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Study on the effect of Mongolian medicine Qiwei Qinggan Powder on hepatic fibrosis through JAK2/STAT3 pathway

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ABSTRACT

This research aimed to evaluate the antihepatic fibrosis effect and explore the mechanism of Qiwei Qinggan Powder (QGS-7) *in vivo* and *in vitro*. Carbon tetrachloride (CCl_4)-treated rats and hepatic stellate cells (HSCs) were used. QGS-7 treatment significantly improved the liver function of rats as indicated by decreased serum enzymatic activities of alanine aminotransferase, aspartate transaminase, and alkaline phosphatase. Meanwhile, the hydroxyproline of liver was significantly decreased. Histopathological results indicated that QGS-7 alleviated liver damage and reduced the formation of fibrosis septa. Moreover, QGS-7 significantly attenuated expressions of Alpha smooth muscle actin, Collagen I, Janus kinase 2 (JAK2), phosphorylation-JAK2, signal transducer and activator of transcription 3 (STAT3), phosphorylation-STAT3 in the rat hepatic fibrosis model. QGS-7 inhibited HSC proliferation and promoted it apoptosis. QGS-7 may affect hepatic fibrosis through JAK2/STAT3 signaling pathway so as to play an antihepatic fibrosis role.

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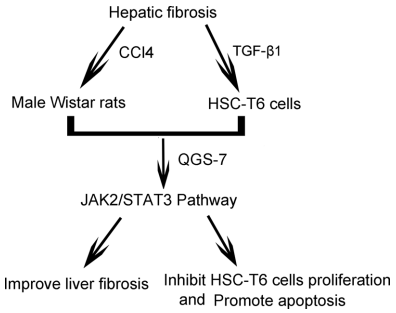
Graphical Abstract



CCl_4-treated rats and TGF-β1-induced HSCs are used. QGS-7 improve the hepatic fibrosis, inhibit HSC proliferation, and promote apoptosis by inhibiting the JAK2/STAT3 pathway.

Keywords: Qiwei Qinggan Powder (QGS-7), hepatic fibrosis, HSC-T6 cells, JAK2/STAT3 pathway

Hepatic fibrosis is an important health problem in the world. About 1.5 million people die of cirrhosis and primary liver cancer every year (Poynard *et al.* 2010). Hepatic fibrosis is mainly related to chronic hepatitis B or C infection, alcoholic steatohepatitis, nonalcoholic steatohepatitis, and biliary diseases (Lee and Friedman 2011). With the progress of fibrosis, cirrhosis will occur and even develop into HCC (Pinzani 2015; Tacke and Trautwein 2015). At present, most of the drugs for the treatment of hepatic fibrosis are expensive, with many side effects, and there is no clearly recognized effective drug for the treatment of various types of hepatic fibrosis (Zhang *et al.* 2016). Therefore, it is urgent to study the therapeutic drugs and mechanism of hepatic fibrosis. Traditional Chinese medicine has the characteristics of low toxicity, less adverse reactions, and good patient tolerance. Mongolian medicine is an important part of traditional Chinese medicine. It is the cream of traditional culture. It has unique theoretical system, special curative effect for many diseases, and great potential for development.

Mongolian medicine QGS-7 is a classic prescription of Mongolian medicine (Wu *et al.* 2014), which can be used to treat various liver diseases (Bai and Surongzhabu 1990). It has been shown that QGS-7 has a good therapeutic effect on acute liver injury (Qi *et al.* 1994; E WJ and Ma 2017). However, its therapeutic effect and mechanism on hepatic fibrosis have not been reported.

We calculated the liver index of rats, measured the contents of ALT, AST, and ALP in serum, HYP in liver, observed the liver injury by HE staining, and counted the collagen content by Masson staining. It was confirmed that QGS-7 has a good effect on repairing liver injury and alleviating hepatic fibrosis. In addition, we also used immunohistochemistry to detect α-SMA in liver tissue, RT-qPCR and Western blot to detect the expression of α-SMA and Collagen I from mRNA and protein levels, and confirmed the change of expression of hepatic fibrosis marker protein.

In order to study the mechanism of QGS-7 in the treatment of hepatic fibrosis, we extracted RNA from the liver tissue of each group and analyzed the transcriptome sequence. Using bioinformatics, we screened out the signaling pathways with significant changes between the model group and QGS-7 group. After a large number of literature review, we selected Janus-activated kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway for research. As the important members of this pathway, JAK2 and STAT3 play an important role in signal transduction. At present, JAK2/STAT3 signaling pathway has made great progress in renal and bone marrow fibrosis (Koike *et al.* 2014; Xu *et al.* 2014; Gupta *et al.* 2017; Mesa *et al.* 2017; Verstovsek *et al.* 2017). At the same time, there are some studies on liver injury and fibrosis (Klein *et al.* 2017; Wang *et al.* 2019). In liver fibrosis, the expression and activity of JAK2 are elevated in experimental rodents and humans (Granzow *et al.* 2014; Lu *et al.* 2017). STAT3 respond to interleukin-6 by upregulating transforming growth factor-β1 (TGF-β1) in primary hepatocytes and human liver cancer cells (HepG2), thereby enhancing liver fibrosis (Ogata *et al.* 2006). The susceptibility of hepatic fibrosis as well as hepatic inflammation in response to high-fat diet (HFD) was significantly increased with aging (Kim *et al.* 2016). High fat diet induces activation of insulin receptor substrate 1/Forkhead box protein O1 (IRS1/FOXO1), JAK2/STAT3, and protein Kinase B/Glycogen synthase kinase 3 beta (AKT/GSK3β) pathways in live (Chen and Liu 2018). This article aims to study whether Mongolian medicine QGS-7 can relieve liver fibrosis induced by high fat through JAK2/STAT3 signaling pathway. We hope to provide experimental data for the effect, target, and mechanism of QGS-7 in the treatment of hepatic fibrosis.

Materials and methods

Composition and preparation of Qiwei Qinggan Powder

QGS-7 is comprised of the following 7 herbs: *Carthami Flos* (Honghua), 180 g; *Scabiosa comosa* inflorescence (Lanpenhua), 60 g; *Dracocephalum moldavica L.* (Xiangqinglan), 60 g; Wulingzhi (Wulingzhi), 60 g; artificial *Bovis Calculus* (artificial Niuhuang), 80 g; *Dianthus superbus L.* (Qumai), 60 g; and *Gypsum Fibrosum* (Shigao), 180 g. In addition to artificial Calculus Bovis, all herbal medicines are crushed, then mixed with artificial Calculus Bovis, sifted, and evenly mixed. The total used was 1.5-3 g which is the common dose for adult humans. All the herbs were purchased from Inner Mongolia Tiansheng Mongolian Traditional Chinese Medicine Co., Ltd (Inner Mongolia, China).

Experimental animals

The animal experiments were conducted in line with the National Institute of Health's Guidelines for the Care and Use of Laboratory Animals and received approval from the Inner Mongolia Medical University. The research was reviewed and

approved by the Ethics Committee for Experimental Animal Management and Animal Welfare of Inner Mongolia Medical University. Male Wistar rats of specific pathogen-free (SPF) grade, weighing 190-220 g, were obtained from the Experimental Animal Center of Inner Mongolia Medical University, China (Certificate of quality No. SCXK [Jing] 2016-0006) and kept in an 18-22 °C and 70% humidity-controlled room with 12 h light-dark cycle. The animals were fed on regular sterile chow diet and water ad libitum.

Hepatic fibrosis model replication and QGS-7 treatment

Fifty rats were randomly divided into the 5 groups (10 rats per group): control group, CCl_4 group, and QGS-7 [135, 270, and 405 mg/(kg/d)] groups. Hepatic fibrosis was generated by 10 weeks of treatment with CCl_4 (CCl_4/peanut oil 1:1 [vol/vol]), a mixture of pure CCl_4 and peanut oil at 2 mL/kg body weight by gavage twice weekly (Wei, Wu and Li 2010; Wollin, Togbe and Ryffel 2020). At the same time, QGS-7 was given once a day. The CCl_4 group and the control group received equal volume of 0.5% sodium carboxymethylcellulose solution. At the end of the experimental period, all rats were sacrificed under chloral hydrate anesthesia. Blood was obtained from the abdominal aorta, and the liver was excised. The liver was immediately frozen for biochemical measurements or fixed in formalin for histochemical examination.

Preparation of QGS-7-containing serum

Wistar rats were randomly divided into 2 groups (8 rats per group): control group and QGS-7 groups. QGS-7 was given once a day according to 10 times of the lowest adult dose [1350 mg/(kg d)]. On the 7th day, the rats fasted 12 h before gavage and carried out the experiment within 1-2 h after gavage. The blood was collected from abdominal aorta, and then placed for 20 min, centrifuged in a centrifuge (4 °C, 999 g, 15 min). After centrifugation, the serum was filtered with 0.22 μM filter membrane, which was called drug serum. In the control group, the drug was replaced by normal saline, and the preparation method was the same as before. After being inactivated at 56 °C for 30 min, the drug serum was stored in a refrigerator at −80 °C.

Cell culture

The hepatic stellate cell line (HSC-T6 cells) was purchased from Beijing Beina Science & Technology Co., Ltd (Beijing, China). Cells were cultured in DMEM supplemented with 10% FBS (Thermo Fisher Scientific, Shanghai, China) at 37 °C with 5% CO_2. TGF-β1 is a characterized cytokine known to initiate HSC activation, and the activation of HSCs is a significant event in liver fibrosis.

Calculations of liver index

Liver index was calculated according to the formula: (liver weight/body weight) × 100%.

Measurements of serum AST, ALT, ALP, and tissue HYP

The activities of ALT, AST, ALP, and HYP content were measured by Visible light colorimetry. An Ultraviolet spectrophotometer and commercial kits (Nanjing Jiancheng Corporation, Nanjing, China) were used for all analyses. ALT, AST, and ALP activities were expressed as U/L and HYP level was expressed as μg/g.

Histopathological changes

liver sections fixed in formalin were embedded in paraffin and cut to a thickness of 4-5 μm. Hematoxylin–eosin and Masson's trichrome was performed according to standard procedure. Sections were visualized by a microscope and the ratio of collagen deposition (blue color area) over the whole field area was quantified by ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Immunohistochemical examination

For Immunohistochemistry, sections were incubated with α-SMA primary antibody (Proteintech Group, Wuhan, Hubei, China) overnight at 4 °C, followed by incubation with secondary antibody (Maixin Biotechnology Development Co., Ltd, Fuzhou, Fujian, China) for 1 h. Finally, the expression of α-SMA was observed under microscope.

Screening signal pathway by transcriptome sequencing

The first step is to extract RNA from liver tissue and evaluate its quality, then purify, fragment and synthesize the first and second strands of cDNA, then repair the end of cDNA and place it in DNA add "A" at the 3′ end, then connect the DNA segment with the connector and purify the cDNA template, then enrich and purify the cDNA template by PCR, and then check the DNA library and sequence after completion; determine the signal path for further research by analyzing the RNA sequencing results (processing the original data, calculating the gene expression amount, and bioinformatics analysis).

RT-qPCR assay

Total RNA was extracted from rat liver and HSC respectively. After the quality test was qualified, the reverse transcription kit (TIANGEN BIOTECH Co., Ltd, Beijing, China) was used for reverse transcription. Then the relative mRNA expression of α-SMA, collagen I, JAK2 and STAT3 in rat liver and HSC was detected by RT-qPCR. The data were processed by $2^{-\Delta\Delta Ct}$ method. The primers (Shanghai Sengon Biological and Technological Company, Shanghai, China) for each gene are shown in Table 1.

Western blot analysis

The protein in rat liver and HSC was extracted respectively. After quantitative analysis by BCA method, the sample buffer was added. After protein boiling, the sample was loaded and electrophoresis was carried out. The protein expression levels of α-SMA, collagen I, JAK2, STAT3 (Proteintech Group, Wuhan, Hubei, China), p-JAK2, and p-STAT3 (BOSTER Biological Technology Co., Ltd, Wuhan, Hubei, China) were detected, and the gray value of the item was analyzed by image studio software. The ratio of the gray value of the target protein band to the gray value of the internal reference band was used as the expression amount of the target protein, each group repeated 3 times, and the follow-up statistical analysis was carried out.

Detection of HSC proliferation by MTT

The logarithmic phase cells were collected and seeded in 96 well plates. Each group was set with 5 duplications and cultured for 12 h. Discard the original culture medium, and add the

Table 1. Objective gene primer design

Gene	Forward primer	Reverse primer
β-Actin	ACCCGCGAGTACAACCTTCT	TTCAGGGTCAGGATGCCTCT
α-SMA	CATCCACGAAACCACCTA	GGGCAGGAATGATTTGGA
Collagen type I alpha 1 chain	TGTTGGTCCTGCTGGCAAGAATG	GTCACCTTGTTCGCCTGTCTCAC
JAK2	GTGCGTGCGAGCGAAGATCC	ACTGCTGAATGAACCTGCGGAATC
STAT3	CCAGTCGTGGTGATCTCCAACATC	CAGGTTCCAATCGGAGGCTTAGTG

Table 2. The expression level of liver index, ALT, AST, ALP, and HYP ($\bar{x} \pm s$, $n = 10$)

Group	n	Liver index (%)	ALT (U/L)	AST (U/L)	ALP (U/L)	HYP (μg/g)
Blank	10	3.06 ± 0.21	11.73 ± 6.16	17.31 ± 2.48	14.63 ± 4.36	352.50 ± 37.30
Model	10	3.91 ± 0.62$^{\#\#}$	34.22 ± 5.15$^{\#\#}$	41.07 ± 9.30$^{\#\#}$	50.84 ± 16.04$^{\#\#}$	1151.00 ± 173.90$^{\#\#}$
High dose	10	3.55 ± 0.48	20.48 ± 4.70**	21.04 ± 7.40**	26.07 ± 6.87**	649.50 ± 62.09**
Middle dose	10	3.47 ± 0.30	19.31 ± 5.93**	23.74 ± 9.34**	40.56 ± 10.42	754.70 ± 64.84**
Low dose	10	3.31 ± 0.25*	20.16 ± 4.74**	21.2 ± 6.49**	42.02 ± 9.43	911.10 ± 139.60**

Notes: Compared with the blank group, $^{\#}P < .05$, $^{\#\#}P < .01$; compared with the model group, $^{*}P < .05$, $^{**}P < .01$.

corresponding concentration of serum culture medium into each well for 24 h. Remove the supernatant, add 90 μL fresh culture medium, add 10 μL MTT solution, and continue to culture for 4 h. Suck off the supernatant, add 110 μL formazan, shake it on the shaking table at low speed for 10 min, and measure the absorbance value of each well at 490 nm of the enzyme labeling instrument.

Apoptosis of HSC-T6 cells detected by Annexin V-FITC and PI double staining

The logarithmic phase cells were collected and seeded in 6 well plates. Each group was set with 3 duplications and cultured for 12 h. Discard the original culture medium, add the corresponding concentration of serum culture medium for 24 h. Trypsin digests cells, centrifuges, discards supernatant. Add 390 μL Annexin V-FITC binding solution and gently resuspend the cells. Add 5 μL Annexin V-FITC and mix gently. Add 10 μL PI and mix gently. Incubate at room temperature in dark for 10-20 min, then place in ice bath. Detection on flow cytometry.

Statistical analysis

All results were presented as mean ± SD. Statistical analysis was performed with SPSS software (version 24). The statistical significance between groups was analyzed using one-way ANOVA. The difference was considered significant at $P < .05$.

Results

QGS-7 attenuated CCl_4-induced liver fibrosis in rats

In order to study the antifibrosis effect of QGS-7, we first studied the therapeutic effect of QGS-7 on CCl_4 induced hepatic fibrosis in rats. At the end of the experiment, no rats in each experimental group died. However, all the rats in the model group had reduced diet, sluggish action, depressed spirit, weight loss, disordered fur and sometimes irregular stool. At the same time, as shown in Table 2, the rats in model group have higher liver index compared with other groups. Meanwhile, the rats in model group had significantly higher levels of serum ALT, AST, and ALP, which represented a decrease in liver function. Liver tissue levels of HYP are surrogate markers of hepatic fibrosis. Model rats also exhibited higher levels of HYP. Each dose group of QGS-7 significantly decreased the elevated ALT, AST, and HYP levels, while low- and middle-dose group (135 and 270 mg/kg) had no obvious effect in ALP, and middle and high dose (135 and 405 mg/kg) had no obvious effect in liver index.

Liver morphology can directly reflect the color, smoothness and hardness of liver surface so as to preliminarily judge the damage of liver. The liver of the control group showed bright red, smooth surface, no rough and granular feeling, and soft texture. In the CCl_4 group, the liver was dark red or even yellow, with rough surface, obvious granular feeling and hard texture. The liver of rats in each dose group of QGS-7 was dark red, with rough surface, but no obvious granule sense. The liver state was between the control group and the hepatic fibrosis model group. The results suggests that QGS-7 can improve the hepatic fibrosis of rats (Figure 1a).

To assess histological changes, hematoxylin and eosin (H&E) and Masson staining of liver tissue sections from each group were examined. H&E staining showed that in the control group, the structure of liver lobule was complete, the hepatocytes were arranged orderly and the plasma was even. In the CCl_4 group, the structure of liver lobule was destroyed, the arrangement of liver plate was disordered, a large number of inflammatory cells infiltrated and the balloon like changes of liver cells could be seen in liver tissue, and some samples even appeared and pseudolobule. The infiltration of inflammatory cells and the decrease of cell degeneration and necrosis in liver tissue of rats in each dose group of QGS-7 (Figure 1b). Masson staining showed that the hepatocytes of the control group were intact without abnormal fibrous tissue proliferation. In the CCl_4 group, a large number of fibroblasts appeared in the liver tissue, the arrangement of liver cords was disordered, the connective tissue and fibrous tissue proliferated obviously, and the pseudolobule was formed. The rats in each dose group of QGS-7 were improved in varying degrees, the proliferation of fibrous tissue was reduced, and the structure of liver tissue tended to be normal (Figure 1c). After statistical analysis of pathological sections stained by Masoon, it can be observed that compared with the control group, the collagen content in the liver of the CCl_4 group increased significantly; compared with the model group, the collagen content in the liver tissue of each group of QGS-7 decreased significantly

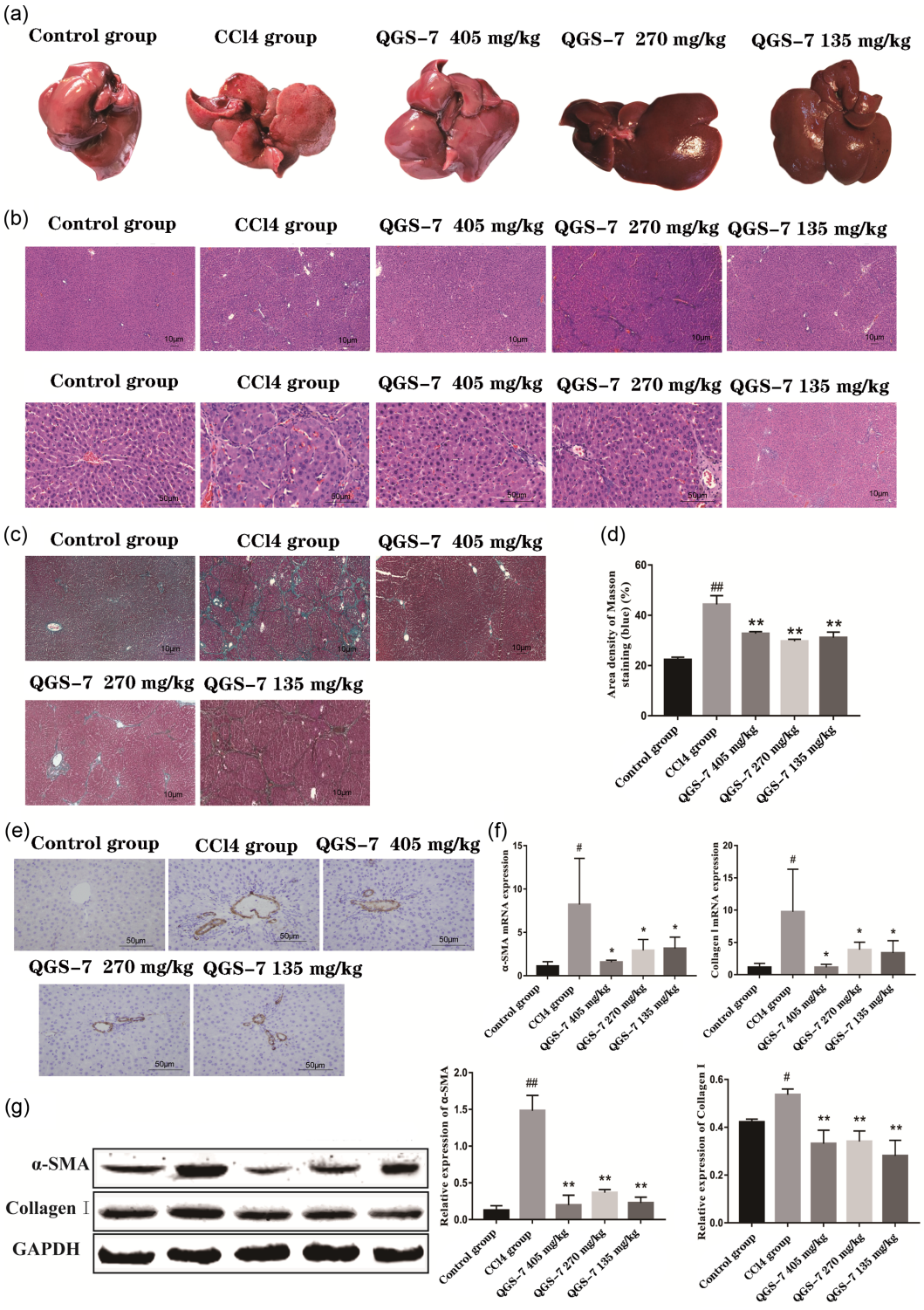


Figure 1. QGS-7 attenuated CCl_4-induced liver fibrosis in rats. (a) Generation of hepatic fibrosis of rats in different groups. (b) Effects of QGS-7 on the histological changes of liver in CCl_4-induced hepatic fibrosis rats (100× and 400×). (c) Representative micrographs of Masson trichrome staining of liver tissues. (d) Quantification of liver fibrosis (ratio of blue color area). (e) QGS-7 ameliorated pathological changes in liver as shown by immunohistochemistry. (f) RT-qPCR for α-SMA and Collagen I. (g) Western blot analysis of α-SMA and Collagen I. Compared with the control group, $^{\#}P < .05$, $^{\#\#}P < .01$; compared with the CCl_4 group, $^{*}P < .05$, $^{**}P < .01$.

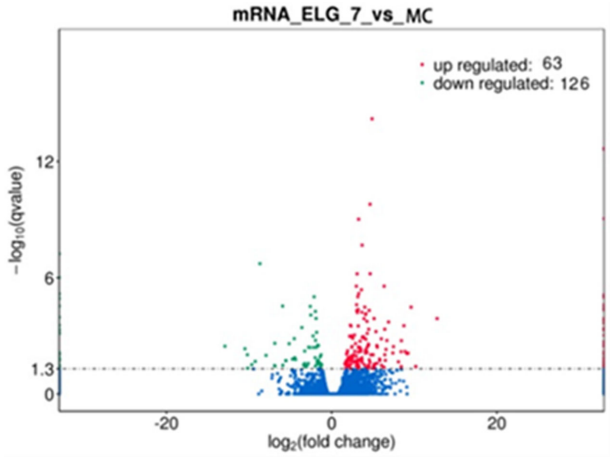


Figure 2. Volcanic map of differential gene comparison between QGS-7 group and model group.

(Figure 1d). Furthermore, intervention with QGS-7 also inhibited the upregulation of α-SMA, collagen I (Figure 1e-g), indicating that QGS-7 treatment inhibited established hepatic fibrosis.

Transcriptome sequencing

The gene expression level of control group, CCl_4 group and QGS-7 group was analyzed by transcription sequencing, and the overall distribution map of different transcripts or genes was obtained (q value <0.05) (Figure 2). Compared with the model group, there were 63 upregulated mRNA and 126 downregulated mRNA in QGS-7 group. Through further enrichment analysis of GO and KEGG, and combined with comparison with the control group, it was found that there were many signal pathways with significant changes. Combined with literature search, the JAK2/STAT3 signaling pathway possibly related to QGS-7 antifibrosis effect was obtained, which was verified by subsequent experiments.

Effect of QGS-7 on iJAK2, STAT3 mRNA expression, and JAK2, p-JAK2, STAT3, and p-STAT3 protein expression *in vivo*

The RT-qPCR results showed that compared with the control group, the expression of JAK2 and STAT3 mRNA in the liver tissue of the model group increased significantly; compared with the CCl_4 group, the expression of JAK2 mRNA in the low-dose group of QGS-7 decreased significantly, and the expression of STAT3 mRNA in the high-dose group of QGS-7 decreased significantly, as shown in Figure 3a. The results showed that QGS-7 could significantly inhibit the expression of JAK2 and STAT3 mRNA in liver tissue.

Western blot results showed that compared with the control group, the expression level of JAK2, p-JAK2, STAT3, and p-STAT3 protein in the liver tissue of the model group was significantly increased; compared with the CCl_4 group, the expression of JAK2, p-JAK2, and STAT3 protein in each group of QGS-7 was significantly decreased, and the expression of p-STAT3 protein in the high- and middle-dose groups of QGS-7 was significantly decreased (Figure 3b). It is suggested that QGS-7 can not only reduce the protein content of JAK2 and STAT3 in liver tissue, but also reduce the expression level of p-JAK2 and p-STAT3.

In vitro experiments verify that QGS-7 has antifibrosis effect through JAK2/STAT3 signaling pathway

The results of RT-qPCR showed that compared with the control group, the relative mRNA expression of α-SMA and collagen I in the low- and high-dose serum group decreased significantly, and the relative mRNA expression of α-SMA and collagen I in the middle-dose serum group decreased significantly (Figure 4a).

Western blot results showed that compared with the control group, the expression level of α-SMA and Collagen I protein in the low-, middle-, and high-dose serum groups decreased significantly (Figure 4b).

The results of RT-qPCR showed that compared with the control group, the relative mRNA expression of JAK2 and STAT3 in each dose serum group decreased significantly (Figure 4c). The results showed that QGS-7 could reduce the expression of JAK2 and STAT3 mRNA in HSC.

Western blot results showed that compared with the control group, the protein expression of JAK2, p-JAK2, STAT3 and p-STAT3 in the low- and middle-dose serum groups decreased significantly; the protein expression of p-JAK2 and p-STAT3 in the high-dose serum group decreased significantly (Figure 4d). This suggests that QGS-7 can significantly reduce the expression level of JAK2 and STAT3 protein, and p-JAK2 and p-STAT3 in HSC.

The results of MTT showed that after 24 h treatment the OD values of the low-, middle-, and high-dose serum groups decreased significantly, and the inhibition rates were 42.95%, 50.89%, and 44.93% respectively; after 48 h treatment, the OD values of the middle- and high-dose serum groups decreased significantly and the inhibition rates were 50.89% and 33.55%; the OD values of the low-, middle-, and high-dose serum groups decreased significantly after 72 h treatment, the inhibition rates were 21.36%, 33.26%, and 33.00% (Table 3).

FITC labeled Annexin and PI double staining can be used to distinguish 3 types of cells: living cells, early apoptotic cells and late apoptotic and necrotic cells. The results showed that compared with the control group, the apoptosis rate of the low-dose serum group was significantly higher; the apoptosis rate of the high-dose group was significantly higher (Figure 4e).

Discussion

Fibrosis is a process closely related to organ damage, which plays a role in preventing organ tissue from disintegration in the process of chronic inflammation. With the repair of tissue damage, fibrosis can be reversed in a few weeks (Atta 2015). However, this ability to reverse fibrosis is limited. When ECM is widely accumulated and cross-linked, fibrinolysis is blocked and cell components that can eliminate scar tissue are lost, it will make hepatic fibrosis difficult to be reversed (Sun and Kisseleva 2015). Although people have a deeper understanding of the pathogenesis of hepatic fibrosis, there is still a lack of effective antihepatic fibrosis drugs (Fagone *et al.* 2016).

QGS-7 is a classic prescription of Mongolian medicine, also known as Eligen-7 (ELG-7) (Wu *et al.* 2014), which can be used to treat various liver diseases in clinical practice (Bai and Surongzhabu 1990). Although QGS-7 has been used in the clinical treatment of hepatic fibrosis, there is no preliminary *in vitro* and *in vivo* experimental data, and the mechanism of its treatment of hepatic fibrosis is not clear, so it is particularly important to supplement this data.

Hepatic fibrosis is an important node in the development of liver diseases. Correct understanding and timely treatment can effectively control the development of liver diseases. It has been

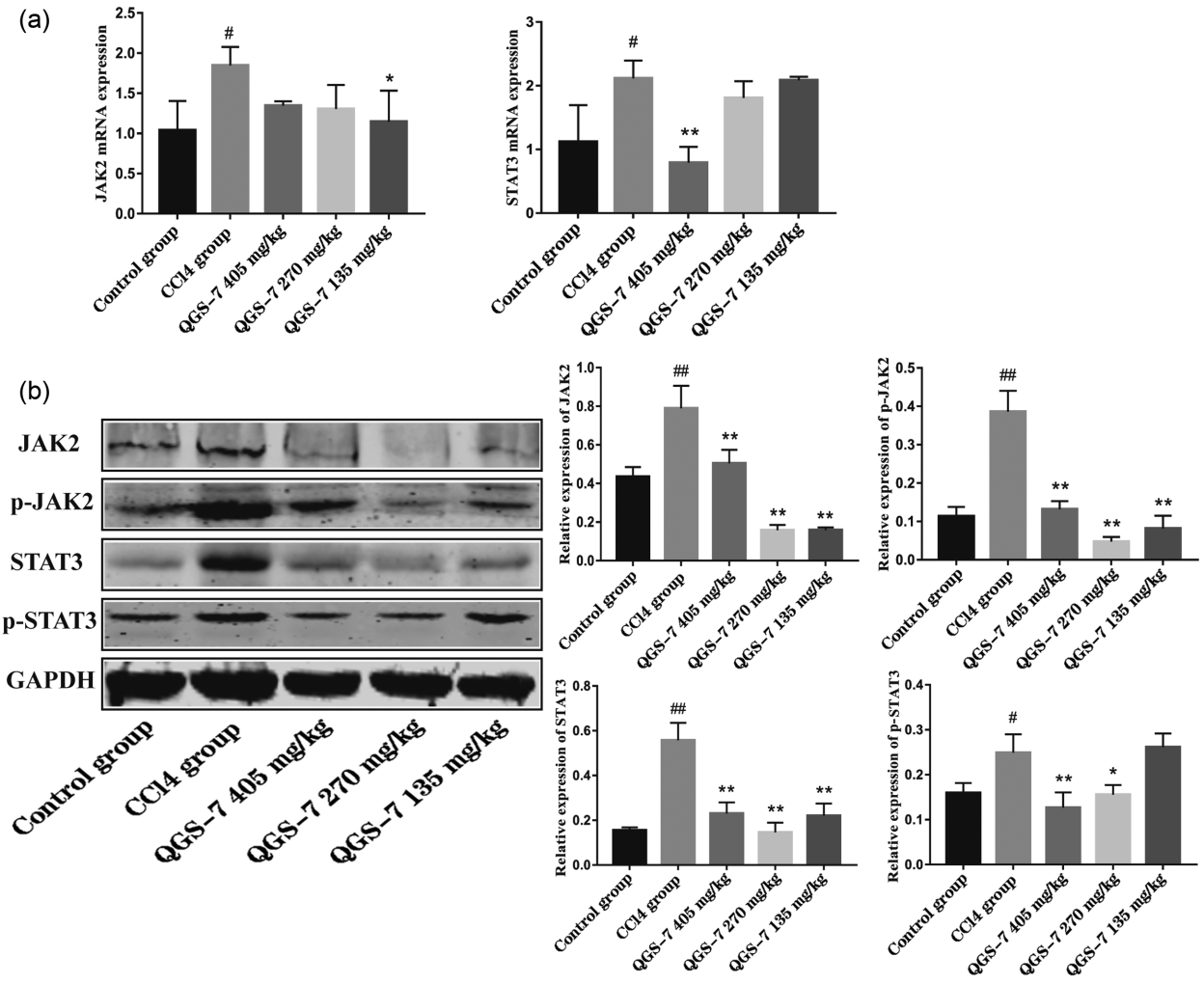


Figure 3. Effect of QGS-7 on JAK2, STAT3 mRNA expression, and JAK2, p-JAK2, STAT3, and p-STAT3 protein expression *in vivo*. (a) JAK2 and STAT3 mRNA expression level. (b) JAK2, p-JAK2, STAT3, and STAT3 protein expression levels. Compared with the control group, $^{\#}P < .05$, $^{\#\#}P < .01$; compared with the CCl_4 group, $^{*}P < .05$, $^{**}P < .01$.

Table 3. Effect of QGS-7 containing serum on proliferation of HSC-T6 cells ($\bar{x} \pm s$, $n = 3$)

Group	OD values	24 h inhibition rates (%)	OD values	48 h inhibition rates (%)	OD values	72 h inhibition rates (%)
Control	1.15 ± 0.15	–	1.49 ± 0.06	–	1.55 ± 0.02	–
High-dose serum	$0.65 \pm 0.11^{**}$	42.95	1.23 ± 0.11	17.67	$1.22 \pm 0.08^{**}$	21.36
Middle-dose serum	$0.56 \pm 0.02^{**}$	50.89	$1.02 \pm 0.23^{**}$	32.03	$1.03 \pm 0.12^{**}$	33.26
Low-dose serum	$0.63 \pm 0.16^{**}$	44.93	$0.99 \pm 0.06^{**}$	33.55	$1.04 \pm 0.10^{**}$	33

Notes: Compared with the blank group, $^{\#}P < .05$, $^{\#\#}P < .01$; compared with the model group, $^{*}P < .05$, $^{**}P < .01$.

reported that CCl_4 induction method is the most classical one among the chemical drug induction methods (Kang *et al.* 2016). The method of gavage is simple in operation and well tolerated by animals, so we choose it to establish hepatic fibrosis model (Wei, Wu and Li 2010; Wollin, Togbe and Ryffel 2020). In the development process of hepatic fibrosis, the liver will increase and become heavier, so the liver index can reflect the status of the liver. Compared with the control group, the liver index of the CCl_4 group increased significantly; compared with the model group, the liver index of the low-dose group decreased significantly. The process of liver injury is accompanied by the degeneration, necrosis and rupture of hepatocytes, and then the enzymes existing in the cells enter into the serum. Therefore, the content of enzymes in the serum can reflect the damage and damage degree of hepatocytes (Ogaly *et al.* 2015). The changes of serum enzymes (ALT, AST, and ALP) are often used to reflect the liver function to judge the degree of liver damage. Therefore, the above indicators are also selected in the study of the therapeutic effect of QGS-7 on hepatic fibrosis. In addition, HYP is a characteristic amino acid component of collagen. When hepatic fibrosis occurs, a large number of ECM accumulates, and the collagen content increases, which leads to a significant increase of HYP, which can be used as one of the indicators to investigate the occurrence of hepatic fibrosis (Zhang *et al.* 2020). The results of this

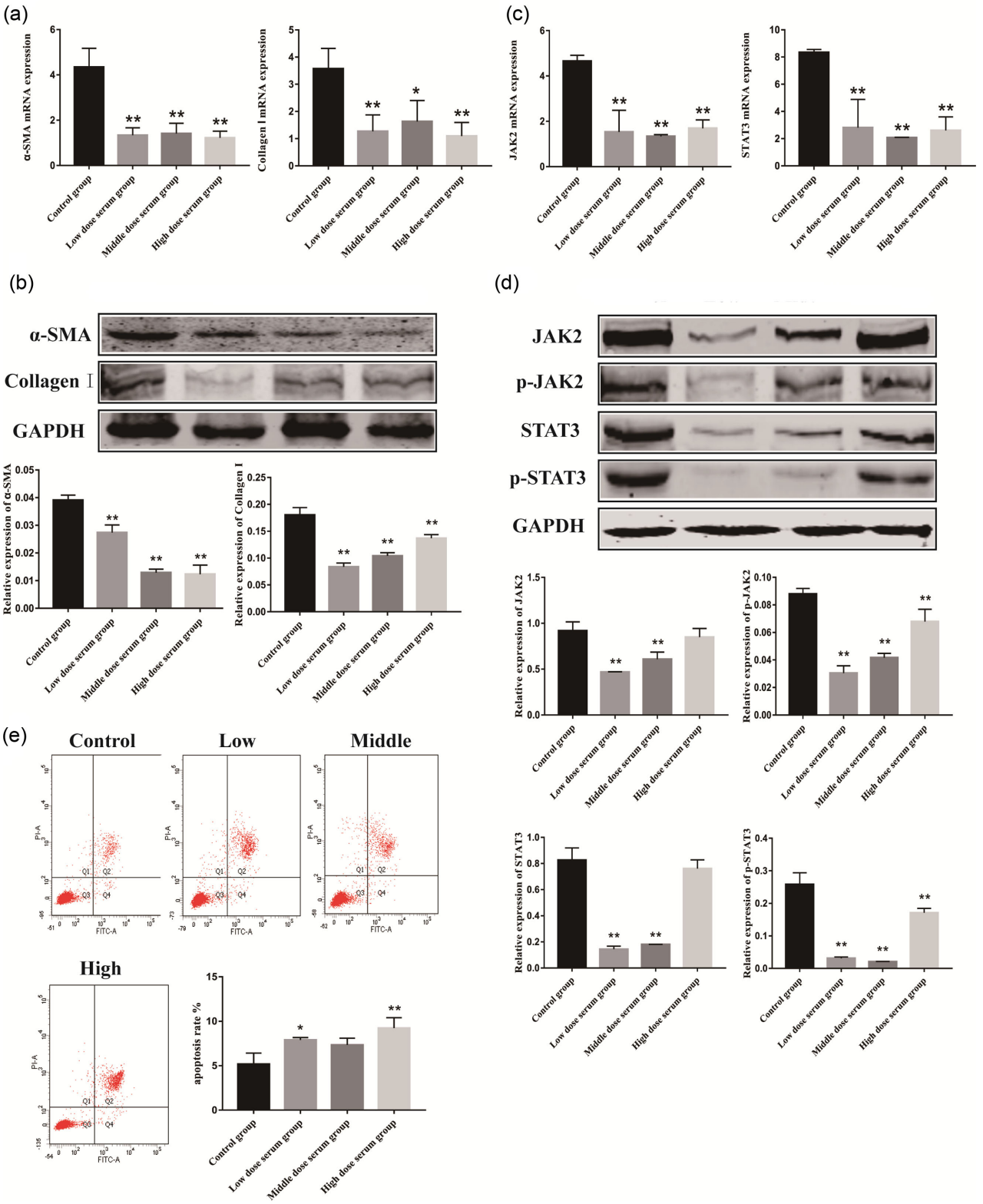


Figure 4. *In vitro* experiments verify that QGS-7 has antifibrosis effect through JAK2/STAT3 signaling pathway. (a) Relative mRNA expression of α-SMA and collagen I. (b) α-SMA and collagen I protein expression level. (c) JAK2 and STAT3 mRNA expression level. (d) JAK2, p-JAK2, STAT3, and STAT3 protein expression levels. (e) HSC apoptosis rate. Compared with the control group, $^{*}P < .05$, $^{**}P < .01$.

study showed that compared with the control group, the liver tissue was hard and felt granule, and the contents of ALT, AST, ALP, and HYP in serum were significantly increased. Compared with the CCl_4 group, the liver condition of rats in each dose group of QGS-7 was improved, the ALT, AST, and HYP content in each dose group and the ALP content in high-dose group were significantly reduced. It shows that QGS-7 has a certain protective effect on liver injury. In addition to the above indicators, pathological section observation can more accurately determine the degree of hepatic fibrosis. Clinically, it is also an important indicator to evaluate liver injury and differential diagnosis of hepatic fibrosis. H&E staining can observe the infiltration of inflammatory cells, balloon like changes, pseudolobules and other pathological states, while Masson staining can intuitively reflect the proliferation of collagen fibers, connective tissue hyperplasia, hepatic cord disorder and pseudolobules and other pathological states in the liver tissue so as to judge the situation of hepatic fibrosis. Compared with the control group, inflammatory cell infiltration, pseudolobule and bridge connection can be seen in the liver pathological section of the CCl_4 group, Masson staining can see a large number of fibrogenesis, wrapping the damaged hepatocyte to form pseudolobule, indicating the success of the hepatic fibrosis model. Compared with the CCl_4 group, the pathological manifestations of QGS-7 group were alleviated, and the blue collagen fibers were also decreased by Masson staining. It is suggested that QGS-7 has a certain therapeutic effect on rats with hepatic fibrosis.

In the process of hepatic fibrosis, HSC is activated, α-SMA is expressed in large quantities, ECM is synthesized and secreted (Higashi, Friedman and Hoshida 2017) and collagen IV is replaced by collagen I, which can form scar tissue. When HSC is activated and proliferated, the expression level of collagen I mRNA is 60-70 times that of resting (Stefanovic *et al.* 1997), which is the most important part of ECM. Therefore, the increase of collagen I can reflect the degree of hepatic fibrosis (Puche, Saiman and Friedman 2013). The results of immunohistochemistry can not only reflect the position of protein expression in cells, but also directly reflect the expression of α-SMA in liver tissue of rats in each group. α-SMA is mainly expressed in HSC cytoplasm activated in the portal area. Compared with the control group, the positive expression area of the model group increased greatly. And the α-SMA in the liver tissue of each dose group of QGS-7 decreased compared with the CCl_4 group. The results of RT-qPCR and Western blot suggested that QGS-7 might play an antifibrosis role by inhibiting the activity of HSC and reducing the secretion of collagen.

Mongolian medicine is similar to other traditional medicine prescriptions which has the characteristics of multi target and multi mechanism. In order to better define the target and mechanism of QGS-7 against hepatic fibrosis, we extracted RNA from rat liver tissue and sequenced the transcriptome gene. The JAK2/STAT3 signaling pathway is one of the possible mechanisms of QGS-7 and antifibrosis by bioinformatics analysis and a large number of literature search. We have carried out subsequent tissue and cell verification.

JAK2/STAT3 signaling pathway is mediated by cytokines, mainly involved in cell proliferation, differentiation, apoptosis and immune regulation (Jatiani *et al.* 2010). JAK2/STAT3 has been widely confirmed to play an important role in the development of organ fibrosis in recent years (Ogata *et al.* 2006; He *et al.* 2008; Zhao, Shi and Duan 2008; Xu *et al.* 2014). Through a large number of literature searches, combined with transcriptome results, we found the JAK2/STAT3 signaling pathway changed significantly after hepatic fibrosis. The results of transcriptome reflect the common effects of all cells in the liver tissue, but the key point for the treatment of hepatic fibrosis is the role of HSC, so it is necessary to detect the activation, proliferation and apoptosis of HSC *in vitro*. At the same time, HSC accounts for about 8%-15% of the total number of liver cells in the normal liver, but with the occurrence of chronic fibrosis injury, HSC rapidly proliferates to several times of the normal state (Shang *et al.* 2018). Therefore, we preliminarily determined that the change of JAK2/STAT3 pathway was related to the change of HSC in the transcriptome sequencing results, and speculated that JAK2/STAT3 signaling pathway was related to the antifibrosis effect of QGS-7.

In JAK2/STAT3 signaling pathway, p-JAK2 and p-STAT3 are the activation forms of JAK2 and STAT3, respectively. In this study, the changes of p-JAK2 and p-STAT3 protein can reflect the activation degree of JAK2/STAT3 signaling pathway. The expression of JAK2, STAT3, p-JAK2, and p-STAT3 could be down-regulated by QGS-7 *in vivo*. Furthermore, JAK2-mediated fibrosis signal is caused not only by the increase of JAK2 expression, but also by the p-JAK2 expression.

HSC is considered to be the main fibroblast type of liver and the main source of ECM (Kaimori *et al.* 2007). At the same time, the activation of HSC is also a key step of hepatic fibrosis. Therefore, in order to confirm that QGS-7 plays an antifibrosis role by affecting HSC, we carried out a series of experiments *in vitro* with HSC-T6 cell line. HSC-T6 is an immortalized rat HSC line transfected by simian virus 40 (SV40). It is known that HSC-T6 has almost all functions of activating HSC, such as expression of α-SMA, collagen I, matrix metalloproteinases (MMP), tissue inhibitor of matrix Metalloproteases (TIMP-1), and produce endogenous TGF-β1. The morphology of fibroblasts was observed under the microscope, which can proliferate rapidly in the process of culture (Vogel *et al.* 2000; Zhuang *et al.* 2007). These characteristics of HSC-T6 are typical of activated astrocytes. We also observed under the inverted microscope that HSC morphology showed stretching state, pseudopodia increased with star like change, and the connection between cells became loose obviously, showing a significant activation state. The expression of α-SMA mRNA and protein was detected by RT-qPCR and Western blot. So, we did a follow-up experiment without induction.

When Mongolian medicine acts on cells, we adopt the drug containing serum administration method, which can simulate the absorption, distribution, metabolism and excretion of oral drugs through a series of processes so that there are not only prototype components of compound formula in serum, but also products after metabolism, which can fully reflect the changes of drug compatibility (Wang *et al.* 2012; Chen and Guo 2016).

The activation of HSC is the central link of hepatic fibrosis and α-SMA is the marker of HSC activation. After HSC activation, α-SMA protein is highly expressed, which will further increase the synthesis and accumulation of ECM dominated by collagen I, and finally lead to hepatic fibrosis (Nishikawa, Wang and Carr 1998). This study found that QGS-7 can inhibit the activity of HSC and reduce the production of ECM by reducing α-SMA and collagen I in HSC.

Subsequently, in order to determine whether the changes of JAK2/STAT3 pathway in transcriptome sequencing results are related to the changes of HSC, we used RT-qPCR and Western blot assay to detect the changes of pathway related factor's mRNA and protein level in HSC. The results of the experiments showed that the effect of QGS-7 on hepatic fibrosis might be influenced by the JAK2/STAT3 signaling pathway in HSC.

According to the literature, JAK2/STAT3 signaling pathway participates in the process of cell differentiation, thus promoting the fibrotic response and leading to HSC activation

(Friedman 2008). In this study, it was also confirmed that the expression of α-SMA in HSC increased significantly after hepatic fibrosis. Meanwhile, the downstream signal of JAK2/STAT3 affects the expression of many genes, including some genes related to cell proliferation, migration, and apoptosis (Bromberg *et al*. 1999; Bromberg and Darnell 2000; Hirano, Ishihara and Hibi 2000; Kisseleva and Brenner 2007; Xu *et al*. 2014). Therefore, we used MTT method to detect the proliferation of HSC-T6 cells. The results showed that the inhibition rate of HSC-T6 cells in the serum containing drugs increased. In addition, Annexin V-FITC and PI double staining technique showed that the apoptosis rate of the low-dose and high-dose groups was significantly higher than control group. This suggests that JAK2/STAT3 signaling pathway can inhibit HSC proliferation and promote HSC apoptosis to produce antifibrosis effect.

There were some limitations to the study as we did not use the HPLC to analysis effective components of QGS-7. A follow-up study using HPLC to analysis effective components of QGS-7 has already been planned.

Conclusions

So far, eliminating the root cause of liver disease is still the most effective way to prevent hepatic fibrosis. However, in the process of hepatic fibrosis, drugs and means for the treatment of HSC and disease molecular mechanism are particularly important. Through the above research, it is confirmed that the Mongolian medicine QGS-7 has the effect of treating hepatic fibrosis; the Mongolian medicine QGS-7 can inhibit the activation, proliferation and promote apoptosis of HSC; the Mongolian QGS-7 can reduce the expression of JAK2, p-JAK2, STAT3, and p-STAT3 in JAK2/STAT3 signaling pathway in the process of antihepatic fibrosis; we speculate that the Mongolian medicine QGS-7 may be through JAK2/STAT3 signaling pathway affects HSC and plays an antifibrosis role.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Author contribution

J.L., H.Y., F.W., and X.B. contributed equally to this work.

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Disclosure statement

Contributions: (I) Conception and design:Jie Liang, Hongwei Yuan,Liping Xu, Feng Wang and Xiaomei Bao; (II) Administrative support: Yuehong Ma; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

References

Atta HM. Reversibility and heritability of liver fibrosis: implications for research and therapy. *World J Gastroenterol* 2015;**21**:5138-48.

Bai QY, Surongzhabu S. *Chinese Medical Encyclopedia · Mongolian Medicine*. Shanghai: Shanghai Science and Technology Press, 1990 (in Chinese).

Bromberg J, Darnell JE. The role of STATs in transcriptional control and their impact on cellular function. *Oncogene* 2000;**19**:2468-73.

Bromberg JF, Wrzeszczynska MH, Devgan G *et al*. Stat3 as an oncogene. *Cell* 1999;**98**:295-303.

Chen HJ, Liu J. Actein ameliorates hepatic steatosis and fibrosis in high fat diet-induced NAFLD by regulation of insulin and leptin resistant. *Biomed. Pharmacother* 2018;**97**: 1386-96.

Chen JM, Guo J. Progress on the serum pharmacology of Chinese medicine. *J Guangdong Pharm Univ* 2016;**32**:390-3 (in Chinese).

E WJ ZY, Ma LJ. Protective effects of Mongolian medicine Eligen-7 on acute liver injury in mice. *J. Inner Mongolia Univ Natl (Nat Sci)* 2017;**32**:258-62 (in Chinese).

Fagone P, Mangano K, Pesce A *et al*. Emerging therapeutic targets for the treatment of hepatic fibrosis. *Drug Discov Today* 2016;**21**:369-75.

Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008;**134**:1655-69.

Granzow M, Schierwagen R, Klein S *et al*. Angiotensin-II type 1 receptor-mediated Janus kinase 2 activation induces liver fibrosis. *Hepatology* 2014;**60**:334-48.

Gupta V, Mesa RA, Deininger MW *et al*. A phase 1/2, open-label study evaluating twice-daily administration of momelotinib in myelofibrosis. *Haematologica* 2017;**102**:94-102.

He SQ, Wen B, Lv ZP *et al*. Influence of "Bao Gan Ning" on liver histomorphology and protein phosphorylation levels of JAK2 and protein phosphorylation levels of JAK2 and STAT3 of liver fibrosis rats. *Shanghai J Tradit Chin Med* 2008;**42**:75-9 (in Chinese).

Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. *Adv Drug Deliv Rev* 2017;**121**:27-42.

Hirano T, Ishihara K, Hibi M. Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene* 2000;**19**:2548-56.

Jatiani SS, Baker SJ, Silverman LR *et al*. Jak/STAT pathways in cytokine signaling and myeloproliferative disorders: approaches for targeted therapies. *Genes Cancer* 2010;**1**: 979-93.

Kaimori A, Potter J, Kaimori JY *et al*. Transforming growth factor-beta1 induces an epithelial-to-mesenchymal transition state in mouse hepatocytes in vitro. *J Biol Chem* 2007;**282**: 22089-101.

Kang YH, Yang Y, Wang BJ *et al*. Progress of animal models' establishment of hepatic fibrosis. *Liaoning J Tradit. Chin Med* 2016;**43**:2229-31 (in Chinese).

Kim IH, Xu J, Liu X *et al*. Aging increases the susceptibility of hepatic inflammation, liver fibrosis and aging in response to high-fat diet in mice. *Age* 2016;**38**:291-302.

Kisseleva T, Brenner DA. Role of hepatic stellate cells in fibrogenesis and the reversal of fibrosis. *J Gastroenterol Hepatol* 2007;**22**:S73-8.

Klein S, Rick J, Lehmann J *et al*. Janus-kinase-2 relates directly to portal hypertension and to complications in rodent and human cirrhosis. *Gut* 2017;**66**:145-55.

Koike K, Ueda S, Yamagishi S *et al*. Protective role of JAK/STAT signaling against renal fibrosis in mice with unilateral ureteral obstruction. *Clin Immunol* 2014;**150**:78-87.

Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. *Best Pract Res Clin Gastroenterol* 2011;**25**:195-206.

Lu C, Xu W, Shao J *et al*. Nrf2 induces lipocyte phenotype via a SOCS3-dependent negative feedback loop on JAK2/STAT3 signaling in hepatic stellate cells. *Int Immunopharmacol* 2017;**49**:203-11.

Mesa RA, Vannucchi AM, Mead A *et al*. Pacritinib versus best available therapy for the treatment of myelofibrosis irrespective of baseline cytopenias (PERSIST-1): an international, randomised, phase 3 trial. *Lancet Haematol* 2017;**4**: e225-36.

Nishikawa Y, Wang M, Carr BI. Changes in TGF-beta receptors of rat hepatocytes during primary culture and liver regeneration: increased expression of TGF-beta receptors associated with increased sensitivity to TGF-beta-mediated growth inhibition. *J Cell Physiol* 1998;**176**:612-23.

Ogaly HA, Eltablawy NA, El-Behairy AM *et al*. Hepatocyte growth factor mediates the antifibrogenic action of *Ocimum bacilicum* essential oil against CCl_4-induced liver fibrosis in rats. *Molecules* 2015;**20**:13518-35.

Ogata H, Chinen T, Yoshida T *et al*. Loss of SOCS3 in the liver promotes fibrosis by enhancing STAT3-mediated TGF-beta1 production. *Oncogene* 2006;**25**:2520-30.

Pinzani M. Pathophysiology of liver fibrosis. *Dig Dis* 2015;**33**:492-7.

Poynard T, Lebray P, Ingiliz P *et al*. Prevalence of liver fibrosis and risk factors in a general population using non-invasive biomarkers (FibroTest). *BMC Gastroenterol* 2010;**10**:40.

Puche JE, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. *Compr Physiol* 2013;**3**:1473-92.

Qi JF, Zhang JL, Sun QN *et al*. Protective function of traditional Mongolian medicines on experimental liver injury. *J Inner Mongolia Med Univ* 1994;**2**:82-7 (in Chinese).

Shang L, Hosseini M, Liu X *et al*. Human hepatic stellate cell isolation and characterization. *J Gastroenterol* 2018;**53**:6-17.

Stefanovic B, Hellerbrand C, Holcik M *et al*. Posttranscriptional regulation of collagen alpha1(I) mRNA in hepatic stellate cells. *Mol Cell Biol* 1997;**17**:5201-9.

Sun M, Kisseleva T. Reversibility of liver fibrosis. *Clin Res Hepatol Gastroenterol* 2015;**39**:S60-3.

Tacke F, Trautwein C. Mechanisms of liver fibrosis resolution. *J Hepatol* 2015;**63**:1038-9.

Verstovsek S, Talpaz M, Ritchie E *et al*. A phase I, open-label, dose-escalation, multicenter study of the JAK2 inhibitor NS-018 in patients with myelofibrosis. *Leukemia* 2017;**31**:393-402.

Vogel S, Piantedosi R, Frank J *et al*. An immortalized rat liver stellate cell line (HSC-T6): a new cell model for the study of retinoid metabolism in vitro. *J Lipid Res* 2000;**41**:882-93.

Wang X, Hao L, Han X *et al*. Hepatoprotective activity of Jiedu Hugan Recipe on acetaminophen-induced hepatotoxicity by activatin. *Pharmacol Clin Chin Mate Med* 2019;**35**:144-9 (in Chinese).

Wang YQ, Yan JY, Shi BM *et al*. Research progress on the material basis of the efficacy of traditional Chinese medicine. *Chin J Inf Tradit Chin Med* 2012;**19**:99-102 (in Chinese).

Wei W, Wu XM, Li YJ. *Methodology of Pharmacological experiment*. 4th edn. Beijing: People's Health Press, 2010 (in Chinese).

Wollin L, Togbe D, Ryffel B. Effects of cc in an animal model of liver fibrosis. *Biomed Res Int* 2020;**2020**:3867198.

Wu BY, Zhao MH, Wu JM *et al*. Clinical observation of Eligengurigumu-7 on treatment of alcoholic liver diseases. *Drugs Clin* 2014;**29**:282-5.

Xu MY, Hu JJ, Shen J *et al*. Stat3 signaling activation crosslinking of TGF-β1 in hepatic stellate cell exacerbates liver injury and fibrosis. *Biochim Biophys Acta* 2014;**1842**:2237-45.

Zhang CY, Yuan WG, He P *et al*. Liver fibrosis and hepatic stellate cells: etiology, pathological hallmarks and therapeutic targets. *WJG* 2016;**22**:10512-22.

Zhang D, Zhang L, Chen G *et al*. Hepatoprotective effect of Xiayuxue decoction ethyl acetate fraction against carbon tetrachloride-induced liver fibrosis in mice via inducing apoptosis and suppressing activation of hepatic stellate cells. *Pharm Biol* 2020;**58**:1229-43.

Zhao S, Shi YH, Duan HJ. JAK/STAT pathway activation is involved in high glucose-induced transdifferentiation in renal proximal tubular epithelial cells *Chin J Pathophysiol* 2008;**24**:325-9 (in Chinese).

Zhuang P, Ma HH, Jiang YS *et al*. Establishment of immortalized rat HSC induced by the genes of Simian Virus 40 large T antigen and study on the morphological and biological characteristics of the new HSC line. *Chin J Pathophysiol* 2007;**10**:2074-7 (in Chinese).