There were no deep ulcers in the gastric mucosa, but there was partially infiltration by inflammatory leukocytes. **e**, **f** The *S. chinensis* (160 and 320 mg/kg) groups are shown, respectively. There were no disruption to the surface epithelium, no edema, and no leukocyte infiltration into the submucosal layer

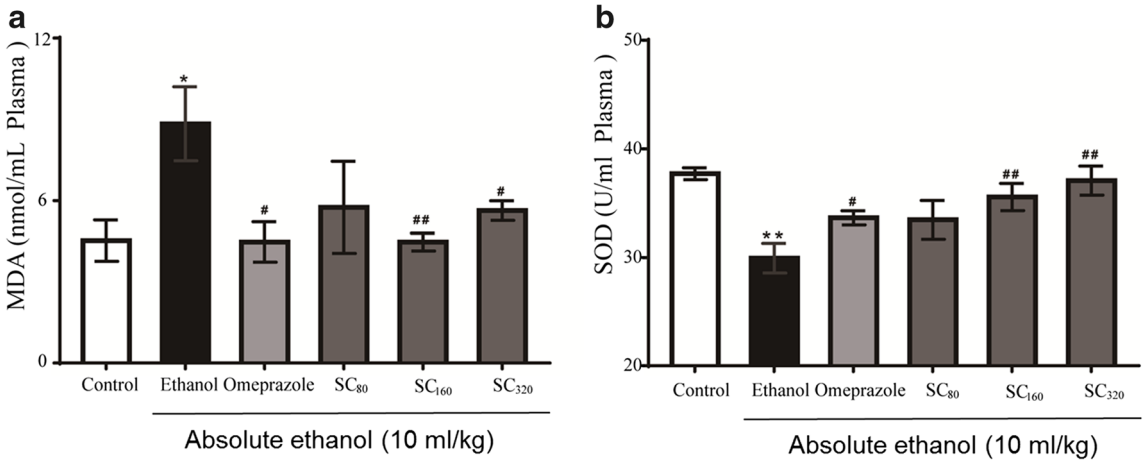

Fig. 4 Effects of the crude extract of *Schisandra chinensis* on the plasma levels of MDA (**a**) and SOD (**b**) in ethanol-induced gastric ulcers in mice. Groups are as defined in Fig. 2. Each value represents the mean ± SEM of 10 mice. Significance is represented as $*p < 0.05$ and $**p < 0.01$ compared with the control group, and $\#p < 0.05$ and $\#\#p < 0.01$ compared with the ulcer control group

areas correlated with mucosal damage (Fig. S3B). Both the crude extract and omeprazole treatment groups showed a strong PAS reaction (Figs. S3C–F). These results demonstrated that gastric mucosal glycoprotein in mice treated with absolute ethanol was markedly depleted. However, pretreatment with the crude extract or omeprazole significantly preserved mucosal glycoprotein. At a dose of 320 mg/kg, the analyzed extract showed the greatest intensity in PAS staining (Fig. S3).

Antioxidative and Anti-inflammatory Effects of Drug Administrations

Oral treatment of mice with absolute alcohol (10 ml/kg) significantly increased MDA levels and reduced SOD levels in plasma compared with those in the control group ($p < 0.05$). Furthermore, in the SC_{160} and SC_{320} groups, the MDA levels showed a significant decrease and the SOD levels displayed a partial prevention of depletion in comparison with these parameters in the model group ($p < 0.001$ and $p < 0.05$). However, the plasma levels of MDA and SOD in the SC_{80} group were not significantly different from those in the ethanol-treatment group. Furthermore, omeprazole at 20 mg/kg prevented the ethanol-induced increase in lipid peroxidation ($p < 0.05$) and decrease in SOD levels ($p < 0.05$) (Fig. 4).

The administration of ethanol significantly increased the levels of plasma TNF-α and IL-1β by 24% and 32%, respectively ($p < 0.001$), when compared with those in the control group. As shown in Fig. 5, the plasma levels of TNF-α and IL-1β were increased markedly in the ulcer control group ($p < 0.01$ and $p < 0.05$, respectively) when compared with those in the control group. In contrast, pretreatment of ethanol-treated mice with either omeprazole or the crude extract at doses of 80, 160, and 320 mg/kg caused a significant decrease in plasma TNF-α levels by 20%, 33%, 41%, and 50%, respectively ($p < 0.01$, $p < 0.01$, $p < 0.001$, and $p < 0.001$) and a decrease

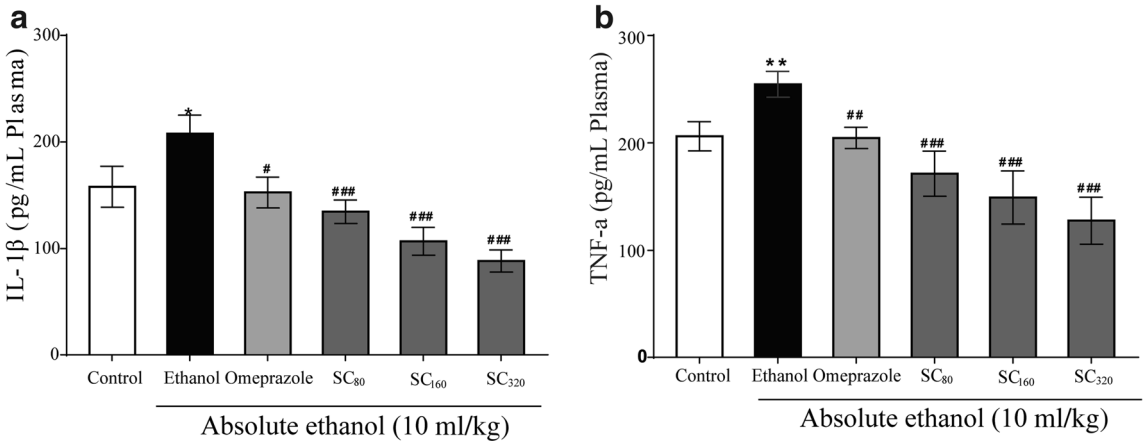

Fig. 5 Effects of the crude extract of *Schisandra chinensis* on the serum levels of IL-1β (**a**) and TNF-α (**b**) in the ethanol-induced gastric ulcers in mice. Groups are as defined in Fig. 3. Each value represents the mean ± SEM of 10 mice. Significance is represented as $*p < 0.05$ and $**p < 0.01$ compared with the control group, and $\#p < 0.05$, $\#\#p < 0.01$, and $\#\#\#p < 0.001$ compared with the ulcer control group

in IL-1β levels by 27%, 35%, 49%, and 56%, respectively ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.001$) (Fig. 5).

Discussion

In the present study, we explored the gastroprotective effects of crude total extracts from magnolia vine berries on ethanol-induced gastric lesions in mice. Our results showed that pretreatment with these extracts dramatically ameliorated hemorrhagic superficial gastric lesions and histological signs of damage, as well as decreased pro-inflammatory cytokines (*i.e.*, TNF-α, IL-1β) and plasma MDA levels while increasing plasma SOD activity and gastric wall mucosal glycoprotein production. Collectively, our findings reveal that ethanol extracts from the berries of *S. chinensis* have potential in mitigating ethanol-induced gastric lesions.

Gastric mucus is the first line of defense against gastric acid, and it prevents gastric mucosa from self-digestion. In the present study, extracts of *S. chinensis* significantly increased the glycoprotein production of mucus (Fig. S3). Our results were further supported by PAS-staining experiments, which confirmed characteristic carmine staining of stomach regions that secrete mucopolysaccharides (Fig. S3). The tissue sections from mice pretreated with extracts showed an intense secretion of mucus in gastric glands, reflecting a higher secretion in these glands. Consequently, the extracts at a dose of 320 mg/kg contributed to the repair of gastric mucosal damage by improving the gastric wall mucus.

Furthermore, it has been widely reported that oxidative stress has an involvement in the pathogenesis of ethanol-induced gastric ulcers (de Araujo et al. 2018; Sidahmed et al. 2019; Mohan et al. 2020). Specifically, oxidative stress plays a key role in both gastric hemorrhages and the formation of ulcers. SOD is an important antioxidative enzyme that converts hydrogen peroxide to oxygen and water. Therefore, the investigation of antioxidants, such as by assessing SOD activity, may help to elucidate the mechanisms of *S. chinensis* extracts, which we found also increased the antioxidative activity of SOD enzymes. Studies in isolated organs, tissues, cells, and enzymes have revealed that *S. chinensis* exhibits strong antioxidative activities. This activity may be attributed to its component ingredient, deoxyschisandrin, which inhibits H_2O_2-induced apoptosis in intestinal epithelial cells through nuclear factor-kappa B (Gu et al. 2010). In the present study, experimental groups had elevated SOD levels after the administration of the crude extracts. Therefore, our present results demonstrate that *S. chinensis* may reduce oxidative stress and enhance antioxidative protection.

Another primary factor in ethanol-induced gastric damage is ROS-mediated lipid peroxidation (Sidahmed et al. 2019; Mohan et al. 2020). As mentioned above, the elimination of ROS has been regarded as one of the mechanisms involved in ulcer healing. In addition, the formation of ROS is related to an increase in lipid peroxidation. MDA is the final product of lipid peroxidation and is widely used to determine the levels of peroxidation lipids; hence, the level of MDA represents a biomarker of oxidative stress. For this reason, we investigated the effects of *S. chinensis* on MDA levels in ethanol-induced ulcerous stomach tissue in mice. We found reduced MDA levels in mice treated with *S. chinensis* extracts compared with those in ethanol group. Thus, *S. chinensis* extracts may generate gastroprotective effects by decreasing lipid peroxidation products. In addition to above factors, the gastroprotective effects of *S. chinensis* extracts may be due to inhibition of inflammatory mediators such as TNF α and IL-1β. Numerous findings have shown that TNF-α and IL-1β levels are dramatically increased in the gastric tissues of ethanol-induced ulcers (Alzokaky et al. 2020; Mohan et al. 2020). These pro-inflammatory cytokines have been reported to play an important role in ethanol-induced gastric ulcer formation, particularly TNF-α, which is a representative inducer of gastric mucosal apoptosis by activating the caspase family of proteases. Much evidence has supported that high-plasma TNF-α levels induce leukocyte adherence, whereas TNF-α antibodies reduce leukocyte adherence and ameliorate gastric mucosal damage (Liu et al. 2016; de Araujo et al. 2018). Similarly, IL-1β, another key pro-inflammatory cytokine participating in inflammation, modulates the expression of genes involved in cell cycle progression and inhibition of apoptosis. Our present study demonstrated that administration of *S. chinensis* extracts inhibited the production of TNF-α and IL-1β in ethanol-induced gastric ulcers and decreased gastric lesions by alleviating gastric mucosal inflammation. In addition, we found that the effects of high-dose *S. chinensis* extracts (320 mg/kg) on TNF-α and IL-1β levels were similar to those of omeprazole.

Conclusions

In conclusion, our present findings suggest that pretreatment with *S. chinensis* extracts suppressed ethanol-induced gastric damage in mice by ameliorating inflammation and oxidative stress, as well as by preserving mucous glycoprotein levels in the gastric mucosa. Furthermore, our findings also indicate that *S. chinensis* may be a promising anti-gastric ulcer drug

to protect the stomach from acute tissue lesions induced by ethanol or other harmful factors.

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Author Contributions WZS, XXH, YHX, HJF, MXL, HFZ, and GYX performed the experiments. WZS and XXH designed the research, analyzed the data, and wrote the paper. Funding acquisitions were derived from JZ and WZS. CS and ZL revised the manuscript. All authors read and approved the final version of the manuscript that was submitted for publication.

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Compliance with Ethical Standards

Protection of Animal Subjects The authors declare that all procedures were in accordance with the regulations of relevant preclinical research ethics and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Conflict of Interest The authors declare that there are no conflicts of interest.

References

Alzokaky AA, Abdelkader EM, El-Dessouki AM, Khaleel SA, Raslan NA (2020) C-phycocyanin protects against ethanol-induced gastric ulcers in rats: role of HMGB1/NLRP3/NF-kappaB pathway. Basic Clin Pharmacol Toxicol. https://doi.org/10.1111/bcpt.13415

Arab HH, Salama SA, Omar HA, El-Shaimaa AA, Maghrabi IA (2015) Diosmin protects against ethanol-induced gastric injury in rats: novel anti-ulcer actions. PLoS One 10:e0122417. https://doi.org/10.1371/journal.pone.0122417

Chen P, Shen Y, Shi H, Ma X, Lin B, Xiao T, Wu F, Zhu J, Li Z, Xiao J, Li X, Zhang H, Geng F (2016) Gastroprotective effects of Kangfuxin-against ethanol-induced gastric ulcer via attenuating oxidative stress and ER stress in mice. Chem Biol Interact 260:75–83. https://doi.org/10.1016/j.cbi.2016.10.021

de Araujo ERD, Guerra DCB, Araujo DFS, Araujo AA, Fernandes JM, Araujo Junior RF, Silva VC, Carvalho TG, Ferreira LS, Zucolotto SM (2018) Gastroprotective and antioxidant activity of *Kalanchoe brasiliensis* and *Kalanchoe pinnata* leaf juices against indomethacin and ethanol-induced gastric lesions in rats. Int J Mol Sci 19:1265. https://doi.org/10.3390/ijms19051265

Dilshara MG, Jayasooriya RGPT, Kang C-H, Lee S, Park SP, Jeong J-W, Choi YH, Seo YT, Jang YP, Kim G-Y (2013) Downregulation of pro-inflammatory mediators by a water extract of *Schisandra chinensis* (Turcz.) Baill fruit in lipopolysaccharide-stimulated RAW 264.7 macrophage cells. Environ Toxicol Pharmacol 36:256–264. https://doi.org/10.1016/j.etap.2013.04.005

Giridharan VV, Thandavarayan RA, Arumugam S, Mizuno M, Nawa H, Suzuki K, Ko KM, Krishnamurthy P, Watanabe K, Konishi T (2015) Schisandrin B ameliorates ICV-infused amyloid beta induced oxidative stress and neuronal dysfunction through inhibiting RAGE/NF-kappaB/MAPK and up-regulating HSP/Beclin expression. PLoS One 10:e0142483. https://doi.org/10.1371/journal.pone.0142483

Gu B-H, Van Minh N, Lee S-H, Lim S-W, Lee Y-M, Lee K-S, Kim D-K (2010) Deoxyschisandrin inhibits H2O2-induced apoptotic cell death in intestinal epithelial cells through nuclear factor-κB. Int J Mol Med 26:401–406. https://doi.org/10.3892/ijmm_00000479

He X, Lian L, Lin L (1997) Analysis of lignan constituents from *Schisandra chinensis* by liquid chromatography-electrospray mass spectrometry. J Chromatogr A 757:81–87. https://doi.org/10.1016/S0021-9673(96)00685-1

Hernandez DE, Hancke JL, Wikman G (1988) Evaluation of the antiulcer and antisecretory activity of extracts of *Aralia elata* root and *Schizandra chinensis* fruit in the rat. J Ethnopharmacol 23:109–114. https://doi.org/10.1016/0378-8741(88)90120-1

Huyke C, Engel K, Simon-Haarhaus B, Quirin KW, Schempp CM (2007) Composition and biological activity of different extracts from *Schisandra sphenanthera* and *Schisandra chinensis*. Planta Med 73:1116–1126. https://doi.org/10.1055/s-2007-981559

Kang YS, Han M-H, Hong S-H, Park C, Hwang H-J, Kim BW, Kyoung KH, Choi YW, Kim CM, Choi YH (2014) Anti-inflammatory effects of *Schisandra chinensis* (Turcz.) Baill fruit through the inactivation of nuclear factor-kappaB and mitogen-activated protein kinases signaling pathways in lipopolysaccharide-stimulated murine macrophages. J Cancer Prev 19:279–287. https://doi.org/10.15430/JCP.2014.19.4.279

Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M (2019) Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. J Clin Med 8:179. https://doi.org/10.3390/jcm8020179

Lee HJ, Kim CY (2010) Simultaneous determination of nine lignans using pressurized liquid extraction and HPLC-DAD in the fruits of *Schisandra chinensis*. Food Chem 120:1224–1228. https://doi.org/10.1016/j.foodchem.2009.11.068

Li Q, Hu X, Xuan Y, Ying J, Fei Y, Rong J, Zhang Y, Zhang J, Liu C, Liu Z (2018) Kaempferol protects ethanol-induced gastric ulcers in mice via pro-inflammatory cytokines and NO. Acta Biochim Biophys Sin 50:246–253. https://doi.org/10.1093/abbs/gmy002

Liu J, Wang F, Luo H, Liu A, Li K, Li C, Jiang Y (2016) Protective effect of butyrate against ethanol-induced gastric ulcers in mice by promoting the anti-inflammatory, anti-oxidant and mucosal defense mechanisms. Int Immunopharmacol 30:179–187. https://doi.org/10.1016/j.intimp.2015.11.018

Mohan S, Hobani YH, Shaheen E, Abou-Elhamd AS, Abdelhaleem A, Alhazmi HA, Abdelwahab SI (2020) Girinimbine from curry leaves promotes gastro protection against ethanol induced peptic ulcers and improves healing via regulation of anti-inflammatory and antioxidant mechanisms. Food Funct 11:3493–3505. https://doi.org/10.1039/d0fo00053a

Panossian A, Wikman G (2008) Pharmacology of *Schisandra chinensis* Bail.: an overview of Russian research and uses in medicine. J Ethnopharmacol 118:183–212. https://doi.org/10.1016/j.jep.2008.04.020

Sidahmed HMA, Hashim NM, Abdulla MA, Ali HM, Mohan S, Abdelwahab SI, Taha MME, Fai LM, Vadivelu J (2015) Antisecretory, gastroprotective, antioxidant and anti-*Helicobcter pylori* activity of zerumbone from *Zingiber zerumbet* (L.) Smith. PLoS One 10:e0121060. https://doi.org/10.1371/journal.pone.0121060

Sidahmed HMA, Vadivelu J, Loke MF, Arbab IA, Abdul B, Sukari MA, Abdelwahab SI (2019) Anti-ulcerogenic activity of dentatin from *Clausena excavata* Burm.f. against ethanol-induced gastric ulcer in rats: Possible role of mucus and anti-oxidant effect. Phytomedicine 55:31–39. https://doi.org/10.1016/j.phymed.2018.06.036

Szopa A, Ekiert R, Ekiert H (2017) Current knowledge of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) as a medicinal plant species: a review on the bioactive components, pharmacological properties, analytical and biotechnological studies. Phytochem Rev 16:195–218. https://doi.org/10.1007/s11101-016-9470-4

Yamada M, Wong FL, Fujiwara S, Tatsukawa Y, Suzuki G (2005) Smoking and alcohol habits as risk factors for benign digestive diseases in a Japanese population: the radiation effects research foundation adult health study. Digestion 71:231–237. https://doi.org/10.1159/000087048

Yang HJ, Kim MJ, Kwon DY, Kang ES, Kang S, Park S (2017) Gastroprotective actions of *Taraxacum coreanum* Nakai water extracts in ethanol-induced rat models of acute and chronic gastritis. J Ethnopharmacol 208:84–93. https://doi.org/10.1016/j.jep.2017.06.045