Ameliorative effect of grape seed proanthocyanidin extract on thioacetamide-induced mouse hepatic fibrosis

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HIGHLIGHTS

- Administration of TAA for 9 weeks led to a serious liver necrosis and collagen deposition.
- GSPE diminishes TAA-induced mRNA expression of TGF-β1, α-SMA and α1(1)-collagen.
- GSPE suppresses the collagen deposition and the activated HSCs induced by TAA.

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ABSTRACT

The present study was designed to examine the effect of the grape seed proanthocyanidin extract (GSPE) on developing hepatic fibrosis that was induced by thioacetamide (TAA) in mice. Administration of TAA for 9 weeks led to a serious necrosis and apoptosis of the parenchymal cells, which resulted in an accumulation of excessive collagen in the liver and an increase of transformed hepatic stellate cells (HSCs). In addition, the mRNA expression of transforming growth factor β1 (TGF-β1), α-smooth muscle actin (α-SMA), as the marker of the activated HSCs, and α1(1)-collagen were all up-regulated significantly when compared with the control. However, combined oral administration of GSPE at 100 mg/kg suppressed the mRNA expression of TGF-β1 and α-SMA, with decreased collagen accumulation as demonstrated by histomorphological evaluation and quantitative RT-PCR. The mRNA expression of the pro-inflammatory factors, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), was remarkably enhanced by TAA treatment. However, their levels displayed a down-regulated trend beyond simultaneous GSPE treatment. Moreover, GSPE administration markedly suppressed lipid peroxidation. In conclusion, as a plant antioxidant, GSPE manifested effective hepatocellular protective action to ameliorate the developing liver fibrosis induced by chronic TAA administration in mice.

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1. Introduction

Liver fibrosis is a wound-healing response to chronic noxious stimuli (Bedossa and Paradis, 2003). The vast majority of hepatocellular carcinomas and cases of hepatic cirrhosis develop in patients suffering from liver fibrosis (Kornek et al., 2006). Damage to the liver tissues can be brought about by long-term administration of various noxious substances which promote serious necrosis and apoptosis of parenchymal cells. When the liver’s defensive capacities are exceeded and the loss of hepatocytes continues, a potent regenerative response is triggered. However, when hepatic injury persists further, the rebuilding process of damaged tissues will be disrupted and the extracellular matrix degradation process will be inhibited (Tipoe et al., 2010). This complex response process requires the activation of the hepatic stellate cells (HSCs). HSCs have been identified as the principal collagen producing cells in the liver, and are mediated by an intricate network of cytokines, mitogens, and growth factors (Friedman et al., 1985; Mann and Marra, 2010). At later stages, an excessive extracellular matrix is accumulated in liver tissues, which may lead to hepatic fibrosis or cirrhosis. As HSC plays a central role in the formation process of liver fibrosis, reducing activated HSCs may represent an effective method of protecting liver against fibrogenesis.

Appropriate animal models are of great importance in the understanding of the mechanisms responsible for the acute or chronic liver injury process. Carbon tetrachloride (CCL4) is the most common agent to cause liver damage and induce liver
fibrosis. However, this is insoluble in water, and usually needs to be dissolved in vegetable or mineral oil and then administrated via repetitive intraperitoneal or subcutaneous injection (Erman et al., 2004; Miyazaki et al., 2005; Abdel Aziz et al., 2007). Its application to mice is inconvenient and associated with an intolerable high mortality rate (Kornek et al., 2006). The approaches of other chemicals to induce hepatic damage such as dimethylnitrosamine (DMN), thioacetamide (TAA), α-naphthyl-isothiocyante, or 3,5-diethoxycarbonyl-1,4-dihydrocollidine has been well described in the literatures (for example Weiler-Norman et al., 2007).

The hepatic toxic chemical TAA has been widely used in the study of the underlying mechanisms of hepatic fibrogenesis and the therapeutic effects of potential anti-fibrosis drugs. TAA is water soluble, and so can be easily administrated orally by being dissolved in drinking water (Salguero Palacios et al., 2008), or utilized in other approaches such as intraperitoneal injection (Baskaran et al., 2010; de David et al., 2011), or injections combined with 10% alcohol in drinking water (Kornek et al., 2006). TAA causes severe centrilobular necrosis and also induces apoptosis and perportal inflammatory cell infiltration in the liver. The initiation of the hepatotoxic effect of TAA requires metabolic activation (Wang et al., 2000; Ramaiah et al., 2001; Chilakapati et al., 2007).

In view of the widespread cases of liver fibrosis, there is a great demand to innovate or to find a potent anti-fibrotic agent for liver fibrosis patients. This aim so far, has remained elusive. Natural products extracted from plants or traditional Chinese medicine exhibit a variety of biological activities. Many of them can be options for the treatment of liver fibrosis. Among various natural plant extracts, grape seed proanthocyanidin extract (GSPE) is a powerful free radical scavenger which has been reported to possess a broad spectrum of biological, pharmacological and therapeutic effects. Proanthocyanidins are a class of phenolic compounds that take the forms of oligomers or polymers of polyhydroxy falvan-3-ol units, such as (+)-catechin, (−)-epicatechin (Yamakoshi et al., 2002). They accumulate predominantly in the lignified portions of grape clusters, especially in the seeds (Kovac et al., 1995). Currently commercial preparations of GSPE are marketed with over 95% standardized proanthocyanidins as dietary supplement due to its health benefits. Proanthocyanidins exhibit anti-inflammatory, anti- allergic and antitumoral activities (Li et al., 2001; Singh et al., 2004; Mantena et al., 2006; Tang et al., 2012), beyond their free radical scavenging and antioxidant activity. Furthermore, they have been reported to modulate the activity of enzymes including phospholipase A2, cyclooxygenase and lipoxygenase (Bagchi et al., 2000). As to fibrosis-related disease, GSPE was demonstrated to decrease the oxidative stress and reduce the fibrogenic effect of silica-induced pulmonary fibrosis (Hemmata et al., 2008). It also had the ability to reduce oxidative stress and fibrosis in experimental biliary obstruction (Dulundu et al., 2007) and exhibited hepatoprotective and anti-fibrogenic effects against DMN-induced liver injury in vivo (Shin et al., 2010). It also was demonstrated to inhibit the arsenic induced-rat liver injury through suppression of NADPH oxidase and TGF-β/Smad activation (Pan et al., 2011).

In light of the observations above, it seems feasible to expect that GSPE might be employed to reduce liver fibrosis and be an effective candidate for the desired anti-fibrotic drug innovation. The present study was designed to determine whether proanthocyanidins have an anti-fibrogenic effect on TAA-induced hepatic fibrosis in mice along with noting any possible changes in mRNA expression of the fibrosis markers of transforming growth factor β1 (TGF-β1), α-SMA and α-1-(I)-collagen, the pro-inflammatory mediators of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). The results would provide a pivotal reference for treatment of liver fibrosis.

2. Material and methods

2.1. Animal model and treatments of chemicals

Female ICR mice weighing 18–22 g were obtained from the Laboratory Animal Center of Zhejiang University. Animals were kept at 22 ± 2 °C and relative humidity (60 ± 10%) under 12-h light/dark cycles. All experimental procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Zhejiang University.

We prepared the TAA-induced liver fibrosis as previously described (Teixeira-Clerc et al., 2006). The dosage of GSPE was based on several reported works (Saada et al., 2009; Pan et al., 2011; Tang et al., 2012). The animals were divided into four groups as follows: (1) control group with the administration of vehicles only. (2) GSPE control group. (3) TAA treatment group received 300 mg/L TAA (Aladdin reagent Co. Shanghai, China) in drinking water for 9 weeks. (4) GSPE + TAA group at a daily dose of 100 mg/kg GSPE by oral administration every day for the length of the study. The GSPE was generously provided by JF-NATURAL Co. (Tianjin, China). The total proanthocyanidins content of the GSPE is over 95% in this experiment.

2.2. Sample preparation

At the end of the treatment period the mice were sacrificed after anaesthesia with sodium pentobarbital. Blood and liver samples were then collected for further analysis. The liver tissue blocks were washed with ice-cold saline, and either frozen in liquid nitrogen and kept at −80 °C for biochemical analyses, or fixed in 4% paraformaldehyde for histological studies. Serum was obtained by centrifugation of blood at 13,000 rpm for 15 min at 4 °C and stored at −80 °C for analysis.

2.3. Histological and immunohistochemical staining

For histomorphological evaluation, a portion of fixed liver tissue was dehydrated, embedded in paraffin and sectioned at 5 μm. The sections were stained with hematoxylin and eosin and Masson’s trichrome stain. The morphological changes were examined under a microscope (Eclipse 80i, Nikon, Japan), and the pictures were captured with a digital camera (DS-Fi1, Nikon, Japan). Grading and staging of hepatic histopathological changes was performed by using a previous reported system (Ishak et al., 1995).

The immunohistochemical stains were carried out by using anti-α-smooth muscle actinin (α-SMA) as previously described (Guexdon et al., 1979). Briefly, five micrometer liver sections were deparaffinized, rehydrated, and dried in 3% H2O2 for 30 min to quench endogenous peroxidase activity. Antigen retrieval was carried out in a citrate buffer (pH 6.0) at 95 °C for 60 min. 5% bovine serum albumin was used to block the nonspecific staining. The histological sections were then incubated with the monoclonal antibody for α-SMA (Boster Bioengineering Co., Wuhan, China) at a dilution of 1:200 overnight at 4 °C. After washing with PBS, sections were incubated with the biotinylated secondary antibodies. Then the immunoreaction was amplified with streptavidin–avidin–peroxidase complex (SBBC), and the sections were visualized by using 3,3′-diaminobenzidine tetrahydrochloride (DAB) and lightly counter-stained with hematoxylin.

2.4. RNA extraction, cDNA synthesis, and quantitative real-time PCR

The extraction of total RNA, preparation of cDNA and the amplification of target genes were carried out according to the manufacturer’s instructions. Briefly, liver tissues were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, USA) to extract total RNA. The RNA purity and concentration were determined spectrophotometrically at 260/280 nm in the range of 1.8–2.0 (Thermo Scientific, Waltham City, MA, USA). Total RNA (3 μg) was reverse transcribed by Fermentas One step RT-PCR kit (MBI Fermentas, Burlington, ON, Canada) and amplified by PCR. The sequences of the primers are listed in Table 1.

The quantitative real-time PCR was carried out on the ABI 7300 HT real time PCR machine (Applied Biosystems, Foster City, CA, USA) with the reaction volume of 20 μl consisting of a 2 or 4 μl cDNA template, 0.4 μl of each of the gene-specific forward and reverse primers and a 10 μl SYBR® Premix Ex Taqtm (TaKaRa Bio Inc. Co., Japan). Experiments were repeated twice. All samples were normalized against 18S rRNA using the comparative CT method (∆∆CT) (Livak and Schmittgen, 2001).

2.5. Biochemical analysis of malondialdehyde and serum aminotransferases

As one endpoint of liver peroxidation, malondialdehyde (MDA) indirectly reflects the level of hepatocellular oxidative injury. MDA concentrations were calculated by detecting the absorbance of thiobarbituric acid reactive substances at 532 nm and were expressed in nmol/mg protein (Agustinho et al., 1997). Frozen liver tissue was homogenized with ice-cold saline. The homogenate was centrifuged for 15 min at 1700 × g and the supernatant was used for further measurements (Bu et al., 2011). The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined with a biochemical analyzer (7180, HITACHI, Japan).
2.6. Statistical analysis

All data were expressed as the mean ± SEM, and analyzed by ANOVA and Duncan’s multiple-range tests using the GLM procedure of SAS 8.1 software. P < 0.05 was considered significantly different.

3. Results

3.1. Effect of GSPE and TAA on liver morphology changes

Chronic administration of TAA induced a significant formation of fibrosis and extensive changes in liver morphology. These phenomena included marked edema, vacuolar degeneration, and necrosis, along with serious inflammation of hepatocytes and obvious infiltration of inflammatory cells (grading 9, staging 3), as observed with H&E staining (Fig. 1C). GSPE significantly ameliorated this fibrosis development in mice after treatment for 9 weeks. The injury and necrosis of the liver parenchyma cells and the infiltration of inflammatory cells were greatly diminished (grading 4, staging 1) (Fig. 1D) as compared to the TAA group (Fig. 1C). In either the TAA or the GSPE + TAA treatment group, the damage area appeared mainly in the periportal region.

Collagen was deposited only in the liver blood vessels in the control and GSPE control groups (Fig. 2A and B). But in the TAA-treated group, the chronic TAA administration caused a markedly increased accumulation of collagen that was deposited mainly in the periportal regions (Fig. 2C). However, simultaneous supply of GSPE for 9 weeks ameliorated the fibrosis formation with only a few tiny, short bundles of collagen found in the GSPE + TAA administration group (Fig. 2D).

3.2. Characterization of HSCs by immunohistochemistry for α-SMA

α-SMA represents a marker of transformed HSCs, and is only expressed in the hepatic vascular smooth muscle cells of the blood vessels in the normal liver. With the exception of that in these vascular smooth muscle cells, there were almost no other α-SMA positive cells apparently in the control and GSPE control groups (Fig. 3A and B). However, in the TAA-treated hepatic damage group, α-SMA was detected by immunohistochemical stain as positive not only in the blood vessels but also concentrated in the periportal areas. These α-SMA positive cells, representing the activated HSCs, were significantly increased in cell number and immunohistochemical staining intensity compared to those in the control group (Fig. 3A and C). However, these were notably decreased in the GSPE + TAA group significantly when compared with the TAA group (Fig. 3C and D).

3.3. Effect of GSPE and TAA on mRNA expression of fibrosis markers TGF-β1, α-SMA and α1-(1)-collagen

There were no differences in mRNA expression levels of TGF-β1, α-SMA and α1-(1)-collagen between the control and GSPE control groups. In the TAA-treated group, expression of TGF-β1, α-SMA and α1-(1)-collagen mRNAs were increased approximately by 2.6-fold, 5-fold and 36-fold, respectively, compared with the control group. But in the GSPE + TAA group, the expression levels of TGF-β1, α-SMA and α1-(1)-collagen mRNAs were markedly reduced from the TAA-treated group to a level of 1.7-fold, 3.4-fold and 15-fold, respectively, after 9 weeks (P < 0.05) (Fig. 4).

3.4. Effect of GSPE and TAA on mRNA expression of pro-inflammatory mediators COX-2 and iNOS

After TAA treatment, the mRNA levels of COX-2 and iNOS were greatly increased by approximately 124-fold and 20-fold, respectively, compared with the control group. However, in the GSPE + TAA group, the expression levels of COX-2 and iNOS were decreased to 98-fold and 13-fold of those in the control group. These down-regulated changes represent a 21% reduction for COX-2 and a 35% reduction for iNOS from the TAA group to the GSPE + TAA group, respectively (Fig. 5).

3.5. Effect of GSPE and TAA on serum AST and ALT levels and liver MDA formation

Treatment of GSPE alone for 9 weeks caused no significant changes in serum ALT and AST levels. However, administration of TAA significantly increased serum ALT and AST levels to 3.8-fold and 2.9-fold of that in the control group, respectively (P < 0.05) (Fig. 6). Simultaneous supplementation of GSPE markedly decreased the serum AST level in the TAA-treated mice (approximately with a 26% reduction from the TAA group to the GSPE + TAA group) (P < 0.05) (Fig. 6B), but the level of ALT was not significantly reduced after treatment with GSPE (approximately with an 11% reduction from the TAA group, Fig. 6A).

As for MDA production, the GSPE control group manifested significantly reduced MDA production with a 26% reduction of that in the control group (P < 0.05) (Fig. 7). After chronic TAA exposure for 9 weeks the MDA level in the liver tissue had not changed significantly (approximately a 7% increase) compared with the control. However, simultaneous GSPE treatment significantly suppressed the lipid peroxidation when compared with the hepatic damaged mice (approximately a 22% decrease) (P < 0.05) (Fig. 7). The SOD activity was not significantly changed in either the TAA or GSPE + TAA group compared with the control or the GSPE control (data not shown).
4. Discussion

The initiation of the hepatotoxic effect of TAA requires metabolic activation through conversion from TAA to TAA sulfoxide (TASO) and further to thioacetamide-S, S-dioxide (TASO₂) via a critical pathway that involves hepatic CYP2E1-mediated biotransformation (Wang et al., 2000; Ramaiah et al., 2001; Chilakapati et al., 2007). The S-oxide metabolite (TASO₂) is an unstable, reactive metabolite, which binds directly and covalently to liver macromolecules with the formation of acetylimidolysine derivatives that are responsible for TAA-induced hepatotoxic effects (Chilakapati et al., 2007; de David et al., 2011). To prevent
such hepatotoxicity, various plant-derived materials have been previously evaluated including GSPE which possesses a variety of pharmacological and therapeutic effects including anti-inflammatory, antiallergic and antitumoral, besides free radical scavenging and antioxidant activities (Li et al., 2001; Singh et al., 2004; Mantena et al., 2006; Tang et al., 2012).

The present study examined the ameliorating effect of GSPE on the developing fibrosis model using standard clinical parameters. The hepatic histological analysis with H&E staining and Masson’s trichrome staining (Figs. 1 and 2), which revealed extensive changes in liver morphology, confirmed the hepatic fibrogenesis in the model group (grading 9, staging 3). Thus our result showed that with the chronic administration of GSPE, hepatocyte necrosis and collagen deposition were significantly diminished in the GSPE + TAA group (grading 4, staging 1) (Figs. 1 and 2). The serum AST level was significantly decreased in GSPE + TAA group, but remained obviously higher than the control group (Fig. 6B). The ALT level in serum, however, was not markedly suppressed by the prolonging administration of GSPE (Fig. 6A).

ALT is a cytosolic enzyme, which is found in many organs and catalyzes the developing of the α-amino group from alanine to α-ketoglutaric acid (Awapara and Seale, 1952). ALT level is particularly high in the liver cytoplasm. Any injury to the cellular membrane of hepatocytes, for example in the ROS-induced peroxidation (Mason et al., 1997), led to molecular disorganization of lipids which resulted in increased membrane permeability and leakage of intracellular enzymes into circulation thus causing a rapid increase of serum ALT level. While AST, an enzyme found in both cytosol and mitochondria (Rej, 1971), is extremely high in mitochondria, only the cytoplasmic AST leaks into the blood when liver injury remains relatively mild. If the hepatic injury persists, and the severe lesion occurs to the hepatocytes, AST in the mitochondria was also successively released into the circulation (Kamiike et al., 1989). Therefore, serum AST activity can reflect the higher degree of the hepatocellular injury. According to the assays of serum ALT and AST levels (Fig. 6), TAA-induced hepatic damage resulted in significant increases in serum ALT and AST levels. Though GSPE manifested a remarkable protective effect on hepatic morphology (Fig. 1), unlike the case of AST, the serum ALT level had not been recovered significantly. The hepatic histological demonstration with H&E staining (Fig. 1) and the assays of serum ALT and AST levels (Fig. 6) imply that the ALT level in serum is not closely associated with liver cell necrosis, but is a sensitive indicator for liver cell injury. Moreover, the AST serum level directly reflects the necrosis of liver cell since the mitochondrial AST is released only when the cells are severely disintegrated (Wang et al., 2012). In this study, GSPE could not markedly suppress the high serum level of ALT, but exerted a resistant effect for the increase of serum AST. These results suggest that the chronic administration of GSPE may exert a protective effect for further deterioration of the mitochondrial membranes of the hepatocytes.

In this experiment, necrosis and fibrosis in the portal area was significantly more serious, as observed with HE and Masson’s trichrome staining (Figs. 1 and 2), indicating that this region suffered more serious injury than did the pericentral region. The concentration of exogenous harmful substances in the blood is higher than that in the hepatic portal area. This is because the blood flows into liver through portal vein, from the hepatic portal region to the central vein. There the hepatocytes might have gradually eliminated the toxin as they have a strong function of detoxification, making the concentration of toxins decrease from the liver portal area to the central vein. Also the activity and concentration of enzymes (for example, CYP2E1) are far higher in the periporal region than that in the centrilobular region. This is the possible explanation for this phenomenon.

As a convenient biomarker for lipid peroxidation (Janero, 1990), MDA indirectly reflects the level of hepatocellular oxidative injury. Among many hepatotoxicants, TAA is known to be somewhat unique in its toxic effect on hepatic injury. Our results show that TAA could neither enhance the production of lipid peroxidation end

Fig. 3. Immunohistochemical stain of liver for α-SMA after treatments with GSPE and TAA. A–D indicate the control, GSPE, TAA and GSPE + TAA treatment, respectively. α-SMA, a marker of activated HSCs, was stained in brown (arrows). Scale bar: 100 μm.
product MDA significantly (Fig. 7), nor markedly inhibit the SOD activities in the liver tissue (data not shown), which indicated that TAA likely caused liver injury in a manner other than by the generation of ROS. This result also demonstrated that the toxic effect of TAA may be induced by the covalent binding of reactive metabolites to liver macromolecules with the subsequent direct formation of acetylimidolysine derivatives (Chilakapati et al., 2007; de David et al., 2011). Further studies are needed to clarify these underlying mechanisms. Administration of GSPE significantly decreased the MDA production in both the GSPE control and GSPE + TAA group when compared with the TAA group or the control. However, the MDA levels were not significantly different between the control and the TAA group (Fig. 7). These results showed that, at least in part, GSPE can be regarded as a free radical scavenger.

As a powerful stimulus for collagen formation in liver, TGF-β1 plays a central role in the cytokine network involved in...
fibrogenesis (Dooley et al., 2001; Zhou et al., 2010). When liver injury takes place, the damaged hepatocytes, activated kupper cells and platelets generate TGF-β1, which in turn induces HSC activation (Kawada, 2011). After exposure to TAA for 9 weeks, TGF-β1 mRNA level increased significantly to 2.6-fold compared to that in the control group. In the GSPE + TAA group, TGF-β1 mRNA expression was markedly decreased (Fig. 4A). This result indicated that the protective effect of GSPE on liver fibrosis might be associated with the inhibition of TGF-β1 production. In fact, many antifibrotic drugs ameliorate hepatic fibrosis by suppressing the expression of TGF-β1 (Miyazaki et al., 2005; Tipoe et al., 2010; Pan et al., 2011).

Activated HSCs and hepatic myofibroblasts (MFB) were identified as the principal collagen producing cells in the liver (Friedman, 2008). When activated, HSCs are transformed into MFBs. Activation of HSCs is associated with cell proliferation, increased contractility and enhancement of matrix production (Ramm et al., 2000; Cassiman et al., 2002; Friedman, 2008). This is responsible for the formation of liver fibrosis. So inhibition of the HSC activation is a key point to block the progression of fibrogenesis. Our results showed that after long-term liver injury, activated HSCs were obviously increased in cell number and the expression level of α-SMA, which was widely accepted as activation marker of HSCs (Figs. 3 and 4B). However, GSPE significantly decreased the number of activated HSCs (Fig. 3). Our results showed that after long-term liver injury, the mRNA expression level of α-SMA increased approximately 5-fold compared with the control. But the GSPE treatment significantly decreased this increase (Fig. 4B). This result demonstrated that GSPE were capable of inhibiting the TAA-induced activation of HSCs, which subsequently resulted in suppressed mRNA level of the α(1)-collagen and decreased collagen accumulation (Figs. 2 and 4C).

The expression of inducible COX-2 causes a production of high concentration of prostaglandins observed in the inflammatory pathology. Meanwhile the iNOS produces large amounts of nitric oxide which displays free radical features and has been suggested to be a very important inflammatory mediator (Li and Billiar, 1999; Hu, 2003; Simmons et al., 2004; Suschek et al., 2004). Our results showed that the long term administration of TAA severely enhanced the expression of iNOS and COX-2 mRNAs (Fig. 5), and that finally caused a severe centrilobular necrosis and apoptosis and periporal inflammatory cell infiltration in the liver (Fig. 1). GSPE treatment depressed the expression levels of these two genes to a certain extent. However, the simultaneous supplementation of GSPE could not diminish these expression levels significantly in the TAA-treated mice (Fig. 5).

In conclusion, chronic exposure of TAA resulted in a significant increase in the expression of iNOS and COX-2 mRNAs, and thus caused severe cellular inflammation and necrosis. The mRNA expression of the fibrotic markers α-SMA and α(1)(1)-collagen were significantly increased, which led to an accumulation of an extracellular matrix. The damaged hepatocytes, activated kupper cells and platelets generated TGF-β1, which strongly activated the principal collagen producing cells HSCs. This will finally results in liver fibrosis or cirrhosis. However, GSPE exhibited a remarkable hepatic protective effect, thus stabilizing the hepatocytes, and significantly suppressing the expression level of TGF-β1 mRNA and thereby decreased the activation of HSCs and collagen accumulation. In summary, GSPE manifested effective hepatocellular protective action and ameliorative effect against chronic liver damage and developing liver fibrosis induced by TAA administration. However, further studies are needed to reveal the underlying mechanisms of the anti-fibrotic effect of GSPE with the aim to develop GSPE as an effective ameliorator for liver fibrosis.

Conflict of interest

The authors declare that there are no conflicts of interest.

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