Xia-yu-xue decoction (XYXD) reduces carbon tetrachloride (CCl₄)-induced liver fibrosis through inhibition hepatic stellate cell activation by targeting NF-κB and TGF-β₁ signaling pathways

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Abstract

Background: Hepatic stellate cell (HSC) activation is activated mainly by endotoxin and transforming growth factor (TGF-β₁) in chronic liver injury, consequently, can be important therapeutic targets. Xia-yu-xue decoction (XYXD), a classical recipe used in China to treat liver fibrosis, and has been revealed to inhibit hepatic fibrosis in animal models, the mechanism of action of XYXD remains elusive. In the present study, we evaluated whether XYXD reduced endotoxin and pro-fibrogenic pathways induced by lipopolysaccharide (LPS) and TGF-β₁ in HSCs.

Methods: The in vivo effect of XYXD on fibrosis progression was assessed in mice model induced by carbon tetrachloride (CCl₄), The in vitro effect of XYXD on mice GFP-Col-HSC cells was evaluated using LPS and TGF-β₁ stimulation.

Results: XYXD treatment reduced CCl₄-induced liver fibrosis and decreased hepatic hydroxyproline (Hyp) content, the mRNA levels of smooth muscle actin (α-SMA) and Col1(α1) in fibrotic liver. XYXD suppressed nuclear factor-κB (NF-κB) activation induced by LPS and TGF-β₁ assessed by using NF-κB-luciferase reporter. The expression of NF-κB target genes, chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-X-C motif) ligand 2 (CXCL2) induced by LPS was suppressed after XYXD treatment. The expression of TGF-β₁ targets genes, Col1(α1) and tissue inhibitor of metalloproteinases (TIMP1) induced by TGF-β₁ was inhibit after XYXD treatment.

Conclusion: XYXD treatment attenuates liver fibrosis by inhibiting HSC activation via inhibition of NF-κB and TGF-β₁ signaling pathway, thereby blocking synthesis of Col1(α1) and TIMP-1. These findings from present study suggest that XYXD may be a therapeutic decoction for liver fibrosis in which NF-κB and TGF-β₁ are thought to take part.

Keywords: Xia-yu-xue decoction, hepatic stellate cells, NF-κB, TGF-β₁

Background

Liver fibrosis, defined by redundant deposition of extracellular matrix (ECM) and resultant loss of soft and liver function, is the result of wound-healing responses stimulated by various liver injury [1, 2]. In response to liver injury, quiescent hepatic stellate cells (HSCs) are activated and develop myofibroblast-like phenotype that expresses profibrogenic genes [3].

HSC activation, the most important event in liver fibrosis, is mediated by many inflammatory and fibrogenic cytokines released from the damaged hepatocytes, circulating system or from Kupffer cells (KCs). The events subsequent to HSC activation, including the augmented production of collagen, are crucial for the hepatic fibrogenesis cascade. Thus, the HSC activation is an appealing target for the development of new antifibrotic drugs [4, 5].

Xia-yu-xue decoction (XYXD) is a classical recipe from Jin Kui Yao Lue (Synopsis of the Golden Chamber) in 200 AD that has a long history in traditional Chinese medicine. XYXD consists of three medicinal herbs, Radix et Rhizoma Rhei (10 g), Semen Persicae (10 g), and Eupolyphaga Seu Steleophaga (6 g). XYXD was used...
widely in clinical for treatment liver fibrosis patients without side effects [6]. It was reported that XYXD could regulate the balance of MMP2,9/TIMP1,2 in response to LPS stimulation in RAW264.7 cells [7] and inhibit KC activation in pig serum induced liver fibrosis in rats [8]. There was reported that XYXD exerts therapeutic effects by inhibiting HSC activation in carbon tetrachloride (CCl4)-induced liver fibrosis in mice [9]. However, scant information is available regarding the antifibrotic mechanism of XYXD action in HSC activation in vitro and in vivo.

Lipopolysaccharide (LPS) level increased in liver fibrosis from portal and systemic circulation owing to changes in the intestinal mucosal permeability [10]. Toll-like receptor 4 (TLR4) signaling pathway is activated upon LPS stimulation, and induces nuclear factor-κB (NF-κB) activation, which leads to the transcription of inflammatory genes, such as chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-X-C motif) ligand 2 (CXCL2) in HSCs. We previously showed that LPS stimulation enhanced the response of HSCs to trans-signaling, thereby attenuating inflammation and profibrogenic growth factor (TGF-β) stimulation enhanced the response of HSCs to trans-signaling, thereby attenuating inflammation and profibrogenic growth factor (TGF-β) signaling in HSCs. Consequently, in the present study, we applied the CCl4 model to examine the antifibrotic effects of XYXD in the mice liver. The anti-fibrotic activities were evaluated by histopathological hepatic hydroxyproline content, and mRNA expression of α-SMA and collagen 1(α1) in vivo. Because the importance of LPS and TGF-β1 in hepatic fibrosis, in vitro we detected the possibility that the anti-fibrotic activities of XYXD might act through the interruption of LPS and TGF-β1 signaling in HSC activation.

Methods

Preparation of XYXD
XYXD consists of crude slices were purchased from Shanghai Huayu Chinese Herbs Co Ltd (China) [12] and from the following ratios of three medicinal herbs: Radix et Rhizoma Rhei 10 g (2 kg, Cat No:140501), Semen Persicae 10 g (2 kg, Cat No:140619), and Eupolyphaga Seu Steleophaga 6 g (1.2 kg, Cat No: 141110), total weight 5.2 kg. The medicines were accredited by a pharmacist. The medicinal mixture was extracted by extracted with 75 % ethanol twice, then infiltration and the resulting ethanol extracts were evaporated and dehydrated under vacuum. The extract powder was weighed (0.585 kg) and used for the experiments by dissolving in pure water or DMEM at the desired concentrations for in vivo and in vitro studies.

Ethics statement

All of the study protocols complied with the current ethical considerations of Shanghai University of Traditional Chinese Medicine’s Animal Ethic Committee and the procedural and ethical guidelines of the Chinese Animal Protection Act, which is in accordance with the National Research Council criteria. All animal experiments and procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai University of Traditional Chinese Medicine and were performed in accordance with the relevant guidelines and regulations.

In vivo CCl4-induced liver fibrosis
Male C57BL/6 mice at 6–8 week (18–20 g) were housed in an air-conditioned room at 25 °C with a 12 h dark/light cycle. The mice received humane care during the study with unlimited access to chow and water. The mice were randomized into two groups: the normal (n = 10) and CCl4-treated group (n = 30). The CCl4-treated mice were treated with 10 % CCl4 (2 mg/kg of body weight i.p.) diluted in corn oil or with corn oil only (normal) for triweekly and distilled water (by gavage) daily. The CCl4-treated mice then divided into CCl4-water (CCl4, n = 20) and CCl4-XYXD (XYXD, n = 10) from the beginning of first CCl4-treatment. At the end of the third week, 10 mice from the CCl4-treated group were sacrificed for the fibrosis development assessment. The XYXD treatment group was exposed to the same level of CCl4 and administered XYXD at a dose of 0.467 g/100 g body weight, which is equivalent to human doses in clinical therapeutics daily for 6 weeks until sacrifice.

Hydroxyproline (Hyp) determination
Hepatic hyp content was used as an indirect measure of tissue collagen content. Hyp from liver tissues (50–100 mg) was determined according to the paper we published previously [13].

In vitro cell culture and treatment

The mouse HSC cell line GFP-Col-HSC was provided by Dr. Ekihiro Seki (School of Medicine, University of California San Diego, CA) and cultured in DMEM with 10 % FBS and 1 % penicillin-streptomycin antibiotics. XYXD was dissolved with vehicle (DMEM). HSCs were serum starved for 12 h, the GFP-Col-HSCs first treated with XYXD (5, 25 μg/ml) for 1 h, the cells then treated with or without LPS (100 ng/ml) or TGF-β1 (10 ng/ml).
Reagents
LPS (Sigma; Escherichia coli serotype 055:B5), recombinant human TGF-β1 (R&D Systems) were used in this study. The antibodies used for the western blot analysis, and are p-JNK (catalog no. sc-81502), JNK (catalog no. sc-7345), p38 (catalog no. sc-398305), p-p38 (catalog no. sc-17852-r), p-Smad2 (catalog no. sc-101801), Smad2 (catalog no. sc-39312), p-Smad3 (catalog no. sc-101154), and Smad3 (catalog no. sc-130218), all purchased from Santa Cruz Biotechnology, Inc. NF-κB inducible reporter plasmid were purchased from InvivoGen (cat no: pnifty2-luc, San Diego, CA). Lipofectamine 2000 transfection reagent was purchased from Invitrogen.

NF-κB luciferase analysis
The GFP-Col-HSC was transfected with the NF-κB inducible reporter plasmid by Lipofectamine 2000 for 12–18 h. The cells were first treated with XXYD (5, 25 μg/ml) for 1 h before treatment with 100 ng/mL LPS or 10 ng/mL TGF-β1. Luciferase activity was measured after 16 h of the treatment with LPS or TGF-β1. Luciferase activity was normalized to the protein concentration of GFP-Col-HSC in each well.

Measurement GFP-Col-HSC activation
The GFP-Col-HSC normal culture and supplement with XXYD (5, 25 μg/ml) for 36 h. The fluorescent signal HSC was then measured by fluorescent microscopy.

Quantitative real-time PCR
Total RNA was extracted using TRIzol (Life Technologies, Grand Island, NY), followed by reverse transcription of total RNA to cDNA. cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster city, CA). cDNA subsequently underwent quantitative real-time polymerase chain reaction (PCR) using the ABI ViiA7™ 7 Dx real-time PCR system (Life Technologies, Grand Island, NY). PCR primer sequences were used were: 18 s rRNA forward 5′-AGTCCC TGCCCTTTGTGCA-3′. 18 s rRNA reverse 5′-CGA TCCGAGGGCCTCACTA-3′. Bambi forward 5′-AGCAG AGCATGAGTAGCTCA-3′. Bambi reverse 5′-CGCC ACGTTGCTTACTTCTT-3′. TIMP1 forward 5′-AAGGG TGGCATGTTGTTCGAT-3′. TIMP1 reverse 5′-GTAAGG GCTTCTGCTGTGCC-3′. CCL2 forward 5′-ATTGGG ATGCTTCTGCTGT-3′. CCL2 reverse 5′-CCTGCTGT TCACAGTTTGC-3′. CXCL2 forward 5′-TCCAGGGTC AGTTACCTGTGC-3′. CXCL2 reverse 5′-CGGTTCAA AAAAGTTTGGCTTG-3′. PPAR-γ forward 5′-AACTCCC TCAAGGTTGCATGTA-3′. PPAR-γ reverse 5′-GCAATT TGAGACATGCCACCC-3′. Col 1α1 forward 5′-TAGGG CATTGCTGTACGAGC-3′. Col 1α1 reverse 5′-ACAT GTTTCAGCTTTTGGACC-3′. α-SMA forward 5′-GTTC AGTGGGTGCTGTCTGCA-3′. α-SMA reverse 5′-ACTG GGAGCAGTGAAAAG-3′. Gene expression was normalized to 18 s RNA as an internal normal.

Western blot
Cell samples were prepared in radio immunoprecipitation lysis buffer containing protease inhibitors. After protein quantification, protein samples at 20 μg/lane were subjected to polyacrylamide gel electrophoresis, and then incubated with antibodies for phospho-JNK, JNK, p38, p38, p-Smad 2, Smad2, p-Smad 3, Smad3 with appropriate secondary horseradish peroxidase (HRP)-conjugated antibodies, and developed. Anti-glyceraldehyde 3-phosphate dehydrogenase monoclonal antibody was purchased from Kangchen and diluted 1:5000 ratio.

Immunohistochemistry
The sections were dewaxed in xylene and dehydrated in alcohol. Antigen retrieval was achieved by microwaving in citric saline, pH 6.0 for 3 min. Thin sections were treated with 3 % hydrogen peroxide for 10 min. The sections were further blocked by 5 % BSA and were then incubated 37 °C with primary antibody against α-SMA (Abcam, UK). The sections were incubated with biotinylated secondary antibody (Boster, Wuhan, China) for 30 min at room temperature. α-SMA expressions were visualized by DAB (Boster, Wuhan, China) staining.

Statistics
Differences between two groups were compared using the two-tailed unpaired student t-test. Differences between multiple groups were compared using one-way ANOVA with a post hoc Dunnett’s test using SPSS 18.0. P values, 0.05 were considered significant. All experiments were performed at least three times and the representative data were presented.

Results
Inhibition of CCl4-induced liver fibrosis by XXYD
CCl4 is known to induce toxicity in the liver by producing highly reactive metabolites, which severely damage hepatocytes and subsequent fibrosis [14]. As shown in Fig 1a, livers of normal mice showed normal lobular architecture with central vein and radiating hepatic cords. After 3 weeks of CCl4 treatment, liver centrilobular necrosis, deposition of lipid droplets in hepatocytes, and inflammatory cells infiltration were observed. After 6 weeks CCl4 treatment, liver sections revealed collagen deposition, severe fatty changes, whereas, concomitant treatments of XXYD significantly inhibited CCl4-induced hepatic damage, as indicated by decreases in hepatocytes degeneration, inflammation, and collagen deposition (Fig 1a).

Sirius red staining revealed that mice treated with CCl4 for 3 weeks showed prominent red staining in collagen
was seen to stretch from portal area to lobular (Fig. 1b). Livers showed marked distortion in architecture, including portal and lobular bridging fibrosis, cirrhotic nodule formation. Collagen fiber percentages in the CCl₄ groups were significantly decreased in XYXD treated mouse livers. HSC activation was inhibited by XYXD in vivo
As sustained deposition of ECM results mainly from HSC activation, α-SMA is a marker of HSCs in hepatic fibrosis [15], and the α-SMA-positive cells are increased gradually in number, mainly located in fibrotic septa following 6 week CCl₄ treatment. In contrast, a marked reduction of α-SMA-positive HSCs was observed in XYXD liver compared with 6 weeks CCl₄ liver (6 wk) (Fig 2a).

The expression of α-SMA in CCl₄-treated liver samples was also detected by real-time PCR analyses (Fig 2b). The expression of α-SMA and Col 1(α1) increased gradually following CCl₄ treatment (Fig 2b and c). Compared to 6 weeks CCl₄ treatment liver (6 wk), XYXD administration resulted in marked reductions in α-SMA and Col 1(α1) (P < 0.05). Hepatic hyp content increased in CCl₄-treated mice gradually, after 3-week CCl₄ administration, the Hyp content was 206 % of that in the normal group (P < 0.05) (Fig 2d). XYXD was found to decrease liver Hyp content significantly (P < 0.01).

The effect of XYXD on HSCs activation in vitro
To reveal the mechanisms responsible for these in vivo observations, we performed in vitro studies using GFP- Col-HSC cells, a well-characterized mouse HSC cell line. First, we tested the cytotoxicity of XYXD, as assessed by cell viability. MTT assay showed no significant difference between normal and XYXD treated cells at concentrations 25 μg/ml (data not shown here). Therefore, we used 25 μg/ml of XYXD in subsequent experiments.

Effects of XYXD on LPS signaling in HSC
We examine whether XYXD inhibits the LPS signaling in HSC. It has been reported that NF-κB activation in HSC is associated with sustained liver inflammation [16]. We investigated the effect of XYXD on NF-κB activity in HSCs. The luciferase array analysis showed that the relative luciferase activity increase significantly 4-fold (P < 0.01) with LPS treatment (Fig. 3a) in HSCs. In contrast, treatments at 5 and 25 μg/ml one hour prior to LPS treatment significantly suppressed the LPS-induced NF-κB activation (P < 0.05 or 0.01) (Fig 3a). In addition to NF-κB, JNK and MAPK are also activated by LPS in HSC. We therefore examined the effect of XYXD on JNK and MAPK activation. JNK and p38 were quickly phosphorylated in HSC in response to LPS stimulation (Fig 3b). The LPS-mediated JNK and MAPK activation was reduced by XYXD treatment (Fig 3b). We previously reported that Bambi decreased in response to LPS stimulation [11], as expected, LPS treatment increased TIMP1 and decreased Bambi in GFP-Col-HSC cells (Fig 3c) (P < 0.05 or 0.01). The XYXD significantly reduced the expression of LPS-induced TIMP1 in HSC (P < 0.05 or 0.01). XYXD could inhibit Bambi decrease induced by LPS stimulation (Fig 3c) (P < 0.05 or 0.01).
Because NF-κB induces an inflammatory response in the liver, we investigated whether XYXD can suppress the induction of inflammatory cytokines in HSC. The pro-inflammatory cytokines of CCL2 and CXCL2 were up-regulated after LPS stimulation (Fig 3d) \((P < 0.01)\). The mRNA expression of CCL2 and CXCL2 was significantly inhibited by XYXD treatment (Fig 3d) \((P < 0.05\) or \(0.01)\). These results demonstrated that LPS-induced signaling was inhibited by XYXD in HSC.

**Effect of XYXD on TGF-β1 signaling in HSC**

TGF-β1 is a classic activator of HSCs and a key mediator in the pathogenesis of liver fibrosis [17]. However, it was rarely reported NF-κB activated in response to TGF-β1 stimulation in HSCs. We assessed NF-κB activity by using the NF-κB luciferase reporter system. TGF-β1 treatment significantly increased NF-κB activity \((P < 0.01)\) in HSCs (Fig 4a) \((P < 0.05\) or \(0.01)\). XYXD treatment 1 h prior to TGF-β1 treatment significantly suppressed the TGF-β1-induced NF-κB activation \((P < 0.05)\).

The TGF-β1-mediated signaling pathway depends on the phosphorylation of Smad 2/3. As shown in Fig 4b, the protein levels of Smad 2/3 were analyzed. Western blot analysis detected increases in the phosphorylation of Smad 2/3 by TGF-β1, and the inhibition of these increases by XYXD (Fig 4b). Also, the mRNA expression of Col1 (α1) and TIMP1 significantly increased in response to TGF-β1 stimulation \((P < 0.01)\). XYXD treatment suppress Col1 (α1) and TIMP1 mRNA expression (Fig 4c) \((P < 0.05\) or \(0.01)\). These results indicated that XYXD inhibited TGF-β1-induced HSC activation.

**Effect of XYXD on fibrogenic response induced by LPS plus TGF-β1 in HSC**

TGF-β1 treatment increased Col 1(α1) mRNA expression in GFP-Col-HSC cells (Fig. 5), LPS treatment further increased Col 1(α1) mRNA expression in GFP-Col-HSC cells (Fig. 5a) \((P < 0.05\) or \(0.01)\). While, XYXD treatment resulted in dose-dependent decrease in collagen synthesis in GFP-Col-HSC cells \((P < 0.05\) or \(0.01)\). Increased TIMP-1 expression was inhibited by XYXD, with a significantly reduction at the 5 and 25 μg/ml dose level in GFP-Col-HSC cells (Fig 5b) \((P < 0.05\) or \(0.01)\).

**Effect of XYXD on full activated HSC**

As shown in Fig 6a, GFP-Col-HSC cell full activated after 36 h culture by measuring GFP fluorescent signal. XYXD treatments at 5 μg/ml and 25 μg/ml suppressed GFP-Col-HSC activation (Fig 6a). The mRNA expression of α-SMA and TIMP1 was decreased significantly by XYXD treatment (Fig 6b and c) \((P < 0.05\) or \(0.01)\). Meanwhile, XYXD increased PPARγ mRNA level compared with vehicle group (Fig 6d) \((P < 0.01)\). These results showed that GFP-Col-HSC auto-activated after 36 h culture, and XYXD could inhibit GFP-Col-HSC activation.

**Discussion**

Xia-yu-xue decoction (XYXD) has used in China for more than 2 thousand years without side effects.
However, the anti-fibrotic mechanism of action of XYXD was very limited. In an effort to investigate the inhibitory effect of XYXD on HSC activation, we used (1) CCl4-induced liver fibrosis in mice in vivo, and (2) an in vitro model based on GFP-Col-HSC cells treated with or without LPS, TGF-β1 or both. The data demonstrated that XYXD treatment inhibited the accumulation of ECM components in CCl4-induced liver fibrosis in vivo. XYXD is capable of inhibiting HSC cellular activated by LPS and TGF-β1 in GFP-Col-HSC lines.

Chronic liver disease commonly leads to liver fibrosis, resulting in development of liver cirrhosis, organ failure, and eventually liver related mortality. Therefore, prevention or treatment of liver fibrosis is the main target in patients with chronic hepatic disorders [18]. Recently, much interest in herbal medicine has been focused on hepato-protective or anti-fibrotic effects. Although lack of strong clinical evidence, many traditional Chinese medicine/recipes/decotions and drugs such as Yin-chen-hao decoction [13], Xiao-chai-hu decoction (sho-saiko-to in Japan) [19, 20] are used widely in China, Korea, and Japan for thousands of years and reported to have antifibrotic properties. Just like Yin-chen-hao decoction (Inchinko-to in Japan) and Xiao-chai-hu decoction (sho-saiko-to, in Japan), Xia-yu-xue decoction first described in Shanghan Lun, while, there were rare lim-ited information about the anti-fibrotic effects of XYXD. So it is very urgent to investigate the mechanism of action of XYXD. In this study, we found XYXD inhibit α-SMA and Col 1(α1) expression, which indicating HSC...
activation were suppressed in CCl₄-induced liver fibrosis in vivo.

LPS levels increase in the systemic circulation owing to changes in the intestinal mucosal permeability after liver injury [21, 22]. LPS plays a key role in hepatic fibrogenesis by enhancing HSC activation [23]. NF-κB activated in response to LPS-mediated TLR4 activation [24]. So we want to know whether XYXD inhibit LPS signaling through NF-κB. In the present study, NF-κB luciferase increased in response to LPS stimulation and was inhibited significantly by XYXD treatment. Our study also demonstrated that XYXD suppressed both JNK and p38 signaling pathways induced by LPS. Furthermore, we approved that the mRNA expression of CCL2 and CXCL2 was also suppressed by pretreatment with XYXD.

In response to liver injury, HSCs undergo activation process and produce ECM [25]. The process is primed by various growth factors, where TGF-β1 is the most
important profibrogenic mediator. It was well reported the pro-inflammatory cytokine through NF-κB enhance TGF-β1 signaling [1, 23]. However, whether TGF-β1 could induce NF-κB activation was largely unknown. Our results showed that NF-κB activity increased almost 2-fold in response to TGF-β1 stimulation, and XYXD could inhibit NF-κB activity induced by TGF-β1. Moreover, the signaling pathway activated by TGF-β1 involves phosphorylations of Smad 2 and Smad 3 [26, 27], which were also inhibited by XYXD. These data suggest that XYXD blocks fibrogenesis as mediated by TGF-β1 signaling pathways.

We used LPS plus TGF-β1 to mimics the complex environment in vivo. The mRNA expression of Col 1(α1) and TIMP1 increased significantly using LPS plus TGF-β1 stimulation compared with TGF-β1 alone in GFP-Col-HSC cell line. However, XYXD treatment decreased the enhancement of TGF-β1 plus LPS-induced Col 1(α1) and TIMP1 mRNA expression. This may be due to the inhibitory effects of XYXD on NF-κB and TGF-β1 signaling, and the molecular mechanism should be studies in future research.

Conclusions
This study demonstrated that XYXD reduce HSC activation in CCL4-induced liver fibrosis in mice. The inhibitory effects of XYXD on HSC activation may be caused, at least in part, by suppressing on NF-κB and TGF-β1 signaling pathway.

Abbreviations
α-SMA: Smooth muscle actin alpha; CCL4: Carbon tetrachloride; CCL2: Chemokine (C-C motif) ligand 2; CXCL2: Chemokine (C-X-C motif) ligand 2; HSC: Hepatic stellate cell; Hyp: Hydroxyproline; LPS: Lipopolysaccharide; NF-κB: Nuclear factor-κB; TGF-β1: Transforming growth factor; TIMP1: Tissue inhibitor of metalloproteinases; XYXD: Xia-yu-xue decoction.

Competing interests
The authors declare that they have no competing interests

Authors’ contributions
Conceived and designed the experiments CL, YX, DX, Performed the revert experiments: CL, LT, ZC, QD, and XS. Analyzed the data: CL. Contributed reagents/materials /analysis tools: ZC. Wrote the paper: CL. All authors read and approved the final manuscript.

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References

1. Seki E, De Micinis S, Sterreicher CH, Kuwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-β1 signaling and hepatic fibrosis. Nat Med. 2007;13(11):1324–32.

2. Chen L, Li J, Zhang J, Dai C, Liu X, Wang J, Gao Z, Guo H, Wang R, Lu S et al. S100A4 promotes liver fibrosis via activation of hepatic stellate cells. J Hepatol. 2014;60(1):55–64.

3. Wang Y, Gao J, Zhang D, Zhang J, Ma J, Jiang H. New insights into the antifibrotic effects of sorafenib on hepatic stellate cells and liver fibrosis. J Hepatol. 2010;53(1):53–64.

4. Xu WH, Hu HG, Tian Y, Wang SZ, Lu J, Li JZ, et al. Bioactive compound reveals a novel function for ribosomal protein S5 in hepatic stellate cell activation and hepatic fibrosis. Hepatology. 2014;60(2):648–60.

5. Chiu YS, Wei CC, Lin YJ, Hsu YH, Chang MS. IL-20 and IL-20R1 antibodies protect against liver fibrosis. Hepatology. 2014;60(3):1003–14.

6. Dai K. Jiang Chunhua uses the experience of Xiayuxue Decoction. Shanxi J Pharmcologiaeque Shanghai. 2010;24(3):56.

7. Zhang Y, Du GL, Chen DX, Han L. Regulative Effect of Drug Serum of Xiayuxue Decoction on MMP1,2/TIMP1,2 Protein Expression in RAW264.7 Cells Stimulated by LPS. Acta Universities Traditionis of Medicalis Sinesis Pharmcologiaeque Shanghai. 2010;24(3):56–9.

8. Chen S, Du G, Lu Y, Tao Y, Chen D. Effect of Xiayuxue decoction and comontal prescription on the expression of COL1α1 and TIMP1 mRNA in rats with immunological hepatic fibrosis: a comparative study. Acta Universities Traditionis of Medicalis Sinesis Pharmcologiaeque Shanghai. 2012;26(3):182–5.

9. Zhang L, Sun M, Ning B, Zhang W, Chen G, Mu Y, et al. Xiayuxue Decoction attenuates hepatic stellate cell activation and sinusoidal endothelium defenestration in CCl4-induced fibrotic liver of mice. Chin J Integr Med. 2014;20(7):516–23.

10. Bai T, Lian L, Wu Y, Wan Y, Nan J. Thymoquinone attenuates liver fibrosis via PK3 and TLR4 signaling pathways in activated hepatic stellate cells. Int Immunopharmacol. 2013;13(5):275–81.

11. Liu C, Chen X, Yang L, Kisseleva T, Brenner DA, Seki E. Transcriptional Repression of the TGF-β1 Pseudoreceptor BAMBI by NF-κB p50 Enhances TGF-β1 Signaling in Hepatic Stellate Cells. J Biol Chem. 2014;289(7):4060–7.

12. Liu C, Sun M, Wang L, Wang G, Chen G, Liu C, et al. Effects of Yinchenhao Tang and related decoctions on DMN-induced cirrhosis/fibrosis in rats. Chin J Integr Med. 2008;14(1):1.

13. Liu C, Sun M, Yan X, Han L, Zhang Y, Liu C, et al. Inhibition of hepatic stellate cell activation following Yinchenhao decoction administration to dimethyltartaric acid-treated rats. J Ethnopharmacol. 2007;112(3):1406–9.

14. Ji L, Xue R, Tang R, Wu W, Hu T, Liu X, et al. Toll-like receptor 2 knock-out attenuates carbon tetrachloride (CCl4)-induced liver fibrosis by downregulating MAPK and NF-κB signaling pathways. Metabolism. 2014;63(12):2095–100.

15. Friedman SL. Closing in on the signals of hepatic fibrosis. Gastroenterology. 1997;112(4):1406–9.

16. Xu T, Pan Z, Dong M, Yu C, Niu Y. Fenolic acid suppresses activation of hepatic stellate cells through ERK1/2 and Smad signaling pathways in vitro. Biochem Pharmacol. 2015;93(1):49–58.

17. Lee J, Jang EJ, Seo HL, Ku SK, Lee JR, Shin SS, et al. Sauchinone attenuates liver fibrosis and hepatic stellate cell activation through TGF-β1/Smad signaling pathway. Chem-Biol Interact. 2014;224(1):67–75.

18. Wang L, Liu C, Sun M, Liu C, et al. The antifibrotic effects of sorafenib on hepatic stellate cells through ERK1/2 and Smad signaling pathways. J Hepatol. 2014;60(1):55–64.

19. Dong N, Topcic T, Sutramaniam N, Sherman MH, Wilson C, Rao R, et al. The Bcl-2 protein/reaper/SMAD Genomic Circuit Gates Hepatic Fibrotic Response. Gastro. 2013;135(3):601–13.

20. Yamashita H, Hirakawa K, Kikuma T, Terai S, Yamakami T, Okita K. Herbal medicine Shao-saiko-to (TJ-9) increases expression matrix metalloproteinases (MMPs) with reduced expression of tissue inhibitor of metalloproteinases (TIMPs) in rat stellate cells. Life Sci. 2004;74(18):2251–63.

21. Chen M, Chen J, Tsai C, Wang W, Chang D, Yu D, et al. The role of TGF-β1 and cytokines in the modulation of liver fibrosis by Sho-saiko-to in rat's bile duct ligated model. J Ethnopharmacol. 2005;97(1):7–13.

22. Shi H, Dong L, Jiang J, Zhao J, Zhao G, Dang X, et al. Chlorogenic acid reduces liver inflammation and fibrosis through inhibition of toll-like receptor 4 signaling pathway. Toxicology. 2013;303:107–14.

23. Schwabe RF, Seki E, Brenner DA. Toll-Like Receptor Signaling in the Liver. Gastroenterology. 2006;130(6):1886–900.

24. Li L, Xue R, Tang R, Wu W, Hu T, Liu X, et al. Toll like receptor 2 knock-out attenuates carbon tetrachloride (CC4)-induced liver fibrosis by downregulating MAPK and NF-κB signaling pathways. Febb Lett. 2014;588(12):2095–100.

25. Friedman SL. Closing in on the signals of hepatic fibrosis. Gastroenterology. 1997;112(4):1406–9.

26. Xu T, Pan Z, Dong M, Yu C, Niu Y. Fenolic acid suppresses activation of hepatic stellate cells through ERK1/2 and Smad signaling pathways in vitro. Biochem Pharmacol. 2015;93(1):49–58.

27. Lee J, Jang EJ, Seo HL, Ku SK, Lee JR, Shin SS, et al. Sauchinone attenuates liver fibrosis and hepatic stellate cell activation through TGF-β1/Smad signaling pathway. Chem-Biol Interact. 2014;224(1):67–75.

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