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Original research article

The hepatoprotective effect of *Sophora viciifolia* fruit extract against acetaminophen-induced liver injury in mice

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Abstract
This research demonstrated the protective effect and possible mechanism of the *Sophora viciifolia* extract (SVE) against acetaminophen-induced liver injury in mice. The levels of ALT and AST in the serum and antioxidant enzyme activity in the liver were measured. We used immunohistochemistry to detect CYP2E1, Nrf2, and Keap1 protein expression in the liver. The mRNA expression in the liver of T*NF-α, NF-κB*, and *IL-6, Nrf2* and its downstream genes *HO-1* and *GCLC* were measured by qRT-PCR. We found that SVE could decrease the ALT and AST levels, promote the activities of SOD, CAT, GSH-Px, and GSH, and ameliorate pathological liver lesions. SVE could down-regulate the mRNA expression of inflammatory factors and up-regulate *Nrf2, HO-1* and *GCLC*. SVE reduced the protein expression of the CYP2E1 and increased the Nrf2 and Keap1. SVE has been shown to have a protective effect against APAP-induced liver injury, possibly through activation of the Keap1-Nrf2 pathway.

Keywords: Acetaminophen (APAP); Keap1-Nrf2; Liver injury; *Sophora viciifolia* fruit extract

Highlights:
- *Sophora viciifolia* fruit extract (SVE) exhibits a protective effect against acetaminophen-induced liver injury.
- This study demonstrated that the extract could significantly inhibit oxidative stress.
- *Sophora viciifolia* fruit extract (SVE) prevents acetaminophen-induced liver injury through activation of the Keap1-Nrf2 pathway.

Introduction

Acetaminophen (APAP) is an over-the-counter (OTC) antipyretic and analgesic drug used widely worldwide, and it is also the most common drug used for clinically induced drug-induced liver injury (DILI). APAP metabolism occurs mainly in the liver, and excessive intake of APAP can lead to the formation of the toxic metabolite N-acetyl-p-benzoquinonimine (NAPQI) mitochondrial dysfunction, oxidative stress, inflammatory responses, and endoplasmic reticulum stress (Bessems and Vermeulen, 2001; Hua et al., 2018; Lorz et al., 2004; Patra et al., 2018; Reshi et al., 2017). However, high-dose or long-term use of APAP leads to saturation of the combined biotransformation process, resulting in excessive production of NAPQI and the depletion of reduced glutathione (GSH) in the liver cells, when unbound metabolic poisons and enzymatic or non-enzymatic proteins combine to form toxic products, which can cause severe liver damage and further deteriorate into fatal liver failure (Xie et al., 2016a). At present, N-acetylcysteine, an antioxidant, is mainly used clinically to cope with APAP-induced liver injury, but the therapeutic effect is unsatisfactory. It is therefore of great urgency to find potential new drugs that can treat APAP-induced liver injury (Shu et al., 2019).

Nuclear factor erythroid-2 related factor 2 (Nrf2) is a transcription factor widely expressed in various tissues of higher animals. It can combine directly with specific cis-acting originals, such as the antioxidant response element (ARE) in the promoter region of downstream genes, and initiate the transcription of genes (Lv et al., 2018). In the state of cells without any stimulation, the Nrf2 protein will bind to its inhibitor, the Kelch-like ECH-associated protein (Keap1) protein, and will be localized in the cytoplasmic matrix (Hu et al., 2016). When this occurs, Nrf2 cannot play a role in transcription activation. When oxidative stress occurs in the cell, the conformation of Keap1 changes and the binding ability of Keap1 and Nrf2 decreases; Nrf2 can then be released from Keap1, enter the nucleus, and start the transcription of its downstream genes (Huang et al., 2017). Typical downstream genes controlled by

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Nrf2 include genes encoding antioxidant enzymes, in particular heme oxygenase (HO-1) and glutamate-cysteine ligase catalytic subunit (GCLC). These factors can resist the oxidative stress contributed to various toxic substances in the environment or endogenously. Research shows that enhancing Nrf2 pathway activity can significantly alleviate APAP-induced liver injury (Jiang et al., 2019). Therefore, targeted activation of the Nrf2 pathway can serve as a potential intervention strategy for APAP-induced liver injury. Many natural products derived from plants have good interference effects on liver injury induced by toxicants (Shu et al., 2019). *Sophora viciifolia* is a semi-evergreen deciduous shrub species in the Leguminosae family. *S. viciifolia* flowers, leaves, stems, fruit, and roots all contain polyphenols, flavonoids, alkaloids, and other active substances that have anti-inflammatory, liver-protective, anti-tumor, hypoglycemic, and antioxidant effects (Li et al., 2017; Lin et al., 2019; Wang et al., 2016; Xiao et al., 1999; Xie et al., 2017). In traditional Chinese medicine, *S. viciifolia* was taken as a cure for indigestion, gastritis and stomach ulcers, lung heat cough, as well as epidermal cancer and leukemia. According to the extant literature, research has yet to be undertaken on the treatment of acute liver injury caused by APAP with *S. viciifolia*.

In this study, the fruit extract of *S. viciifolia* was used as a therapeutic drug. The serum AST and ALT levels in mice were measured, along with the antioxidant enzyme activity in their livers, their cytochrome P4502E1 (CYP2E1), Nef2, and Keap1 protein expression, Nrf2 and its downstream genes HO-1 and GCLC, inflammatory factors [nuclear factor κB (NF-κB), tumor necrosis factor (TNF-α), and interleukin-6 (IL-6)], and their mRNA expression levels, to examine the protective influence of *S. viciifolia* on APAP-induced acute liver injury in mice and to explore whether it plays a role in liver protection by activating the Keap1-Nrf2 pathway.

Materials and methods

Materials

For this study, the fruits of *S. viciifolia* were gathered in the south region of the Qinling Mountains in September 2017 (110° 54' east longitude, 33° 32' north latitude) and identified by Dr. Yong Wang from the College of Biological Science and Engineering, Shaanxi University of Technology. A total of 42 Kunming mice (SPF level, 22 ± 5 g in weight) were received from the Chengdu Dasuo Experimental Animal Co., Ltd. (Chengdu, China).

Chemicals

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px) and reduced glutathione (GSH) kits were bought from the Jiancheng Bioengineering Institute (Nanjing, China). Rapid extraction kits of animal total RNA and Vc were purchased from the Sangon Biotech Co., Ltd. (Shanghai, China). Acetaminophen (APAP) was obtained from the Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Silybin was purchased from the Tianjin Tasly Co., Ltd. (Tianjin, China). A BSA protein assay kit was purchased from Beyotime Biotechnology (Shanghai, China). A DAB reagent kit was purchased from the Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China). CYP2E1, Nrf2, and Keap1 antibodies were acquired from Bioss (Beijing, China). A cDNA reverse transcription kit and a PCR kit were purchased from TaKaRa (Dalian, China). Hematoxylin, eosin, and an immunohistochemistry pen were obtained from the Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China).

Preparation of SVE

The *S. viciifolia* fruits were dried at 45 °C and then smashed. The smashed fruits were weighed, and we then added 60% ethanol (40 ml to 1 g) for a material to liquid ratio (m : *V*) of 1 : 40. The fruit was then extracted using ultrasonic waves (the KQ5200DE CNC ultrasonic instrument, Jiangsu, China) for 30 min, subjected to suction filtration, and then added to the 40 ml of 60% ethanol mixed with the filter residue. The fruit was then subjected to another 30 min of ultrasound, the collection of two filtrates, enrichment using a rotary evaporator, and vacuum drying at 45 °C. The resulting *S. viciifolia* extract (SVE) was stored at 4 °C, the phytochemicals content of SVE was reported in our previous study (Lin et al., 2019), with total polyphenol content of 103.04 mg GAE/g DW, total flavonoid of 2.14 mg RE/g DW, and total alkaloid of 48.78 mg/g DW.

Construction of animal models

The mice were kept at temperatures of 25 ± 2 °C with a humidity of 55% ± 5% and a 12-hour light and dark cycle. They were allowed to eat food and water freely and were fed adaptively during the experiments. All animal procedures were performed in accordance with the Animal Ethics Committee of the Shaanxi University of Technology (2019-006, Chinese-German Joint Laboratory for Natural Product Research). The mice were divided into seven groups (n = 6).

1. Normal group (NC group): mice intragastric with normal saline.
2. APAP group: mice intragastric with normal saline.
3. Low-dose group (SL group): mice intragastric with SVE 125 mg/kg BW/day.
4. Medium-dose group (SM group): mice intragastric with-SVE 250 mg/kg BW/day.
5. High-dose group (SH group): mice intragastric with SVE 500 mg/kg BW/day.
6. Positive control group (SPC group): mice intragastric with silybin 80 mg/kg BW/day.
7. SVE control group (SC group): mice treated with SVE 500 mg/kg BW/day.

After continuous treatment for seven days, the liver injury model was established by intraperitoneal injection of APAP. One hour after the last treatment, each group was injected intraperitoneally with APAP at 300 mg/kg of body weight (Xie et al., 2016b), with the exception of the NC and the SC groups, which were injected with an equal volume of normal saline. Silybin was used clinically for the acute or chronic hepatitis and toxic liver injury treatment, especially acetaminophen-induced liver injury (Li et al., 2019).

All mice were then fasted overnight, anesthetized by inhalation of 2% isoflurane (0.41 ml/min, 4 l/min) and blood collected and liver collected after euthanasia. Then, the blood was centrifuged at 3000 r/min at 4 °C for 10 min (Eppendorf, Hamburg, Germany) to obtain blood serum, which was then kept at –80 °C for further analysis. Part of the liver of each mouse was fixed in a 4% paraformaldehyde solution for histomorphology observation. The remaining liver tissues were stored at –80 °C for mRNA detection.

Determination of serum indices

The serum activities of AST and ALT were measured in accordance with the instructions of commercial kits (Nanjing Jiancheng Biological company, Nanjing, China).

Measurement of antioxidation activity in the liver

To determine the SOD, CAT and GSH-Px activity, as well as the GSH and MDA contents in the mouse liver, we added ice and normal saline in the proportion of 1 : 9 to the livers, homogenated the livers (OMNI, Kennesaw, GA, USA), centrifuged them at 4 °C, 3000 r/min for 10 min, and then took the supernatant and packed it separately at –20 °C for storage.

Histomorphological observation and MDA content of the liver

The livers fixed with paraformaldehyde solution were embedded in paraffin and then sectioned with a 5 μm tissue slicer (Leica, Oskar Barnack, Germany) and stained with hematoxylin-eosin (H&E). Pathological changes in the liver tissues were observed under a microscope (Leica, Oskar Barnack, Germany).

Immunohistochemistry

The expression of the CYP2E1, Nrf2, and Keap1 proteins in the liver was detected using immunohistochemistry (Qi et al., 2019a). The liver sections were dewaxed with xylene and gradient alcohol, and then the Triton X-100 broke the membrane for 30 min. The liver sections were then incubated with 3% H_2O_2 for 30 min, sealed with 3% BSA for 20 min, incubated with anti-CYP2E1, anti-Nrf2, and anti-Keap1 antibodies for 2 h (1 : 250, antibodies: PBS-T), incubated with horseradish perioxidase (HRP) for 1.5 h (1 : 200, HRP : PBS-T), stained with 3,3'-Diaminobenzidine (DAB) solution for 5 min, neutral-gum sealed, and counterstained with hematoxylin. Image Pro Plus 5.0 analysis software (Maryland, USA) was used to quantitatively analyze the percentage of CYP2E1, Nrf2 and Keap1 positive staining areas under a microscope (Leica, Oskar Barnack, Germany). Stain with 3,3'-diaminobenzidine (DAB) for 5 minutes.

Quantitative real-time PCR

Rapid extraction of the total RNA from the liver tissues was conducted using a total RNA extraction kit. The first-strand cDNA was reverse-transcribed using the PrimeScript RT reagent kit. The transcription levels of *Nrf2, TNF-α, NF-κB, IL-6, HO-1*, and *GCLC* were quantified using qRT-PCR with the *GAPDH* gene as an internal reference. qRT-PCR was analyzed on a real-time PCR detection system using SYBR Green (StepOnePlus, ABI, Carlsbad, California, USA). The primer sequences of the target and reference genes are shown in Table 1.

Table 1. qRT-PCR amplification primer

Target gene	Upstream primer (5'-3')	Downstream primer (5'-3')
Nrf2	ACACGGTCCACAGCTCATCAT	TTGGCTTCTGGACTTGGAAC
TNF-α	TATGGCTCAGGGTCCAACTC	GCTCCAGTGAATTCGGAAAG
NF-κB	ACGATCTGTTTCCCCTCATCT	TGGGTGCGTCTTAGTGGTATC
IL-6	CAAAGCCAGAGTCCTTCAGAG	GTCCTTAGCCACTCCTTCTG
HO-1	AGGTACACATCCAAGCCGAGA	CAGTGAGGCCCATACCAGAAG
GCLC	CTACCACGCAGTCAAGGACC	CCTCCATTCAGTAACAACTGGAC
GAPDH	ACAGTCCATGCCATCACTGCC	GCCTGCTTCACCACCTTCTTG

After quantitative real-time PCR, the relative expression level of each gene was calculated using GAPDH as the internal reference gene using the formula $2^{-(\Delta\Delta Ct)}$.

Statistical analysis

The resulting data were processed using SPSS 19.0 software (SPSS Inc., Chicago, Illinois, USA). GraphPad Prism 5 software (San Diego, California, USA) was used for drawing. One-way analysis of variance (ANOVA) was conducted using Duncan's Multi-Range test, and $P < 0.05$ was considered statistically significant. Three repetitions in each experiment were displayed as the mean ± standard deviation (SD).

Results

AST and ALT levels in the serum

The AST and ALT levels in the APAP group were significantly higher (430.03% and 643.71%) than those in the NC group ($P < 0.05$; Table 2), indicating the successful construction of APAP liver-injury model. After different doses of SVE, the AST and ALT levels were significantly reduced ($P < 0.05$). The AST and ALT levels of the SVE control group and the NC group were almost the same, indicating that high-dose SVE will not cause liver damage.

SOD, CAT, GSH-Px, and GSH levels in the liver

As exemplified in Fig. 1, in contrast to the NC group, the levels of SOD, CAT, GSH-Px, and GSH in the APAP group were significantly decreased by 31.79%, 29.44%, 34.86%, and 74.95% ($P < 0.05$) respectively, indicating that the body was in a state of oxidative stress. The SVE treatments significantly enhanced the activities of SOD, CAT, and GSH-Px in the liver tissues of the test mice, and the GSH levels also returned to normal.

Table 2. AST and ALT levels in mice serum

Group	AST (U/L)	ALT (U/L)
NC	14.73 ± 1.82	11.89 ± 3.47
APAP	76.14 ± 12.79#	88.78 ± 5.39#
SL	19.40 ± 9.64*	16.29 ± 3.06*
SM	17.33 ± 2.89*	14.52 ± 0.76*
SH	15.62 ± 4.18*	13.69 ± 1.37*
SPC	15.29 ± 2.30*	12.26 ± 3.16*
SC	14.85 ± 0.75*	12.03 ± 3.73*

Note: Compared to NC group, # $P < 0.05$; Compared to model group, * $P < 0.05$. NC, normal group; APAP, APAP group; SL, low-dose group; SM, medium-dose group; SH, high-dose group; SPC, positive control group; SC, SVE control group; Six mice in each group.

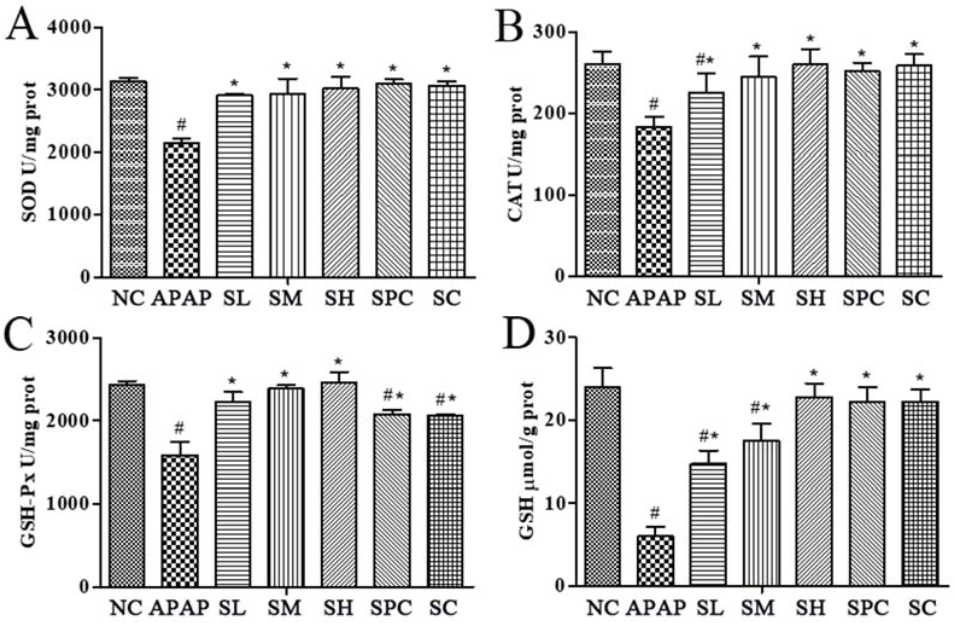

Fig 1. Effect of *S. viciifolia* extract on the activity of antioxidant enzymes in mice liver. Compared to NC group, # $P < 0.05$; Compared to model group, * $P < 0.05$. NC, normal group; APAP, APAP group; SL, low-dose group; SM, medium-dose group; SH, high-dose group; SPC, positive control group; SC, SVE control group; Six mice in each group.

Histological changes and MDA content in the liver

As shown in Fig. 2, the liver cells in the NC were neatly arranged, and the nuclei were large and round. The hepatocytes are arranged neatly, and the cell morphology is complete. Compared with the NC, the APAP group demonstrated disordered liver tissue cells, liver lobular-structure destruction, deeper nuclear staining, and severe loss of liver-cell morphology (shown by the arrows in the Fig. 1). After seven days of preventive treatment, APAP was used to establish a liver injury model. Compared with the NC group, the liver injury in the APAP-induced model group was significant, while that in the low-dose SVE group was slight. After medium-dose and high-dose treatment of SVE, the liver cells were intact and neatly arranged, comparable to those in the NC group, with the difference that liver damage was significantly improved.

As exemplified in Fig. 2, the MDA content in the livers of the APAP group members increased significantly by 90.04% ($P < 0.05$), indicating that APAP caused damage to the liver antioxidant system. After treatment with SVE, the MDA content in the liver was significantly decreased in a dose-dependent manner ($P < 0.05$). In contrast to the APAP group, the MDA content in the livers of the high-dose group decreased by 50.64%. Therefore, SVE effectively reversed the APAP-induced liver pathological changes in the test mice.

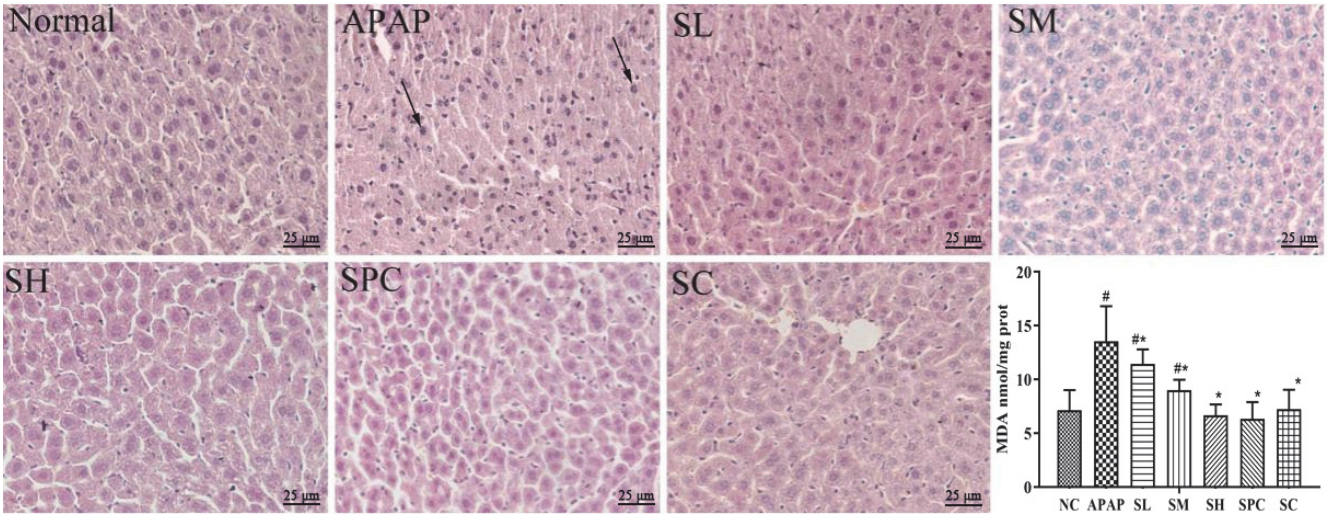

Fig 2. Effect of *S. viciifolia* extract on APAP-induced hepatic histopathological changes and MDA content in mice (HE, ×400). NC, normal group; APAP, APAP group; SL, low-dose group; SM, medium-dose group; SH, high-dose group; SPC, positive control group; SC, SVE control group; Six mice in each group.

CYP2E1, Nrf2, and Keap1 protein expression in the liver

As shown in Fig. 3 and 4, the liver CYP2E1 protein expression levels of the mice in the APAP group significantly exceeded those of the mice in the NC group ($P < 0.05$). Compared with the APAP group, the results from the SVE groups indicate that SVE can protect against the increase of CYP2E1. In addition, SVE can significantly intensify the expression of Nrf2 and Keap1 ($P < 0.05$), indicating that SVE can increase the antioxidant capacity of the body by activating the Keap1--Nrf2 signaling pathway and repair the liver damage caused by APAP.

mRNA expression of TNF-α, NF-κB, IL-6, Nrf2, HO-1 and GCLC in the liver

Inflammation is one of the pathogeneses of acute liver injury caused by APAP. TNF-α, NF-κB, and IL-6 are typical inflammatory factors. In the APAP group, the mRNA expression levels of inflammatory factors increased significantly, indicating that there was an inflammatory reaction in the liver and that there was liver damage (Fig. 5A–C). After treatment with SVE, the mRNA expression levels of inflammatory factors were significantly lower ($P < 0.05$), and there was a statistical difference. These results show that pre-treatment with SVE can relieve APAP-induced inflammation.

Both HO-1 and GCLC are typical downstream genes of Nrf2. The expression levels of Nrf2, HO-1, and GCLC in the livers of the APAP group mice decreased, but not significantly (Fig. 5D–E). There was no significant difference in the expression of Nrf2, HO-1, and GCLC between the low-dose group and medium-dose group compared with that of the NC group and APAP group. In the high-dose and positive control groups, the expression levels of Nrf2, HO-1, and GCLC were significantly increased ($P < 0.05$), showing that SVE can significantly increase the expression of Nrf2, HO-1, and GCLC at the mRNA level.

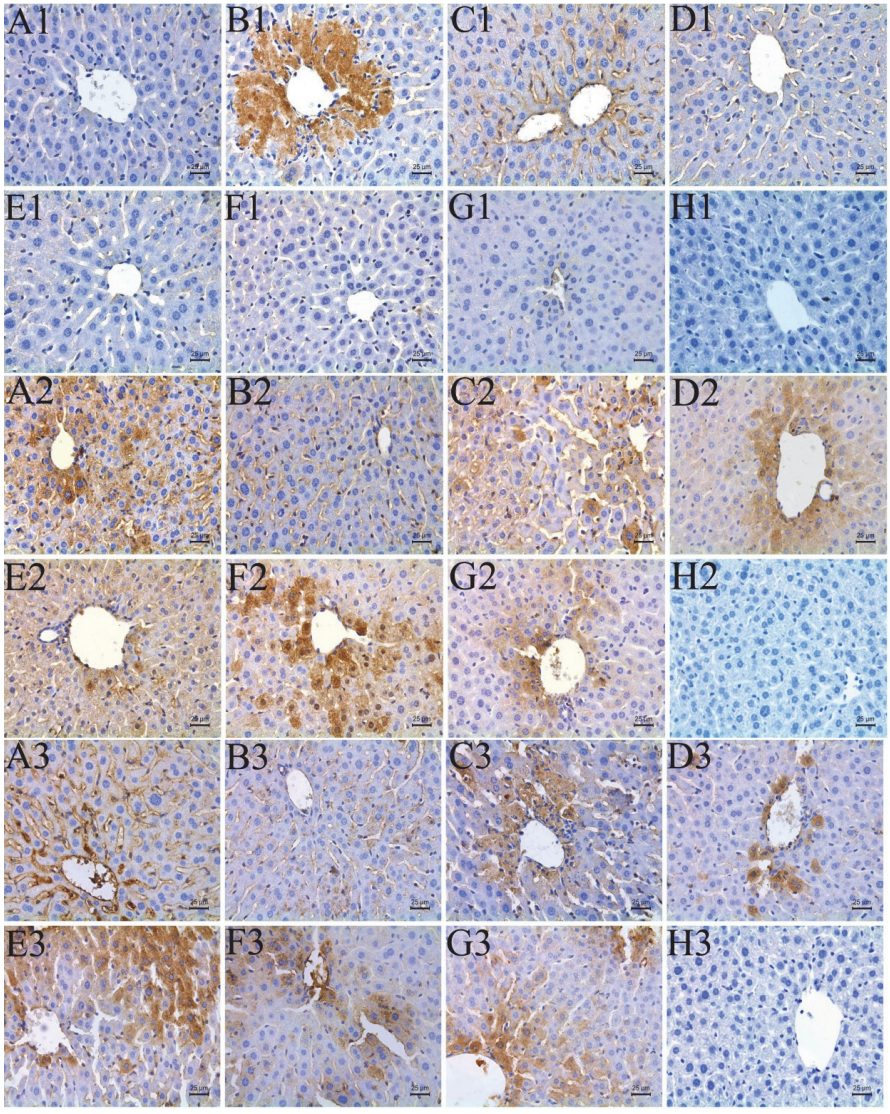

Fig 3. Immunohistochemical staining in liver tissue (×400). CYP2E1 (A1–H1), A1, NC group; B1, APAP group; C1, low-dose group; D1, medium-dose group; E1, high-dose group; F1, positive control group; G1, SVE control group; H1, negative control. Nrf2 (A2–H2), A2, NC group; B2, APAP group; C2, low-dose group; D2, medium-dose group; E2, high-dose group; F2, positive control group; G2, SVE control group; H2, negative control. Keap1 (A3–H3), A3, NC group; B3, APAP group; C3, low-dose group; D3, medium-dose group; E3, high-dose group; F3, positive control group; G3, SVE control group; H3, negative control.

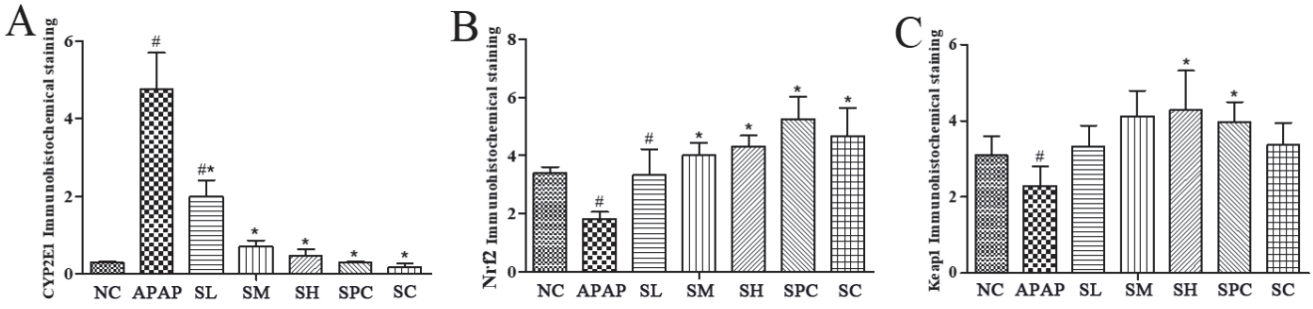

Fig 4. The average optical density of immunohistochemical staining in mice liver. Compared to NC group, # $P < 0.05$; Compared to model group, * $P < 0.05$. NC, normal group; APAP, APAP group; SL, low-dose group; SM, medium-dose group; SH, high-dose group; SPC, positive control group; SC, SVE control group; Six mice in each group.

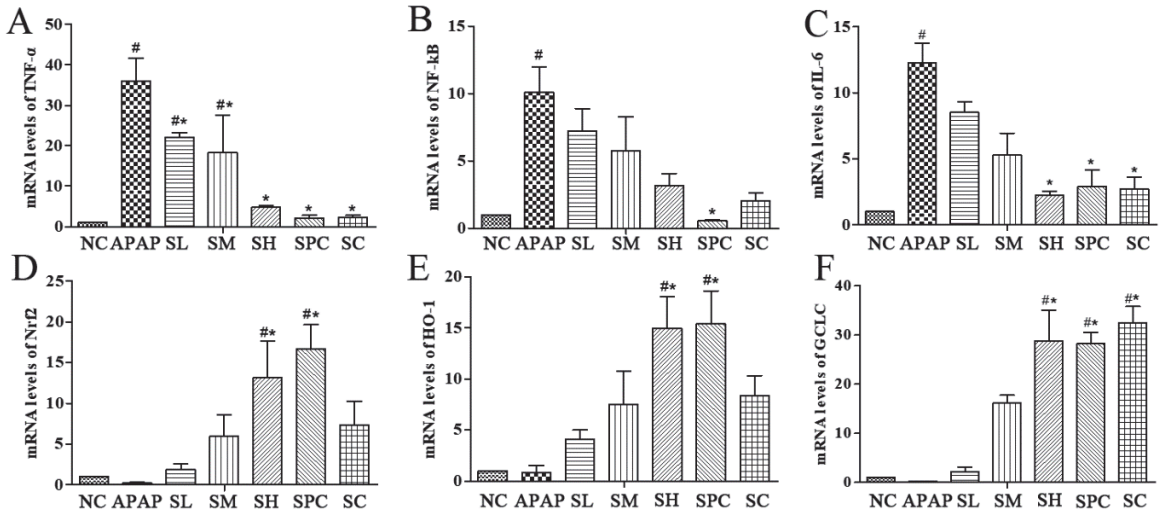

Fig 5. mRNA levels of TNF-α, NF-κB, IL-6, Nrf2, HO-1 and GCLC in mice liver. Compared to NC group, # $P < 0.05$; Compared to model group, * $P < 0.05$. NC, normal group; APAP, APAP group; SL, low-dose group; SM, medium-dose group; SH, high-dose group; SPC, positive control group; SC, SVE control group; Six mice in each group.

Discussion

The liver is the main organ of drug metabolism and the main target organ of drug damage. Pre-treatment with hepatoprotective drugs to reduce the toxicity of hepatotoxic compounds is an attractive strategy for future drug development. AST and ALT are considered sensitive biomarkers for appraising liver toxicity in the clinic practices (Jiang et al., 2016). Significant differences of ALT levels were observed between the Normal and APAP groups, as well as the SVE and APAP groups. The pathological observation of liver tissue showed that there were hepatocyte injury, inflammatory cell infiltration and other lesions in the APAP group, indicating liver injury in the APAP group. CYP2E1 is a major phase I poison-metabolizing enzyme metabolizing exogenous and endogenous compounds. The expression of the CYP2E1 protein was significantly increased, NAPQI increased, hepatocellular toxicity increased, and the livers of the mice showed significant damage. With SVE pre-treatment, the activity of AST and ALT and the expression of the CYP2E1 protein can be significantly reduced. These results are consistent with the results of Hong et al. (2012), indicating that post-treatment can significantly inhibit expression of the CYP2E1 protein, reduce the increase of Nrf2, and reduce liver-cell toxicity.

Excessive intake of APAP will cause the depletion of GSH, destruction of the body's oxidative balance, and reduction of the antioxidant capacity of liver cells, causing intracellular oxidative stress and liver damage (Brown et al., 2012; Lin et al., 2018). SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, and then GSH-Px converts hydrogen peroxide to oxygen and water, thereby generating reduced GSH as a substrate. MDA is an important index of lipid peroxidation (Kong et al., 2018). In the APAP group, the MDA content in the liver tissues increased, GSH content decreased, and SOD, CAT, and GSH-Px activity decreased, which was significantly different from the NC group. In the SHgroups, MDA in the liver tissues decreased and GSH content and SOD, CAT, and GSH-Px activity remained near to normal levels, indicating that SVE can secure the body's oxidative balance, relieve liver oxidative stress, and cure APAP-induced acute liver injury. Our results are consistent with the results of Xie et al. (2015), indicating that SVE has the same effects as the extract of *Apocynum venetum* extract, which can treat APAP-induced liver injury by improving the antioxidant system in mice.

APAP-induced liver injury is characterized by liver cell necrosis, which causes the release of intracellular substances, including nuclear DNA fragments (McGill et al., 2012), high mobility box-1 proteins (Antoine et al., 2012; Sharifi-Rigi et al., 2019), and mitochondrial DNA. These immune-stimulat-

ing factors can transcribe and activate pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-10) and then concentrate them in the liver, causing acute liver inflammation (Wu et al., 2018). It is also reported that after APAP treatment, the expressions of the human nuclear transcription factor peptide (NF-κBp65) and the nuclear factor κB inhibitor (IκBα) in the liver are up-regulated, the stability of NF-κB/IκBα is destroyed, and NF-κB is activated, leading to the release of inflammatory factors, and causing inflammation. In our study, the levels of inflammatory factors TNF-α, NF-κB, and IL-6 in the liver tissues of the APAP mice were significantly increased. Pre-treatment with SVE can significantly reduce the inflammatory factor content in the livers of mice, verifying that SVE can relieve APAP-induced liver injury by inhibiting the occurrence of inflammatory reactions. A previous study has found that *Phyllanthus emblica* extract can reduce the high expressions of inflammatory factors and the liver damage caused by APAP (Zhang et al., 2018). Combined with the conclusion of this research, it can be shown that both SVE and *P. emblica* extract can inhibit the expression of inflammation factors and can thereby prevent APAP-induced liver injury.

Kelch-like ECH-associated protein (Keap1) is a regulatory factor in cellular oxidative stress responses. It is a polypeptide containing 624 amino acids and is a binding protein for Nrf2 in the cytoplasm. Nrf2 (nuclear factor erythroid-2 related factor 2) is a basic leucine zipper (bZIP) transcription factor that mediates the expression of more than 100 oxidative stress-related genes, including HO-1, GCLC, and quinone oxidoreductase 1 (NQO1). The Keap1-Nrf2 signaling pathway plays a critical role in the anti-inflammatory and anti-tumor responses of cells, neuroprotection, drug resistance, and so on (Lu et al., 2017). In our research, SVE increased the expression levels of the Nrf2 and Keap1 proteins, indicating that SVE may improve the body's oxidative stress and inflammation levels by activating the Keap-Nrf2 signaling pathway. As downstream genes of Nrf2, HO-1 and GCLC play key roles in the Keap-Nrf2 signaling pathway. HO-1 is an inducible type that can induce the expression of HO-1 and enhance its enzyme activity in conditions of oxidative stress, endotoxin, and cytokine stimulation, and so on. HO-1 can effectively improve the body's antioxidant capacity and is also an important anti-inflammatory protein in the body (Qi et al., 2019b). GCLC is the main antioxidant molecule in glutathione synthesis. In our study, SVE was demonstrated to exert its antioxidant effect by up-regulating the mRNA expression of Nrf2 and HO-1; it also increased the mRNA expression levels of GCLC, resulting in an increase in GSH levels. Elevated GSH levels can not only improve the body's response to oxidative stress but also reduce the toxicity of NAPQI and protect APAP-induced acute liver injury in mice. The results of this study are consistent with those of Zuo et al. (2018).

Subramanya et al. have reported that there are a variety of plant extracts available to ameliorate acetaminophen-induced hepatotoxicity (Subramanya et al., 2018), including *Astragalus corniculatus, Trifolium alexandrinum, Dalbergia paniculata*, and *Vigna angularis*, all from the *Leguminosae* family. *S. viciifolia* also belongs to Leguminosae, and may also have liver-protective effects. Our study proves this hypothesis and that *S. viciifolia* can prevent acetaminophen-induced hepatotoxicity. SVE can significantly reduce the activity of AST and ALT, down-regulate MDA content and the mRNA expression of TNF-α, NF-κB, and IL-6, increase GSH content, improve the activity of SOD, CAT, and GSH-Px, inhibit the expression of the CYP2E1 enzyme, and activate the Keap1-Nrf2 signaling pathway to prevent APAP-induced liver injury.

Conclusions

We have demonstrated that SVE could prevent APAP induced liver injury in mice, and possibly through activation of the Keap1-Nrf2 pathway. SVE could decrease the ALT and AST levels; promote the activities of SOD, CAT, GSH-Px, and GSH; down-regulate the mRNA expression of inflammatory factors and up-regulate Nrf2, HO-1, and GCLC, and ameliorate pathological liver lesions. However, further studies of the active ingredient of SVE are needed as a candidate for the development of hepatoprotective drugs.

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Conflict of interests
The authors have no conflict of interests to declare.

Data availability statement
The data that support the findings of this study are available from the corresponding author (CC) upon reasonable request.

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