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ORIGINAL ARTICLE

Hepatoprotective effect of gastrodin against alcohol-induced liver injury in mice

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Abstract

Alcoholic liver disease (ALD) is a common and serious threat to human health worldwide. In this study, the hepatoprotective effect of gastrodin against alcohol-induced liver injury in mice was examined. Mice with alcohol-induced hepatotoxicity were treated intragastrically with gastrodin (50, 80, or 100 mg/kg). The mice treated with gastrodin experienced better outcomes than those who received only one dose of alcohol (50%, 10 mL/kg b.w.). Gastrodin treatment reduced the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), decreased hepatic malondialdehyde (MDA) content, and increased hepatic superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities in a dose-dependent manner. Gastrodin also alleviated histopathological changes induced by alcohol. Gastrodin protected against alcohol-induced increases in expression levels of the cytochrome P450 2E1 (CYP2E1) and mRNA levels of chemokine (C-X-C motif) ligand 1 (CXCL-1), interferon-γ (IFN-γ), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), vascular cell adhesion molecule 1 (VCAM-1), nuclear factor-kappa B (NF-κB), Toll-like receptor 4 (TLR-4), and activator of transcription 3 (STAT-3). Moreover, gastrodin-increased nuclear transcription factor 2 (Nrf2) translocates to the nucleus and enhanced the activity of anti-oxidant enzymes, and could thereby ameliorate alcohol-induced liver injury in mice. This study demonstrated that gastrodin may be an effective therapeutic agent against alcohol-induced liver injury.

Keywords Gastrodin · Alcohol · Hepatic injury · Anti-oxidant · Inflammation

Introduction

Alcoholic liver disease (ALD), which refers to changes within the liver caused by excessive alcohol, is a common disease worldwide [4]. ALD is caused by over-production of reactive oxygen species (ROS), lipid peroxidation injury, cytokine damage, and inflammation [27]. In recent years, the incidence of ALD has increased, posing a serious threat to human health [4]. Thus, research on drugs that are effective in interfering with the course of ALD is vital.

ALD has a complex pathogenesis, and the molecular mechanisms underlying its progression remain unclear [4, 7]. A number of studies showed that oxidative stress and inflammation played key roles in the development of ALD and that compounds with anti-oxidative or anti-inflammatory activities ameliorated the progression of ALD in animal models [2, 28]. Silibinin has been used routinely as a positive control in many studies of liver injury in both animal [6, 15] and cell [8, 29] models.

Gastrodin, a small molecule obtained from *Gastrodia elata* Bl., has long been used in natural medicine [11]. Previous research reported that gastrodin had sedative, hypnotic, anti-convulsant [14, 30], anti-osteoporosis [9], and anti-cancer immune regulatory properties [31]. Research also showed that gastrodin improved memory [14], cholestasis-induced liver fibrosis [33], and nonalcoholic fatty liver disease [21, 31].

Alcohol dehydrogenase and cytochrome P450 (CYP450) can decompose alcohol, and a large amount of ROS is

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produced during the decomposition process [25, 27]. The release of ROS causes pathological changes in hepatocytes, leading to alcoholic fatty liver disease [2]. Nuclear transcription factor 2 (Nrf2) is regarded as a master regulator of oxidant defense, and it directly scavenges free radicals or indirectly increases endogenous cellular anti-oxidant defense by activating Nrf2 transcription factor pathways [12]. Qu et al. found that gastrodin activated the AMP-activated protein kinase (AMPK), and stimulates AMPK/Nrf2 pathway, thereby improving liver oxidative stress and pro-inflammatory responses [21].

Currently, it is unclear whether gastrodin can suppress oxidative stress and inflammation in ALD. The signaling pathways related to ALI are also unclear. The detailed mechanisms underlying the modulation of alcohol metabolism by gastrodin need to be investigated. The present study was to examine the hepatoprotective effect of gastrodin against alcohol-induced liver injury in a murine model of ALD.

Material and methods

Experimental animals

Four-week-old Kun-Ming female mice (25 ± 0.5 g) [10] were purchased from the Experimental Animal Center of the Air Force Military Medical University (Xi'an, China). The mice were kept under controlled conditions at a temperature of 22 °C and humidity of 70%, with a 12-h light-dark cycle, and allowed free access to food and water. All animal experimental protocols were reviewed and approved by the Ethics Committee of Shaanxi University of Technology for the use of laboratory animals.

Animals and treatments

In the study, 49 female mice were randomly assigned to one of seven groups ($n = 7$ in each group).

Normal control (NC) group Mice in the NC group were treated with a physiological saline solution and received a standard pellet diet twice a day for 3 days (10 mL/kg b.w.).

Gastrodin high concentration (Gas_H) group Mice in the drug control group were treated with a physiological saline solution and fed an isocaloric control liquid diet every day throughout the experimental period. Two hours after feeding, the mice were treated with gastrodin orally (100 mg/kg b.w.), and this step was repeated each day for the 3 days of the experiment.

Alcohol model (ALI) group Mice in this group received alcohol every day by gavage for the 3 days of the experimental period. The amount of ethanol in the diet was increased from 35 to 50% over the 3-day experimental period in increments of 5% each time (10 mL/kg b.w.).

ALI-silbinin (ALI-SL) group The mice in this group were fed an ethanol-containing diet for 3 days. Two hours after feeding, the animals received silbinin in ultrapure water p.o. The concentration of silbinin was 80 mg/kg b.w. in accordance with that used in a previous study [5, 20].

ALI-gastrodin low concentration (ALI-Gas_L) group

The mice in this group were treated with ethanol by gavage. Two hours later, gastrodin was administered by oral gavage at a concentration of 50 mg/kg b.w.

ALI-gastrodin medium concentration (ALI-Gas_M) group The mice in this group received gastrodin by oral gavage at a concentration of 80 mg/kg b.w., on all 3 days of the experiment.

ALI-gastrodin high concentration (ALI-Gas_H) group The mice received alcohol and gastrodin twice daily via the intragastric route on all 3 days of the experimental period. In this group, gastrodin (100 mg/kg b.w.) was administered 2 h after the administration of alcohol.

At the end of the 3-day experimental period, the mice were fasted overnight and sacrificed, and blood samples and liver tissue samples were obtained. The blood samples were allowed to coagulate at room temperature for 30 min and were then separated by centrifugation at 3,000 rpm, 4 °C, for 10 min. Liver tissues from each animal were fixed in 4% para-formaldehyde for histological observations. The remaining liver tissue was stored in an ultra-low temperature freezer (− 80 °C) for later biochemical analysis.

Assessment of serum alanine aminotransferase and aspartate aminotransferase activities

Serum alanine aminotransferase (ALT) and asparate aminotransferase (AST) activities were measured using commercial diagnostic kits (Nanjing Jiancheng Institute of Biotechnology, Nanjing, China).

Measurement of superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde content

Liver tissues were homogenized in ice-cold phosphate buffer. This mixture was centrifuged at 4 °C for 10 min, at 3,000 rpm, and the supernatants were collected. The protein concentrations in the supernatants were determined using a standard curve derived from bovine serum albumin (BSA) and the Bradford protein assay (Tiangen Biotech, Beijing, China). Malondialdehyde (MDA) levels, in addition to superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities, were tested using commercially available assay kits, in accordance with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Histological analysis

Fresh liver tissues were fixed in 4% para-formaldehyde, embedded in paraffin blocks, and sectioned (5 μm) in a microtome. The sections were placed on microscopic slides and stained with hematoxylin and eosin (H&E). Photomicrographs of the central veins were then captured under a bright-field light microscope (Olympus, Tokyo, Japan).

Western blot analysis

CYP2E1 expression in microsomal fractions, in addition to Nrf2 protein expression in cytosolic and nuclear extracts, was examined. The microsomal fraction was isolated using a method reported previously [16]. The liver homogenate was prepared in ice-cold buffer and centrifuged at 4 °C for 20 min at 9000×*g*. The supernatant was then removed and placed in a tube with 88 mM calcium chloride ($CaCl_2$). In cold conditions, the mixture was centrifuged at 27, 000×*g* for 20 min at 4 °C. The precipitate was collected, suspended in 50 mM Tris-HCl, and mixed with 20% glycerol. Cytosolic and nuclear extracts were prepared using a nuclear and cytoplasmic extraction kit (Vazyme Biotech, Nanjing, China), and the protein concentration was determined using a BSA assay kit (Beyotime Institute of Biotechnology, Beijing, China) as standard. Equal amounts of extractive protein were separated on 12% sodium dodecyl sulfate polyacrylamide gelelectrophoresis and transferred to a vinylidene fluoride PVDF membrane under ice-cold conditions at 200 mA for 40 min. The membranes were incubated in a blocking solution (Tris-buffered Saline with Tween 20) containing 5% non-fat milk powder for 2 h at room temperature. Subsequently, the membranes were incubated overnight at 4 °C with primary antibodies (cytochrome P450 2E1 [CYP2E1], 1:500, Boster Wuhan, China; Nrf2, 1:1000, Boster Wuhan, Wuhan, China). The membranes were then incubated with secondary antibodies (Boster Wuhan, Wuhan, China) for 2 h at room temperature. Signals were visualized by the Emitter-Coupled Logic (ECL) reaction (Advansta, CA, USA). Before the Gel-Pro Analyzer software analysis, protein levels were quantified and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lamin B using Gel-Pro Analyzer software (Media Cybernetics, MD, USA).

Real-time polymerase chain reaction assays

Total mRNA from the liver tissues of the different groups was obtained using an RNA extraction kit (Tiangen Biotech, Beijing, China), according to the manufacturer's instructions. The mRNA samples were then reverse-transcribed into cDNA using HiScript™ RT SuperMix for Real-Time Polymerase Chain Reaction (PCR) (Vazyme Biotech, Nanjing, China) and a Veriti Thermal Cycler (Applied Biosystems, MA, USA). The oligonucleotide primers are listed in Table 1. The real-time PCR was performed on a StepOnePlus™ Real-Time PCR Detection System (Thermo Fisher Scientific, MA, USA) with SYBR® Green Master Mix (Vazyme Biotech, Nanjing, China). The relative expression of mRNA was expressed by the $2^{-(\Delta\Delta Ct)}$ formula and normalized to that of GAPDH, an internal control gene.

Statistical analysis

All the experimental data were expressed as mean ± SD, and SPSS Statistics, version 20.0 (IBM Corporation, NY, USA) was used for statistical analysis. The Shapiro-Wilk test was used to verify whether the data was normally distributed. Levene's test was used to verify the homogeneity of variances. A one-way analysis of variance (ANOVA) or Kruskal–Wallis non-parametric test was used, depending on data distribution and variance homogeneity. The Kruskal–Wallis test followed by the post hoc analysis (Mann–Whitney *U* test) was used to evaluate pairwise differences among the adjusted means. Values of $p < 0.05$ were considered statistically significant.

Results

The protective effect of gastrodin against alcohol-induced hepatotoxicity

Serum ALT and AST enzymatic activities were significantly increased in the ALI group as compared with those in the NC group, indicating alcohol-induced hepatic injury (Fig. 1). By contrast, ALT and AST enzymatic activities of the ALI-Gas_H group were reduced by 96% and 78% when compared with those of the NC group. The increase in serum ALT and AST activities induced by alcohol was inhibited by SL treatment.

The impact of gastrodin on alcohol-induced histopathological changes in the liver

Histological sections of liver tissue obtained from the NC group had a normal lobular architecture. The hepatic cord was normal, central veins showed a radiated orderly arrangement, and liver sinusoids were clearly observed. Also, the morphological structure of the liver cells was normal, with a clear boundary between the nucleus and cytoplasm, and cytoplasm was uniform (Fig. 2). On the other hand, the liver tissue

Table 1 Primers used for real-time PCR

Target gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
TNF-α	TATGGCTCAGGGTCCAACTC	GCTCCAGTGAATTCGGAAAG
IFN-γ	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG
NF-κB	ACGATCTGTTTCCCCTCATCT	TGGGTGCGTCTTAGTGGTATC
VCAM-1	AGCCTCAACGGTACTTTGGA	GCGTTTAGTGGGCTGTCTAT
CXCL-1	GATTCACCTCAAGAACATCCAGAG	GAAGCCAGCGTTCACCAGAC
TLR-4	TTCTTCTCCTGCCTGACACC	CCATGCCATGCCTTGTCTTC
STAT-3	TGCAGAGCAGGTATCTTGAG	TGCTGCTTCTCTGTCACTAC
IL-6	CAAAGCCAGAGTCCTTCAGAG	GTCCTTAGCCACTCCTTCTG
Nrf-2	ACACGGTCCACAGCTCATCAT	TTGGCTTCTGGACTTGGAAC
GAPDH	ACAGTCCATGCCATCACTGCC	GCCTGCTTCACCACCTTCTTG

of the ALI group showed a large area of degeneration and an abnormal hepatic lobule structure. In addition, liver sinusoids could not be seen. Furthermore, liver cell degeneration, characterized by swelling, volume increases, cytoplasm loss, and nuclear pycnosis of liver cells were observed. Cellular size was inconsistent, the nucleus was absent, and cell necrosis was also observed in the ALI group. As shown in Fig. 2, in the ALI-Gas$_L$ group, the hepatic lobule structure was clearly abnormal as compared with that of the ALI group. Also, sparse cytoplasm, cell degeneration, and liver degeneration were visible. In the ALI-Gas$_H$ group, the number of lobular lesions was reduced as compared with that in the ALI group, and the liver had a normal cell structure (Fig. 2). These findings indicated that gastrodin inhibited alcohol-induced liver injury in this murine model of ALD.

Gastrodin inhibited alcohol-induced anti-oxidant enzyme activity in the liver

MDA levels were significantly increased in the ALI group as compared with those in the NC group, pointing to alcohol-induced oxidative stress in the liver. Treatment with alcohol and gastrodin inhibited the increase in MDA levels. In the ALI group, SOD, GPx, and CAT activities were reduced by 86%, 38%, and 80%, respectively, as compared with those in the NC group. In the ALI-GasH group, the administration of gastrodin increased the anti-oxidant enzyme (SOD, GPx, and CAT) activity, improved the defense capability of the anti-oxidant system, and prevented the collapse of the redox balance caused by alcohol hepatotoxicity. Also, gastrodin treatment ameliorated the effects of alcohol exposure in a dose-dependent manner (Fig. 3). Relative to the NC group, gastrodin treatment induced the pronounced restoration of CAT activity in the ALI-Gas$_H$ group. Gastrodin-induced effects were more noticeable in the ALI-Gas$_H$ group than in the ALI-SL group.

Gastrodin suppressed the metabolic expression of CYP2E1

CYP2E1 protein expression levels of experimental mice revealed the dose-dependent effects of gastrodin, with the expression level significantly enhanced in the ALI group. CYP2E1 protein expression levels were suppressed in the ALI-Gas$_H$ group (Fig. 4).

Gastrodin-induced Nrf2 expression

As shown by Western blot and real-time PCR analyses of Nrf2 expression in liver tissue from the different groups,

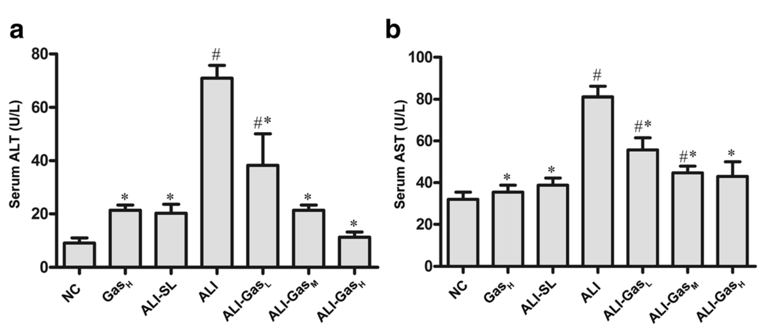

Fig. 1 Effect of gastrodin on alcohol-induced increases in serum transaminase levels. Following the treatment of mice with alcohol and various concentrations of gastrodin (50, 80, or 100 mg/kg b.w.) for 3 days, serum ALT (**a**) and AST (**b**) enzymatic activities were measured using commercial kits. The significance of the differences was estimated by one-way ANOVA test. #$p < 0.05$ versus NC group; *$p < 0.05$ versus ALI group ($n = 7$)

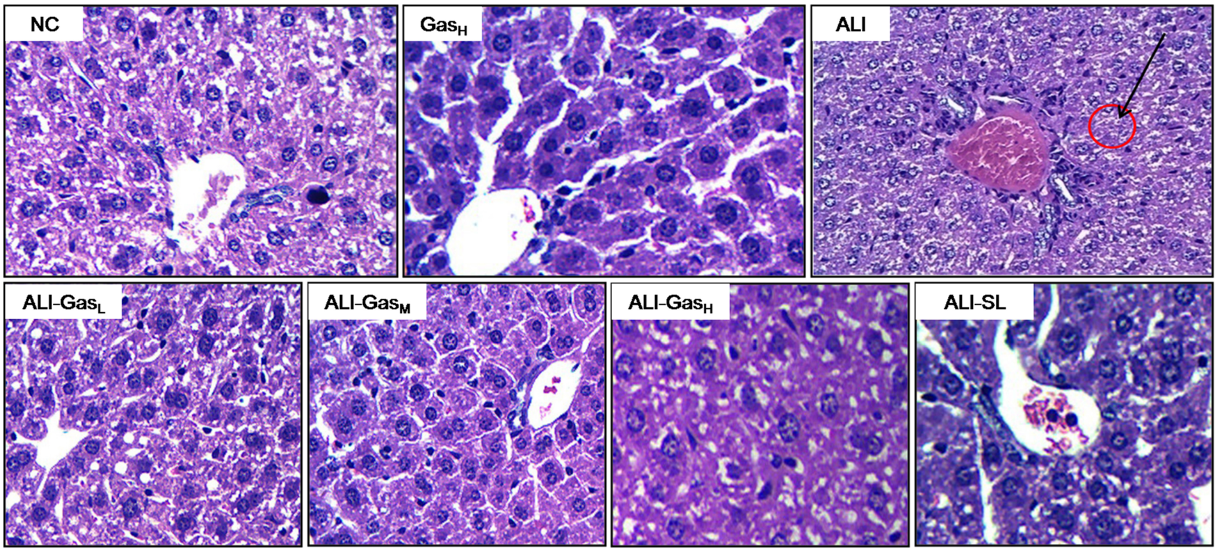

Fig. 2 Effect of gastrodin on alcohol-induced hepatic histopathological changes in mice. Liver H&E stain, × 400. In the NC group, the liver tissue had a normal lobular architecture, liver sinusoids were clearly observed, a clear boundary existed between the nucleus and cytoplasm, and cytoplasm was uniform. The ALI group showed large areas of liver degeneration and necrosis and an abnormal hepatic lobule structure. Liver tissue was plunged into chaos, and liver sinusoids were absent. The arrow denotes a blurred nucleus. The ALI group was also characterized by cytoplasm loss and nuclear pycnosis of liver cells. Improvements in the gastrodin treatment group were dose-dependent

nuclear protein levels of Nrf2 increased in ALI-Gas_L, ALI-Gas_M, and ALI-Gas_H groups as compared with those in the NC group. The trend observed in mRNA levels was consistent with that of nuclear Nrf2 protein levels (Fig. 4c). In ALI-Gas_L, ALI-Gas_M, and ALI-Gas_H groups, protein expression levels and mRNA levels were increased as compared with those in NC group. In contrast, protein expression levels were suppressed in the cytoplasm of the ALI group. The results clearly demonstrated that gastrodin stimulated nuclear Nrf2 expression in a dose-dependent manner (Fig. 4).

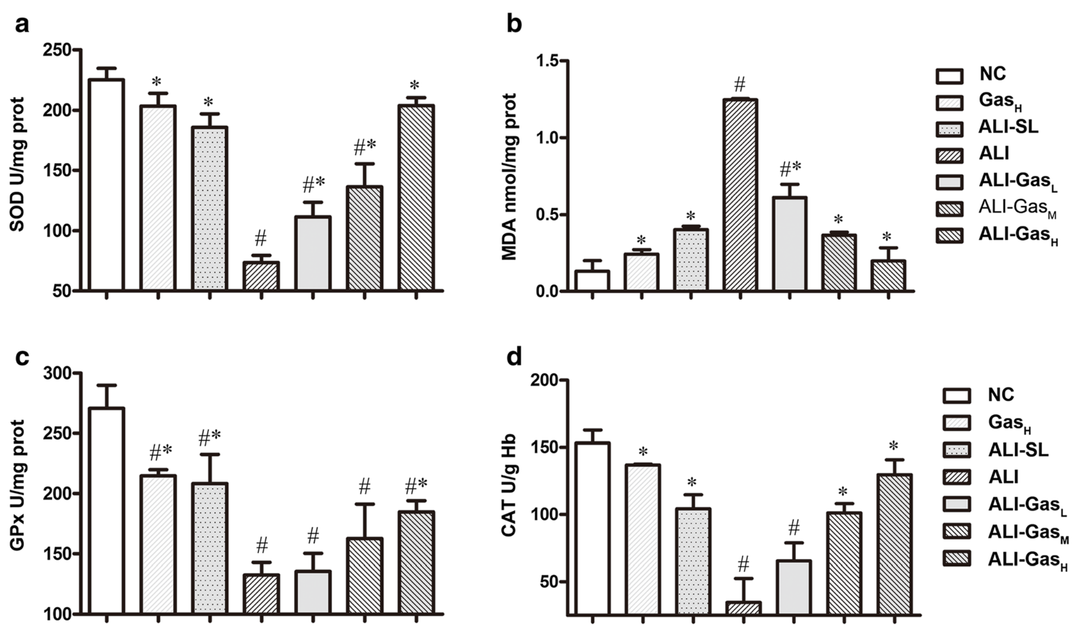

Fig. 3 Effect of gastrodin on oxidative stress induced by alcohol. For anti-oxidative capacity assays, a commercial kit was employed. SOD (**a**), GPx (**c**), and CAT (**d**) activities and MDA (**b**) levels in liver homogenate from the NC, Gas_H, ALI-SL, ALI, ALI-Gas_L, ALI-Gas_M, and ALI-Gas_H groups. (**a**, **c**, and **d** significance of differences was estimated by one-way ANOVA test; **b** significance of differences was estimated by a Kruskal–Wallis non-parametric test followed by a Mann-Whitney U test, $p = 0.013$). #$p < 0.05$ versus NC group; *$p < 0.05$ versus ALI group ($n = 6$)

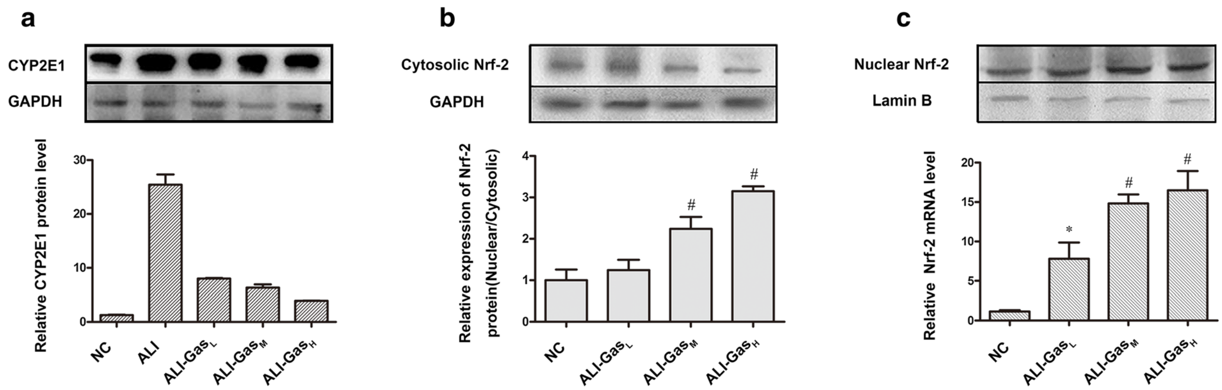

Fig. 4 Effect of gastrodin on expression levels of CYP2E1 and Nrf2 in the ALI group. **a** Western blot analysis of CYP2E1 protein levels. GAPDH was used as a loading control. **b**, **c** Cytosolic and nuclear Nrf2 protein levels were analyzed by a Western blot, and Nrf2 mRNA levels were detected by real-time PCR (**a** significance of differences was estimated by a Kruskal–Wallis non-parametric test, $p = 0.068$; **b**, **c** significance of differences was estimated by one-way ANOVA test). #$p < 0.05$ versus the NC group ($n = 3$), *$p < 0.05$ versus the ALI-Gas$_H$ group ($n = 3$)

Gastrodin suppressed mRNA expression of cytokines and chemokines

mRNA expression of cytokines in the liver was examined using real-time PCR. As indicated in Fig. 5, mRNA levels of VCAM-1, IL-6, TNF-α, IFN-γ, NF-κB, TLR-4, STAT-3, and CXCL-1 were significantly increased in the livers in the ALI group as compared with those in the NC group. In contrast, the ALI-GasH group was characterized by low mRNA expression levels of cytokines.

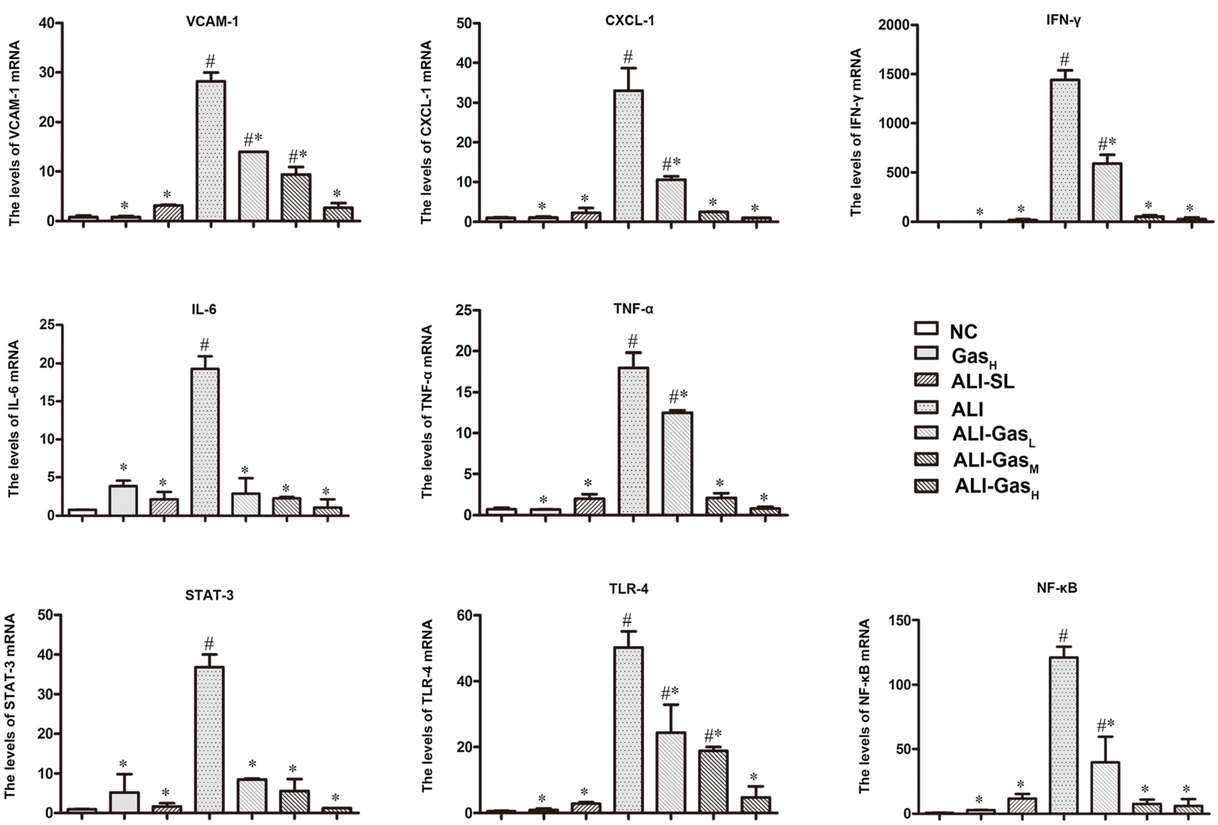

Fig. 5 Inhibitory effect of gastrodin on alcohol-induced cytokine mRNA expression in mice. After 3 days of treatment with alcohol and various concentrations of gastrodin (50, 80, or 100 mg/kg b.w.), the total mRNA of mice liver tissue in each group was obtained by kit, and mRNA was reverse-transcribed into cDNA. mRNA levels of CXCL-1, IFN-γ, IL-6, TNF-α, VCAM-1, TLR-4, STAT-3, and NF-κB were determined by the real-time PCR assay. Statistical analysis was performed using the Kruskal–Wallis non-parametric test followed by the Mann–Whitney *U* test. Corresponding $p = 0.006$ (CXCL-1), $p = 0.010$ (IFN-γ), $p = 0.016$ (IL-6), $p = 0.006$ (TNF-α), $p = 0.008$ (VCAM-1), $p = 0.005$ (TLR-4), $p = 0.006$ (STAT-3), $p = 0.005$ (NF-κB). #$p < 0.05$ versus the NC group; *$p < 0.05$ versus the ALI group ($n = 4$)

Discussion

Alcohol metabolism is considered key in the development and progression of ALD, which can injure multiple organs and multiple systems [6]. Alcohol is mainly metabolized by cytochrome P450 (CYP450) and alcohol dehydrogenase in vivo [27]. The metabolic reactions of ethanol and its derivatives lead to liver toxicity, induce an inflammatory response, and produce oxygen free radicals, resulting in liver damage [2]. The current study focused mainly on the effect of gastrodin on alcohol-induced ALD in mice and investigated levels of anti-oxidant enzymes and cytokines, in addition to expression levels of CYP2E1 and Nrf2.

Clinically, the activities of AST and ALT are regarded as sensitive indicators of hepatotoxicity [10]. In this study, gastrodin treatment suppressed alcohol-induced elevations in ALT and AST enzymatic activities and ameliorated histopathological lesions.

Healthy human cells have an effective anti-oxidative and anti-inflammatory defense system, in which SOD, GPx, and CAT are the main enzymes [27]. This anti-oxidant defense system can effectively remove free radicals and prevent peroxide-mediated damage [7, 19]. However, when ethanol intake exceeds that of the body's scavenging capacity, oxidative damage occurs, especially in the liver [3, 24]. MDA, an end product of membrane lipid peroxidation, can change the permeability of the membrane, thereby inducing a series of physiological and biochemical reactions, which can potentially lead to severe liver cell necrosis [7]. MDA content can be used as an indicator of the severity of cell damage [10]. In the present study, as compared with the normal group, the activities of anti-oxidant enzymes (SOD, GPx, and CAT) were significantly reduced in the ALI group. In addition, MDA levels were eight times higher in the ALI group as compared with those in the normal group. Furthermore, liver TNF-α, IFN-γ, CXCL-1, VCAM-1, TLR-4, STAT-3, and NF-κB mRNA levels were significantly higher in the alcohol-treated groups than the normal group. These findings were consistent with those of a previous study [10, 17]. Previous studies reported that the consumption of alcohol activated innate immune responses and that this resulted in the production of inflammatory factors, such as TNF-α, IFN-γ, VCAM-1, IL-6, and CXCL-1, leading to liver damage [17, 26]. In the present study, after gastrodin treatment, activity levels of anti-oxidant enzymes (SOD, GPx, and CAT), as well as MDA levels, returned to normal. Also, mRNA levels of TNF-α, IFN-γ, CXCL-1, VCAM-1, TLR-4, STAT-3, and NF-κB in the liver returned to normal.

Previous studies showed that Keap1-Nrf2-anti-oxidant response element (ARE) signaling was involved in protecting cells from endogenous and exogenous stresses [32]. Under basal intracellular redox conditions, Nrf2 bundled with the actin-anchored Keap1 protein, and Keap1 mediates proteasomal degradation of Nrf2, which resides mainly in the cytoplasm. However, under conditions of stress (chemical/oxidative), the regulation of Nrf2 becomes complex, involving both Keap1-dependent and Keap1-independent mechanisms. In the Keap1-dependent mechanism, proteasomal degradation of Nrf2 is blocked, and Nrf2 is released from Keap1 and translocates to the nucleus. Additionally, other mechanisms of Keap1-independent regulation may occur under both redox-sensitive and insensitive conditions and may aid in the fine-tuning of the regulation of Nrf2 levels under basal conditions [1]. Recent studies showed that the anti-oxidant activity of gastrodin was closely related to the activation of the AMPK/Nrf2 pathway [21, 31]. Gastrodin increased the activity of SOD, reduced the production of ROS, and activated AMPK. AMPK stimulates Nrf2 phosphorylation, Nrf2 is released from Keap1 and translocates to the nucleus, inhibiting the oxidative and inflammatory damage, thereby promoting cell survival [13] and inhibiting diseases such as brain damage and liver damage caused by oxidative stress [14, 21]. Our results showed that Nrf2 is highly expressed in the nucleus and its expression in the cytoplasm is significantly decreased, which is consistent with the results of Qu et al. [21]. Therefore, we speculated that the cause of this phenomenon may be that gastrodin stimulated the Nrf2 pathway by activating AMPK in the liver, resulting in nuclear translocation of Nrf2.

Silibinin is a commercially available hepatoprotective drug, which has been clinically proven to have better hepatoprotective effects and fewer side effects than glucurolactone, azathioprine, and colchicine [8, 18]. Silibinin exerts protective effects on the liver via the following mechanisms: (i) it scavenges free radicals (anti-oxidant activity); (ii) it regulates membrane permeability, thereby providing membrane protection against drug injury; and (iii) it stimulates DNA polymerase-I and regulates DNA transcription, which increases the synthesis of ribosomal RNA [26]. In doing so, silibinin plays an important role in the regeneration of liver cells and protein synthesis [26]. Studies showed that silibinin activated AMP-activated protein kinase, thereby protecting neuronal cells from oxygen and glucose deprivation/re-oxygenation [34]. Research also demonstrated that silibinin inhibited nonalcoholic fatty liver disease by activating the NAD-dependent protein deacetylase sirtuin-1 (SIRT1)/AMPK pathway [23]. In a murine model of acute ethanol-induced injury, silibinin removed free radical intermediates (hydroxyethyl free radicals) produced by the metabolism of CYP2E1, increased glutathione levels, and regulated membrane permeability, which inhibited alcohol-induced liver injury [5].

Compared with the positive control, our experiments showed that gastrodin not only increased anti-oxidant enzyme activity in the livers of mice but also inhibited high expression of the CYP2E1 protein induced by alcohol and stimulated the nuclear translocation of Nrf2, thereby blocking the development of inflammation. Our experiments demonstrated that gastrodin improved alcohol-induced liver injury.

In humans, alcoholic liver disease is complex and involves a variety of mechanisms (e.g., alcohol consumption, sex, genetic characteristics, metabolism, nutrition, and infections) [22]. Therefore, screening of compounds for the treatment of ALD has attracted significant research attention. Traditional natural medicine has a wide range of sources and a long history, and there are many reports on the use of traditional medicines for the treatment of ALD [6, 10, 28]. There are no reports on the chemical structures of natural monomer drugs that could be used to treat ALD. The present study shows that gastrodin, a natural monomer compound, has potential in providing protection against alcohol-induced liver injury.

Conclusions

This study indicates that gastrodin could significantly alleviate alcohol-induced hepatotoxicity by increasing the anti-oxidative defense and anti-inflammatory responses. Our findings will provide a scientific basis for the clinical application of gastrodin to treat ALD in the future.

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Compliance with ethical standards

All animal experimental protocols were reviewed and approved by the Ethics Committee of Shaanxi University of Technology for the use of laboratory animals.

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

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