Food and Chemical Toxicology 126 (2019) 277–284

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Protective effects of ginsenoside Rk3 against chronic alcohol-induced liver injury in mice through inhibition of inflammation, oxidative stress, and apoptosis

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ARTICLE INFO

Keywords:
Alcoholic liver disease
Ginsenoside Rk3
Oxidation
Inflammation
Apoptosis

ABSTRACT

Alcoholic liver disease (ALD), as one of the most common diseases, has become a global threat to human health. The aim of this study was designed to investigate the hepatoprotective effects of ginsenoside Rk3 against ALD and to discover the potential mechanisms of these protective effects. Mice were intragastrically administered 50% alcohol and treated with ginsenoside Rk3 (25 and 50 mg/kg) once per day for 6 weeks. The results indicated that ginsenoside Rk3 promoted hepatic function through significant downgrading AST and ALT levels in the serum, attenuating oxidative stress, and restoring antioxidant balance in hepatic tissue. Additionally, ginsenoside Rk3 significantly reduced the expression of inflammatory cytokines, such as NF-κB, TNF-α, IL-6, and IL-1β in the mice. Furthermore, ginsenoside Rk3 supplementation significantly inhibited apoptotic protein expression in the liver. The present study clearly demonstrates that ginsenoside Rk3 exerts a protective effect against ALD-induced liver injury because of its antioxidant, anti-apoptotic, and anti-inflammatory activities. The findings from the present investigation show that ginsenoside Rk3 might be a promising candidate treatment agent against ALD.

1. Introduction

Alcoholic liver disease (ALD) induced by chronic heavy drinking has become a growing public health problem in the world today (Panyod et al., 2016). Worldwide, approximately 3.3 million people die each year from the overuse of alcohol, which accounts for approximately 5.9% of all deaths, and the overconsumption of alcohol can lead to many metabolic diseases (WHO, 2018). The early stage of ALD usually manifests as fatty liver, which in turn can develop into hepatic inflammation, necrosis, progressive fibrosis, and even hepatocellular carcinoma (Altamirano and Bataller, 2011).

Even though much research has focused on understanding the pathological features of ALD, the cellular mechanisms that defend against the detrimental effects of alcohol remain unclear (You et al., 2018). Under normal circumstances, alcohol is absorbed through the stomach and small intestine andenters the liver through the blood. In the liver, ethanol is oxidized into acetaldehyde through alcohol dehydrogenase. Acetaldehyde is metabolized into acetic acid by aldehyde dehydrogenase and then excreted through the urethra. Consuming large quantities or chronic consumption of alcoholic drinks can exceed the detoxification capacity of the liver, resulting in the accumulation of alcohol and acetaldehyde in the blood and liver. Excessive oxidative metabolites of acetaldehyde and ethanol in the liver activate the microsomal cytochrome P450 system and increase the activity of cytochrome P4502E1 (CYP2E1), resulting in a large number of reactive oxygen species (ROS). These peroxides, such as ROS, directly attack

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https://doi.org/10.1016/j.fct.2019.02.032
Received 5 December 2018; Received in revised form 31 January 2019; Accepted 23 February 2019
Available online 28 February 2019
0278-6915/

organelles and DNA, causing damage to liver cells and liver dysfunction (Ding and Yin, 2017). Oxidative damage is considered to be one of the main hazards of alcohol consumption and also a key target in the pathogenesis of ALD (Hao et al., 2015). The transcription factor nuclear factor-kappa B (NF-κB) and its corresponding pathway play a major role in pro-inflammatory signaling (Zhao et al., 2017). ALD results in increased expression of the transcription factor NF-κB, producing multiple cytokines and participating in the inflammatory response (Ilaiyaraja and Khanum, 2011). Additionally, acetaldehyde can reduce the concentration of reduced glutathione (GSH) and induce lipid peroxidation, thereby increasing the toxicity of free radicals and promoting cell apoptosis (Popovic et al., 2008).

Panax ginseng CA Meyer (ginseng) is a famous medicinal herb at home and abroad. Research regarding ginseng and its application has been recognized worldwide, ginsenosides are the major component of ginseng, and they exert numerous pharmacological actions, including anti-diabetic, anti-cancer, anti-anemia, and anti-non-alcoholic fatty liver disease properties (Deng et al., 2017; Peng et al., 2015; Wei et al., 2018; Wu et al., 2017). Ginsenoside Rk3, as one of the major rare saponins in heat-treated ginseng, plays a variety of biological roles, containing anti-apoptotic, anti-anemia, anti-cancer and protective effects against cisplatin-induced acute kidney injury (Baek et al., 2017; Duan et al., 2017; Sun et al., 2013; Wei et al., 2018). It is reported that *Panax notoginseng* saponins can effectively improve ethanol-induced hepatic steatosis and reduce oxidative stress in the liver, revealing that *Panax notoginseng* saponins may become a new effective agent to prevent alcoholic hepatitis (Ding et al., 2015). Another study showed that Korean red ginseng extract (RGE) and its ginsenosides could improve alcohol-induced hepatotoxicity and hepatic steatosis by activating the adenosine monophosphate-activated protein kinase (AMPK)/sirtuin 1 (SIRT1) pathway in *vivo* and in *vitro* (Han et al., 2015). It has been demonstrated that ginsenoside Rg1 may protect hepatocytes against alcohol-induced liver injury by preventing excessive inflammation *via* the NF-κB pathway and hepatocellular apoptosis (Li et al., 2018). However, the hepatoprotective effect of ginsenoside Rk3 on ALD has not been researched in *vivo* until now. Here, the ameliorative potential effect of ginsenoside Rk3 was explored for ALD in mice. To the best of our knowledge, the hypothetical mechanisms in association with the protective effect of ginsenoside Rk3 on ALD were found for the first time.

2. Materials and methods

2.1. Reagents and materials

The standard ginsenoside Rk3 (purity ≥ 99.0%) was purchased from Chengdu Puruifa Technology Co., Ltd. (Sichuan, China). Ginsenoside Rk3 was obtained from ginseng leaves by hydrolyzing the ginsenoside Re (Toh et al., 2011). The purity of the standard and separated ginsenoside Rk3 (> 97%) were detected by HPLC (SSI, USA) (Supplemental Figs. 1 and 2). Then, ginsenoside Rk3 was dissolved in 50% deionized water and 50% polyethylene glycol 400 (PEG-400) for subsequent gavage. The chemical structure of Rk3 was shown in Fig. 1A. Primary antibodies against Bcl-2, Bax, cleaved caspase 3, cleaved caspase 8, cleaved caspase 9, and cleaved PARP were purchased from Abcam (Cambridge, UK). Primary antibodies against CYP2E1, NF-κB, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) were obtained from Proteintech Group, Inc. (Chicago, USA). Primary antibody against GAPDH was purchased from Cell Signaling Technology (Danvers, MA, USA). RevertAid first strand cDNA synthesis kits were purchased from Thermo Scientific (Wilmington, DE, USA). The FastStart Essential DNA Green Master was bought from Roche (Roche, Germany). Hematoxylin and eosin (H&E) staining kit was bought from Beijing Solarbio Technology Co., Ltd (Beijing, China). PEG-400 was purchased from Sigma Chemical Co. (St Louis, MO).

2.2. Animals and treatments

Male ICR mice (n = 40; 6-week-old; body weight 18–22 g) were obtained from the Experimental Animal Center of Xi'an Jiaotong University. Mice were housed in standard polypropylene transparent cages at a controlled temperature (20–25 °C) and humidity (50% ± 5%) with a 12 h light/dark cycle and free access to water. The experiment was approved by the Animal Ethics Committee of Northwest University and met the requirements of the Laboratory Animal Act of the People's Republic of China. After a week of adaptation, the mice were divided randomly into 4 groups with 10 mice per group: normal group, mice received PEG-400 solution; ethanol (EtOH) group, mice received PEG-400 solution and were orally administered 50% ethanol (10 mL/kg body weight) 2 h later; and two ginsenoside Rk3 groups, mice received either 25 mg/kg or 50 mg/kg Rk3 and were orally administered 50% ethanol (10 mL/kg body weight) 2 h later. The experimental design was shown in Fig. 1B and the entire experiment lasted for 6 weeks. Body weight was monitored weekly. After the experiment, all mice were fasted overnight and then anesthetized. Blood samples were collected for serum preparation and stored at −80 °C for future use. The livers were obtained and stored at −80 °C.

2.3. Determination of the liver index

The mouse liver index was calculated as follows: liver index = liver weight/final body weight × 100%.

2.4. Serum biochemical analyses

Blood samples were centrifuged at 1000 *g* for 15 min at 4 °C to obtain the serum. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), TNF-α, IL-6, and IL-1β were determined using commercial reagent kits according to the manufacturer's protocols (Nanjing Jiancheng Biology Engineering Institute, Nanjing, China).

2.5. Liver biochemical analyses

The contents of malondialdehyde (MDA), superoxide dismutase (SOD), and GSH in liver homogenates were measured using corresponding commercial kits according to the manufacturer's instructions (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China).

2.6. Histological analysis

For histopathological analysis, liver tissue was fixed in 4% paraformaldehyde and embedded in paraffin for the preparation of tissue sections (5 μm thickness). After staining with H&E, the sections were evaluated for histopathological changes using a Nikon TE 2000 fluorescence microscope (Nikon, Japan).

2.7. Western blot analysis

Liver tissue was lysed in RIPA buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) (Beyotime, Shanghai, China) with protease inhibitor cocktail for 30 min on ice. The lysates were then centrifuged at 12000 *g* at for 20 min at 4 °C, the supernatants were collected carefully, and the protein concentrations were determined using a BCA™ Protein Assay Kit (Solarbio, Beijing, China). The supernatants of the preserved subsamples (25 μg) were used for western blot analysis. In short, after boiling, proteins were separated by SDS-PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane. The imprinted membrane was blocked with 5% skimmed milk for 2 h, incubated overnight with a primary antibody at 4 °C, and incubated with a second antibody at room temperature for 1.5 h. An ECL system (Perkin Elmer, Waltham, MA, USA) was used to visualize the imprinted protein.

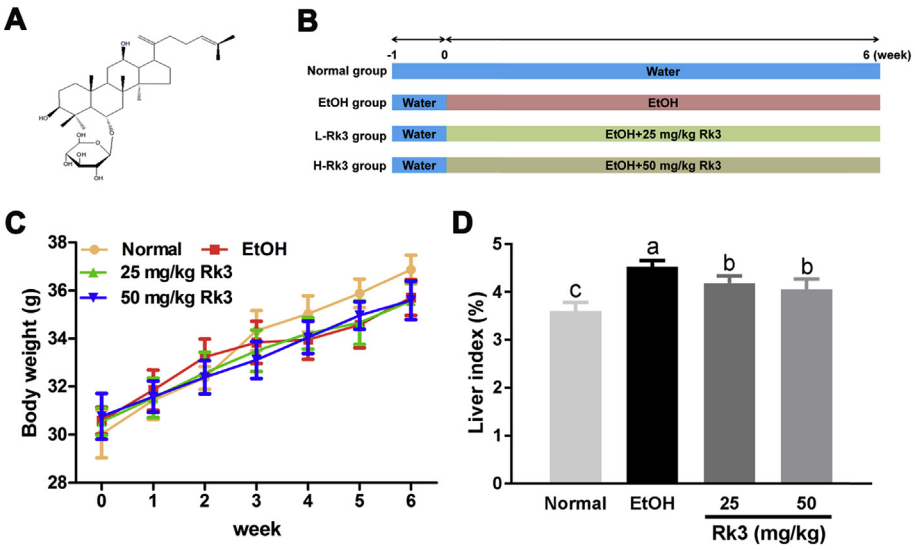

Fig. 1. Effect of ginsenoside Rk3 on bodyweight and liver index in alcohol-induced mice. (A) The chemical structure of Rk3. (B) Scheme of the study design describing several groups of animals treated in 6 weeks. (C) The body weight of mice. (D) The liver index of mice. Values are means ± SD (n = 8). Values marked with different letters are significantly different from each other at $p < 0.05$.

2.8. RNA extraction and mRNA quantification by real-time quantitative reverse-transcription PCR (qRT-PCR)

qRT-PCR was used to analyze relative mRNA expression changes. Total RNA was isolated from frozen livers using TRIzol reagent (Ambion, MA, USA) according to the manufacturer's instructions. Then, the quality of total RNA was evaluated using a Nano-Drop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). First strand cDNA was generated using 1 μg of total RNA as a template with a RevertAid first strand cDNA synthesis kit. qRT-PCR was performed using SYBR Green mix on a CFX thermocycler system (Bio-Rad, Hercules, CA, USA). PCR amplification was performed as follows: predenaturation at 95 °C for 10 min, followed by 45 cycles for amplification consisting of denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s, and extension at 72 °C for 15 s. Relative quantification was calculated using the $2^{-\Delta\Delta CT}$ method. The primers used are shown in Table 1.

2.9. TUNEL assay

Apoptotic cells were determined with TUNEL assay. In short, apoptotic cells in liver tissue were detected by in situ apoptosis assay kit (Roche Applied Science, Germany; No. 11684817910). The liver sections were fixed with distilled protease K (20 mg/mL). The slides were treated with a balanced buffer and end deoxynucleotide transferase, and then anti-digoxin-peroxidase conjugate was added to the liver peroxidase activity immobilized in each liver tissue section. Diaminobenzidine was dyed and used for redyeing sections. The sections were evaluated for histopathological changes using a Nikon TE 2000 fluorescence microscope (Nikon, Japan).

2.10. Statistical analysis

The experimental data were expressed as means values ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's test using SPSS version 19.0 software (SPSS Inc., IL, USA). Statistical significance was considered at $p < 0.05$. Graphs were created using GraphPad Prism (Version 5.00, GraphPad Software Inc., San Diego, CA, USA).

Table 1
Sequences of primers used in the quantitative real-time reverse transcription-polymerase chain reaction.

Genes	Forward primer (5′-3′)	Reverse primer (5′-3′)
GAPDH	gctgagtatgtcgtggagt	gttcacccatcacaaac
TNF-α	gtctactgaacttcggggtgat	ggctacaggcttgtcactcg
IL-6	ctctgcaagagacttccatcc	gaattgcattgcacaactc
IL-1β	ccaacaagtgatattctccatgag	actctgcagactcaaactcca

3. Results

3.1. Effect of ginsenoside Rk3 on body weight and liver index

As shown in Fig. 1C, there was no significant difference in body weight for 6 weeks in the four groups. Mice in the EtOH group had a higher liver index than mice in the normal group (Fig. 1D) ($p < 0.05$), whereas mice in the ginsenoside Rk3 supplementation groups had a lower liver index than those in the EtOH group ($p < 0.05$).

3.2. Effect of ginsenoside Rk3 on serum biochemical factors

For transaminase levels, including ALT (Fig. 2A) and AST (Fig. 2B), intragastric alcohol administration resulted in a significant increase in ALT and AST activity in the EtOH group. However, ginsenoside Rk3 supplementation lowered ALT and AST activity in a dose-dependent manner. These data indicated that ginsenoside Rk3 could alleviate ethanol-induced liver swelling and hepatic function. Meanwhile, we detected inflammatory factors, including TNF-α, IL-6, and IL-1β, in serum using commercial kits. As shown in Fig. 2, compared to the normal group, ethanol administration in mice significantly increased TNF-α (Fig. 2C), IL-6 (Fig. 2D), and IL-1β (Fig. 2E) concentrations, while different doses of ginsenoside Rk3 reduced the levels of these inflammatory factors to varying degrees.

3.3. Effect of ginsenoside Rk3 on hepatic antioxidant capacity

SOD, MDA, and GSH activities in liver were measured to investigate changes in oxidative stress. As shown in Fig. 3, compared to EtOH treatment, 25 mg/kg ginsenoside Rk3 increased SOD (Fig. 3A) and GSH (Fig. 3C) activity, but no significant ($p > 0.05$); compared to EtOH group, 50 mg/kg ginsenoside Rk3 significantly increased SOD and GSH activity ($p < 0.05$), but there was no significant difference between two ginsenoside groups ($p > 0.05$). Ethanol administration, compared with normal treatment, significantly increased MDA (Fig. 3B) activity ($p < 0.05$), whereas ginsenoside Rk3 significantly lowered MDA activity in a dose-dependent manner.

Because CYP-mediated biological activation plays a key role in liver

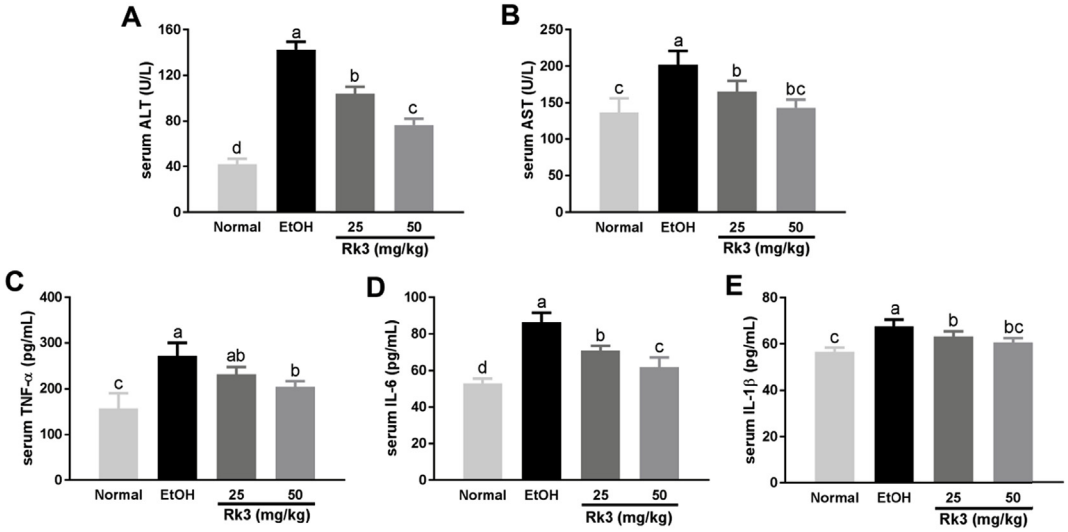

Fig. 2. Effect of ginsenoside Rk3 on serum biochemistry in alcohol induced mice. (A) Alanine aminotransferase. (B) Aspartate aminotransferase. (C) Tumor necrosis factor α. (D) Interleukin 6. (E) Interleukin 1β. Values are means ± SD (n = 8). Values marked with different letters are significantly different from each other at $p < 0.05$.

antioxidant capacity, CYP2E1 protein expression was detected in liver tissue by western blotting. As expected, compared with the normal group, ethanol administration resulted in the overexpression of CYP2E1. However, treatment with ginsenoside Rk3 decreased the protein expression of CYP2E1 (Fig. 3D). These data demonstrated that ginsenoside Rk3 ameliorated ethanol-induced oxidative stress injury.

3.4. Liver histology analysis

H&E staining was used to evaluate the pathological liver changes in mice (Fig. 4). The results indicated that liver tissue showed hepatocyte degeneration, vacuolation, and necrosis in the EtOH group. However, ginsenoside Rk3 significantly reduced alcohol-induced liver degeneration and lipid vacuolation. These histological analyses showed that ginsenoside Rk3 partially protected the liver from injury at the histological level.

3.5. Effect of ginsenoside Rk3 on inflammatory stress in mouse liver

Next, we examined the levels of inflammation markers, including NF-κB, TNF-α, IL-6, and IL-1β, in the livers of mice to assess the effect of alcohol on the inflammatory response. As shown in Fig. 5A, alcohol administration increased the protein expression of NF-κB, TNF-α, IL-6, and IL-1β, whereas ginsenoside Rk3 decreased the protein expression of these inflammation factors in a dose-dependent manner. Moreover, the mRNA expression levels of TNF-α, IL-6, and IL-1β were also analyzed by qRT-PCR. Comparing with the normal group, the EtOH group had higher mRNA expression of TNF-α, IL-6, and IL-1β in their livers. However, ginsenoside Rk3 supplementation decreased the expression of these inflammatory cytokines (Fig. 5B). Ginsenoside Rk3 decreased ethanol-induced inflammatory stress in mouse livers.

3.6. Effect of ginsenoside Rk3 on apoptotic proteins in the mouse liver

Since long-term alcohol administration resulted in hepatocyte apoptosis, the protein expression levels of Bax, Bcl-2, cleaved caspase 3, cleaved caspase 8, cleaved caspase 9, and cleaved PARP, which play key roles in apoptosis, were detected in mouse liver using western blot assays. The results showed that alcohol administration increased the protein expression of Bax, cleaved caspase 3, cleaved caspase 8, cleaved

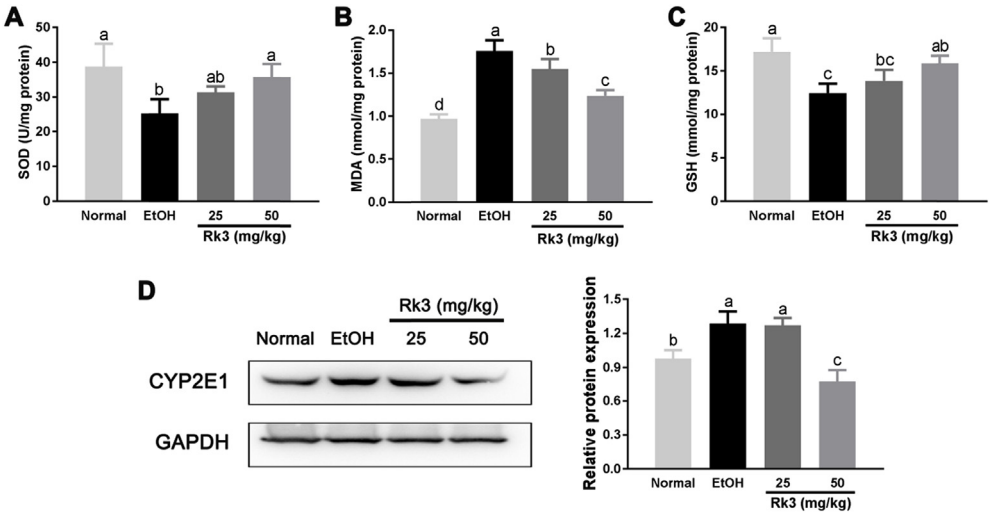

Fig. 3. Effect of ginsenoside Rk3 on liver oxidative stress in alcohol induced mice. (A) Superoxide dismutase. (B) Malondialdehyde. (C) Glutathione. (D) The protein expression of CYP2E1. Values are means ± SD (n = 8). Values marked with different letters are significantly different from each other at $p < 0.05$.

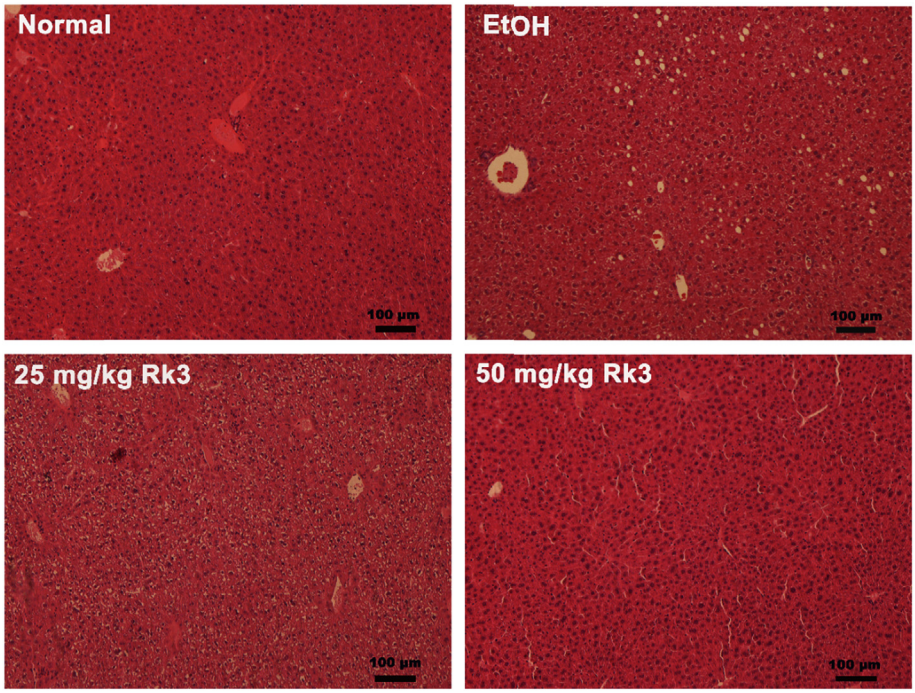

Fig. 4. Effect of ginsenoside Rk3 on hematoxylin and eosin (H&E) staining of liver tissue in alcohol induced mice.

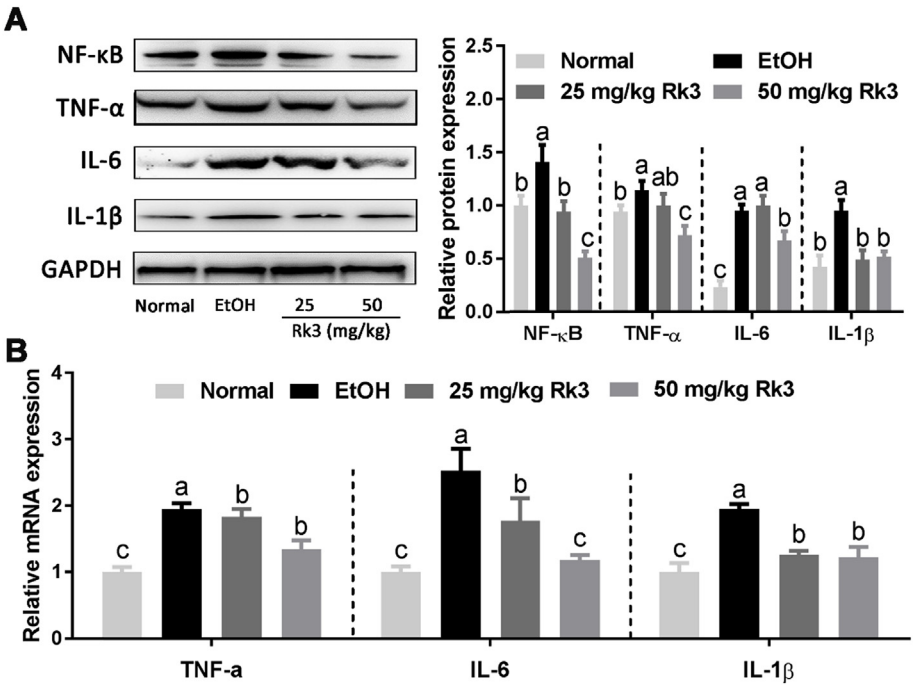

Fig. 5. Effect of ginsenoside Rk3 on hepatic inflammation in alcohol-induced mice. (A) The protein expression of inflammatory factors (NF-κB, TNF-α, IL-6, IL-1β). (B) The mRNA expression of inflammatory factors (TNF-α, IL-6, IL-1β). Values are means ± SD (n = 8). Values marked with different letters are significantly different from each other at $p < 0.05$.

caspase 9, and cleaved PARP. However, ginsenoside Rk3 supplementation lowered the protein expression of these pro-apoptotic proteins in a dose-dependent manner (Fig. 6A). Bcl-2, as an anti-apoptotic protein, was up-regulated by ginsenoside supplementation. To further analyze liver cell apoptosis more clearly, the TUNEL staining was performed. The results showed that almost no apoptotic cells were observed in the normal group, and a large number of apoptotic cells were observed in the EtOH group, whereas ginsenoside Rk3 pretreatment reversed this evaluation (Fig. 6B). Taken together, these data suggested that ginsenoside Rk3 could effectively improve liver cell apoptosis induced by chronic alcohol consumption.

4. Discussion

ALD is a chronic liver injury caused by heavy drinking and can progress to inflammation and hepatocellular damage, and patients eventually develop liver fibrosis deposition, cirrhosis, and hepatocellular carcinoma (O'Shea et al., 2010). Ginseng, as the first of the "three treasures of traditional Chinese medicine" and "three treasures of northeast China", has a long history of more than 4000 years in the Chinese nation and has been favored by doctors and rulers throughout the ages because of its powerful pharmacological activity, such as anticancer, anti-obesity, anti-inflammation, and anti-diabetes properties (Attele et al., 1999). In this study, the effects of ginsenoside Rk3 on

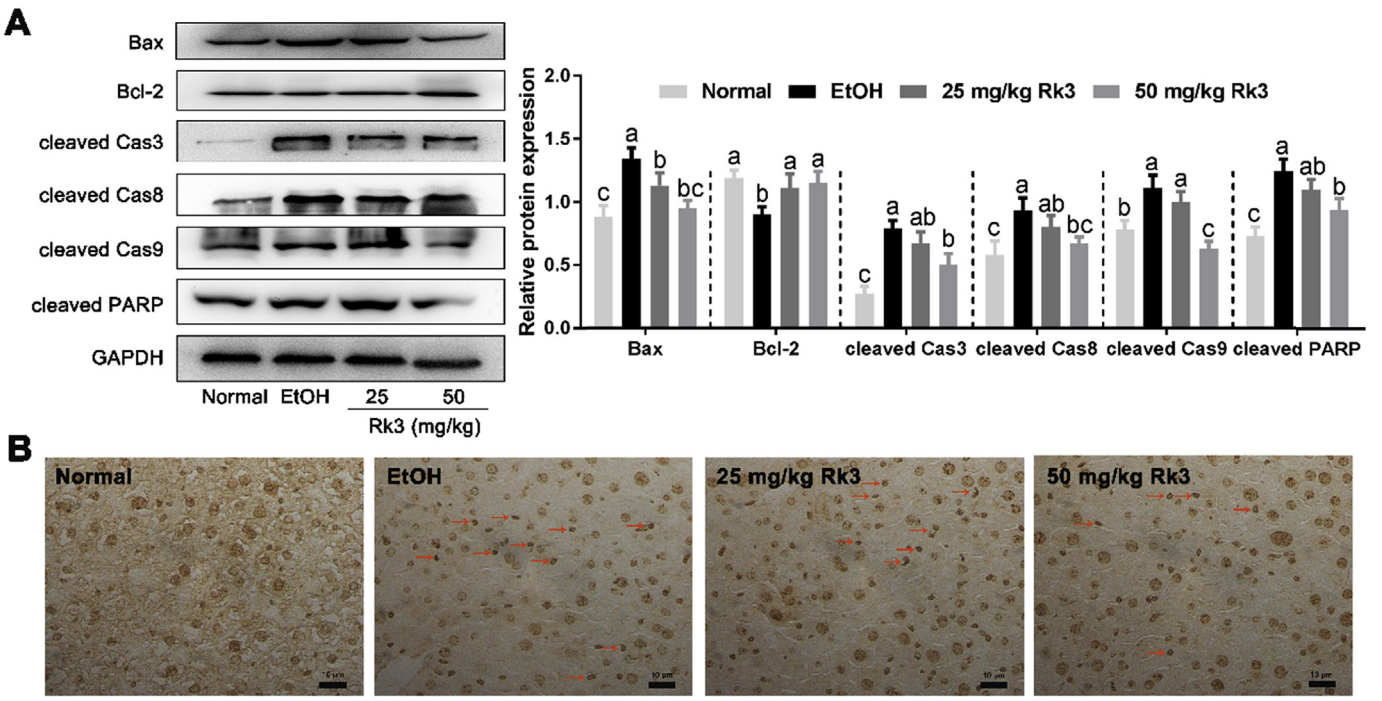

Fig. 6. Effect of ginsenoside Rk3 on hepatic apoptosis in alcohol-induced mice. (A) The protein expression related to apoptosis including Bax, Bcl-2, cleaved caspase 3, cleaved caspase 8, cleaved caspase 9, cleaved PARP in liver tissue of alcohol-induced mice. (B) TUNEL staining of liver tissue. Arrows indicated the apoptotic cells. Values are means ± SD (n = 8). Values marked with different letters are significantly different from each other at $p < 0.05$.

hepatic injury induced by chronic alcohol consumption were explored in animal studies. We evaluated the underlying mechanisms of the protective effect of ginsenoside Rk3 by examining hepatic functional (ALT and AST) and anti-inflammatory (NF-κB, TNF-α, IL-6, and IL-1β), pathological (H&E staining), anti-oxidative (MDA, SOD, CYP2E1, and GSH), and anti-apoptotic (Bax, Bcl-2, cleaved caspase 3, cleaved caspase 8, cleaved caspase 9, and cleaved PARP) activities.

The liver is the most important organ for metabolizing alcohol; therefore, it is the organ that is mainly affected by long-term alcohol consumption (Yip and Burt, 2006). In our study, the ethanol consumption-induced increase in the liver index was improved by ginsenoside Rk3 supplementation. We also detected two main transaminases (ALT and AST) in mouse serum, and the results indicated that ginsenoside Rk3 ameliorated ethanol-induced increases in ALT and AST activity in a dose-dependent manner. ALT and AST are two sensitive indicators of hepatocyte injury and the most important parameters in routine clinical liver tests (Mu et al., 2012). Similar to our study, another study has revealed that rats in *Notoginseng* Radix groups showed significant reductions in liver ALT and AST activity (Zhang et al., 2013). Histological analysis of the liver showed that dietary ginsenoside Rk3 supplementation effectively attenuated hepatic necrosis and vacuolization in mice. Thus, ginsenoside RK3 could attenuate ethanol-induced liver damage.

Ethanol-induced oxidative stress seems to play a major role in mechanisms by which ethanol causes liver injury (Lu and Cederbaum, 2008). In the liver, ethanol consumption generates oxidative stress through excess ROS produced by CYP2E1 and decreased mitochondrial reduced GSH levels (Xiao et al., 2017). At the same time, this oxidative damage induces mitochondrial dysfunction and cell apoptosis, which in turn facilitates the advancement of ALD. GSH, as an important antioxidant in the body, can scavenge free radicals from the body and is of great importance in the liver antioxidant system (Zubkova and Robaire, 2004). Increased MDA levels in the liver can cause tissue damage and interfere with antioxidant defense (Adaramoye et al., 2010). SOD can eliminate harmful substances produced by metabolic processes in organisms, keep cells from oxidative damage of superoxide free radicals and hydrogen peroxide free radicals, act a pivotal part in maintaining the balance between oxidation and anti-oxidation, and is one of the important indicators reflecting free radical metabolism in the human body. The level of SOD indirectly reflects the ability of the body to scavenge free radicals, and SOD is considered a key scavenger of ROS (Firuzi et al., 2011). In our results, ginsenoside Rk3 supplementation, compared to ethanol administration, increased SOD and GSH activity and decreased MDA activity in serum in a dose-dependent manner. Meanwhile, the protein expression of CYP2E1 was also down-regulated by ginsenoside Rk3 supplementation. Consistent with our research, *Panax notoginseng* saponins (PNS) remarkably ameliorated the overexpression of antioxidant enzymes, including GSH and SOD, induced by ethanol; in addition, the protein expression of CYP2E1 was significantly inhibited by PNS in a dose-dependent manner (Ding et al., 2015). To conclude, ginsenoside Rk3 ameliorated ethanol-induced oxidative stress injury.

In addition to oxidative stress, inflammation plays a key role in the pathogenesis of ALD. NF-κB is an important inflammatory transcription factor that plays a key role in regulating the subsequent expression of genes related to pathological liver changes (Liu et al., 2014). Additionally, NF-κB promotes the expression of a series of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, and regulates cell responses, including apoptosis (Barman et al., 2016). TNF-α promotes the migration of the central vein and infiltration of inflammatory cells and triggers the production of ROS (Xu et al., 2017). Overproduction of inflammatory cytokines may be a marker of liver injury, and lowering the expression of these inflammatory factors may help restore liver function (Hsu et al., 2006). The results in our investigation showed that the levels of TNF-α, IL-6, and IL-1β in serum were remarkably increased in the ethanol group; however, ginsenoside Rk3 administration effectively improved this situation. Consistent with the serum results, the hepatic mRNA and protein expression levels of NF-κB, TNF-α, IL-6, and IL-1β were high in ethanol group, whereas these inflammatory cytokines were downregulated after ginsenoside Rk3 administration. According to our results, ginsenoside Rk3 decreased ethanol-induced inflammatory stress in mouse serum and liver.

Previous studies have shown that pro-apoptotic protein such as Bcl-2 in the liver of ALD patients are up-regulated, and it can be activated by oxidative stress, thereby promoting the induction of apoptotic pathways (Altamirano and Bataller, 2011; Ramalho et al., 2006; Ribeiro et al., 2004). In our study, ginsenoside Rk3 administration significantly decreased the protein expression of pro-apoptotic proteins (Bax, cleaved caspase 3, cleaved caspase 8, cleaved caspase 9, and cleaved PARP) and reversed the protein expression of an anti-apoptotic protein (Bcl-2), showing that ginsenoside Rk3 pretreatment resulted in the prevention of pro-apoptotic injury in ethanol hepatotoxicity.

Chronic ethanol abuse leads to the induction of microsomal ethanol oxidation system, thereby increasing the expression of CYP2E1 and the accumulation of ROS, induces lipid peroxidation and oxidative stress, and further damages liver cells (Nordmann et al., 1992; Lieber, 2010). In addition, ROS promote the chronic inflammatory stage and the release of inflammatory factors, such as IL-1, IL-6, TNF-α, and IL-8 (Ceni et al., 2014). Hepatocytes caused by increased oxidative stress and ethanol-induced tissue inflammation could release the Inflammatory cytokines, including TNF-α, IL-6, and IL-1β (Choi et al., 2018). Moreover, the release of inflammatory cytokines, such as COX-2, TNF-α, and IL-6, induces hepatocyte apoptosis (Gyeong-Ji et al., 2017). Apoptotic hepatocytes have been shown to co-localize with infiltrating neutrophils, and thus it is likely that apoptosis and inflammation are correlated (O'Shea et al., 2010; Gao and Bataller, 2011). Moreover, the release of inflammatory cytokines, including TNF-α and IL-6, can directly lead to hepatocyte apoptosis, as well as ROS accumulation and oxidative stress (Hong et al., 2002; Hye et al., 2018).

5. Conclusion

In summary, our study clearly shows that ginsenoside Rk3 can prevent alcohol-caused liver injury in mice through attenuating oxidative stress injury and inhibiting liver inflammation and cell apoptosis. Importantly, these data strongly emphasize that ginsenoside Rk3 appears to be an attractive complementary drug candidate for treating and preventing liver injury.

Acknowledgement

This project was supported by the National Natural Science Foundation of China (21776228, 21878246, and 21676212).

Abbreviations

ALD	alcoholic liver disease
CYP2E1	cytochrome P4502E1
ROS	reactive oxygen species
NF-κB	nuclear factor-kappa B
GSH	glutathione
Ginseng	Panax ginseng CA Meyer
RGE	korean red ginseng extract
AMPK	adenosine monophosphate-activated protein kinase
SIRT1	sirtuin 1
PEG-400	polyethylene glycol 400
AST	aspartate aminotransferase
ALT	alanine aminotransferase
MDA	malondialdehyde
SOD	superoxide dismutase
H&E	hematoxylin and eosin

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.02.032.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.02.032.

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