The extract of a Traditional Chinese Medicine alleviates Hepatic Fibrosis of Rats by inhibiting NF-κB activation and upregulating BAMBI in Hepatic Stellate Cells

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Research

Keywords: BAMBI, DaHuangZheChong pill, hepatic fibrosis, LPS, NF-κB, HSCs

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Abstract

Background

DaHuangZheChong pill (DHZCP) is a formula of traditional Chinese medicine, which has been written into the guideline for the prevention and treatment of hepatic fibrosis in China. The study was aim to investigate the anti-fibrotic effects and the potential mechanisms of DHZCP revolving around the TGF-β pseudo receptor, bone morphogenic protein and activin membrane-bound inhibitor (BAMBI) in hepatic stellate cells (HSCs).

Materials and Methods

Wistar rats were given with CCL4 for four weeks to establish hepatic fibrosis model. Then the rats were given normal saline or DHZCP decoction six weeks. the pathology of liver tissue was analyzed, the expression of Toll-like receptor 4 (TLR4), myeloid differentiation factor 88 (MyD88), BAMBI, and NF-κB were detected. In vitro, the associated signal molecules about LPS-activated NF-κB were also analyzed by immunohistochemistry, western blot, or electrophoretic mobility shift assay (EMSA) in cultured HSC-T6 cells.

Results

The DHZCP showed significant effects on improving fibrosis stage of liver tissue and inhibiting primary HSCs activation. The protein expression of TLR4/MyD88 was lower (P was both < 0.05), BAMBI was higher in DHZCP group than model control (MC) group (P< 0.05) in primary HSCs. In HSC-T6 cells, the activity of NF-κB was lower (P< 0.001), and BAMBI was higher (P< 0.05) in DHZCP added LPS group than in LPS group.

Conclusion

These results suggested that DHZCP alleviates hepatic fibrosis that was maybe associated with inhibiting activation of NF-κB induced by LPS, and upregulating BAMBI expression in HSCs.

Background

Chronic liver disease is often accompanied by persistent liver damage such as chronic viral infections, widespread alcohol abuse, fatty liver diseases and cholestasis. The liver injuries contribute to a wound-healing response, excessive deposition of extracellular matrix (ECM) and reconstruction of tissue structure resulting in liver fibrosis[1].
Hepatic stellate cells (HSCs) were essential to the occurrence and development of liver fibrosis. Transforming growth factor-β (TGF-β) was a powerful fibrogenic cytokine and HSCs activator \[2\]. Lipopolysaccharide (LPS), an exogenous Toll-like receptor 4 (TLR4) ligand, activated the TLR4-myeloid differentiation factor 88 (MyD88)-nuclear factor-κB (NF-κB) signal in HSCs, and led to the decrease of TGF-β pseudo receptor bone morphogenic protein and activin membrane-bound inhibitor (BAMBI) in HSCs \[3, 4\], which made HSCs become more sensitive to TGF-β, that is the main mechanism of endotoxin-induced liver fibrosis.

Hepatic fibrosis alleviation and cirrhosis degradation have been reported in recent years \[5, 6\], clinical and experimental evidence also revealed hepatic liver could be reversible \[7, 8\]. Anti-fibrosis has become an indispensable step in the development of chronic liver disease. Chinese herbal medicine has the pharmacological characteristics of multi-pathway, multi-level and multi-target to alleviate hepatic fibrosis \[9, 10\]. DaHuangZheChong pill (DHZCP) was a classic recipe in traditional Chinese medicine (TCM), and was officially approved and recommended for treat liver fibrosis \[11\]. Many clinical and experimental studies had confirmed the effect on hepatic fibrosis \[12, 13\], and definitied that the anti-fibrotic mechanism was about inhibiting the activation of HSCs \[14\]. However, it was not enough to elucidate the mechanism for treating hepatic fibrosis.

We hypothesized that the possible anti-fibrosis mechanism of DHZCP was to suppress the activation of NF-κB by LPS, and increase the expression of BAMBI in HSCs.

**Materials And Methods**

**Drug preparation**

DHZCP includes Shudahuang (Chinese rhubarb), Tubiechong (Steleophaga), Shuizhi (Aulastomum gulo), Mengchong (Tabanus), Qiao (grub), Ganqi (Dried Lacquer), Taoren (peach kernel), Kuxingren (Semen Armeniacae Amaranum), Huangqin (Scutellaria baicalensis), Dihuang (Rhizome rehmanniae), Baishao (Paeoniae Alba Radix), Gancao (Radix Glycyrrhizae), the dose ratio was 3:0.3:0.6:0.45:0.45:0.3:1.2:1.2:0.6:0.3:1.2:0.9. All the herbs were purchased from Guangxi Liuzhou baicaotang Pharmaceutical Co. Ltd., batch number: gui 20160152 (Liuzhou, China). The crude drugs were crushed into coarse powder and immersed in 10 times amount of water 1h, and then boiled 2h after adding water in a total of 3 times according to clinical treatment. After the combination of decoction, the total extract was obtained by decompressing, concentrate and cryodesiccate in ultrasonic extract, It was dissolved in normal saline when used.

**Reagents**

Identification immunocytochemical reagents on primary HSCs: α-SMA (no. ab108424) were from Abcam, USA. HRP-labeled secondary antibody (no.ab7090) were from Abcam, USA. Western blot reagents on primary HSCs: TLR4 (no. ab13556), MyD88 (no. ab2068), BAMBI (no. ab203070), GAPDH (ab9485) were from Abcam, USA; goat anti-rabbit IgG (no. 41155) was from sigma, USA. Immunocytochemical and
western blot reagents on HSC-T6 cells: TLR4 (no. ab83444), MyD88 (no. ab131071), BAMBI (no. ab203070), GAPDH (no. ab9485) were from Abcam, USA; goat anti-rabbit IgG (no. 41155) was from sigma, USA. HRP-labeled secondary antibody (no.ab7090) were from Abcam, USA.

Animal experiments

Wistar rats, weighing 250-300g, provided by Beijing Hua Fukang Biological Technology Co., Ltd. The experiment was divided into three groups, 8 rats in each group: normal control (NC) group, model control (MC) group, and DHZCP group. The rats in normal control group were subcutaneously injected with normal saline, the rats in model control and DHZCP group were subcutaneously injected with 40% CCl₄ solution (5 ml/kg) at the first time, then were subcutaneously injected with 40% CCl₄ solution (3 ml/kg) every 3 days for 4 weeks to establish the hepatic fibrosis model[15]. At the end of the 4th week, two rats in each group were randomly executed to confirmed the formation of hepatic fibrosis by pathological examination. The rats in DHZCP group were given intragastric administration 3.3 g/kg DHZCP from the fifth week six weeks. The rats in NC and MC groups were given intragastric administration equivalent volumes of normal saline. By the end of the experiment, no rat death occurred in each group. The experiment were approved by the Ruikang Hospital Ethical Committee.

Liver tissues were routinely excised, then washed, dehydrated, embedded in paraffin, and sliced. The slices were stained with hematoxylineosin (H&E). A Ishak scoring system was applcated to evaluate the liver fibrosis[16].

In order to isolate primary HSCs for western blot, the liver were perfused with 0.05% enzyme protease and 0.025% collagenase, re-digested with DNase for 45 min. Centrifugation of liver tissue was performed to remove hepatocytes and to get hepatic Non-parenchymal cells (NPC). Hepatic NPC solution was added with 70% and 30% diluted Percoll cell separation solution. After centrifugation, HSCs are located between hepatic NPC solution and 30% Percoll solution, and Kupffer cells are located between 30% Percoll solution and 70% Percoll solution. Primary HSCs were identified by immunocytochemical staining of α-SMA. Four fields were selected in each slice to evaluate the average optical density by Image-Pro Plus 6.0(Media Cybernetics, Rockville, USA).

Preparation of DHZCP containing serum

Wistar rats, in drug-containing serum group were intragastrically administered with 3.3 g/kg DHZCP for 7 days, and rats in normal serum group were given equivalent volumes of normal saline. Blood was collected 1h after the last Administration from the rats abdominal aorta and placed in a 10ml centrifuge tube. After the blood clot is well contracted, the serum was aseptically separated at 3000 r/min for 15 min, then the serum was inactivated at 55°C for 30 min and stored at −20°C for use.

HSC-T6 cells culture and cell grouping
The HSC-T6 cells were provided by the cell bank of the Chinese Academy of Sciences in Shanghai. HSC-T6 cells were cultured in culture media containing 10% high-quality fetal bovine serum and 1% penicillin-streptavidin. HSC-T6 cells were subcultured when confluent at a 1:3 split ratio, and cultured at 37°C, 5% CO₂ and saturated humidified incubator.

HSC-T6 cells were randomly divided into NC group, DHZCP group, LPS group, and DHZCP+LPS group. The NC group were cultured regularly for 48 h; the DHZCP group were given 20% DHZCP containing serum for 48 h; the LPS group were cultured for 44 h at first, then were given 100 ng/ml LPS for 4 h; the DHZCP+LPS group were given 20% DHZCP containing serum for 44 h at first, and then were given 100 ng/ml LPS for 4 h. The experiment was repeated 2 times, each group had three samples.

**Western blot analysis**

Primary HSCs and HSC-T6 cells were collected and subjected to electrophoresis (Bio-Rad, Hercules, USA). Then the cells were transferred to a membrane (Bio-Rad, Hercules, USA) from gel, blocked, and incubated with a diluted primary antibody 90 min, and incubated with secondary antibody 90 min. Membranes were exposed with chemiluminescence (PIERCE, Holmdel, USA). The relative density of each protein band is the ratio to GAPDH.

**Immunohistochemical analysis**

HSC-T6 cells of all groups were prepared. Slices were treated with 3% H₂O₂ to inhibit the endogenous peroxidase activity, then were blocked with 10% goat serum. Primary antibodies targeting TLR4, MyD88 and BAMBI (dilution 1:400) were applied. After phosphate buffered saline (PBS) poaching, the secondary antibody and peroxidase were used. The activity of peroxidase was shown with diaminobenzidine. Then the slices were stained with hematoxylin. Four fields were selected in each slice to evaluate by semi-quantitative integration method[17]. The semi-quantitatively scores is the sum of the integral of the tinctorial strength and the integral of proportion of positive cells. The proportion of positive cells is < 5% for 0, 5% to 25% for 1 point, 26% to 50% for 2 points, 51% to 75% for 3 points, and > 75% for 4 points. Integral of the tinctorial strength: 0 is for colorless, 1 point is for light yellow, 2 points is for brown, and 3 points is for tan.

**Electrophoretic mobility shift assay (EMSA) analysis**

The nuclear protein components extracted from HSC-T6 cells and the light shift Chemiluminescent EMSA Kit (PIERCE, Holmdel, USA) was used for gel migration assay. The probe was synthesized and purified by Shanghai Bioengineering Co., Ltd. The sequence of NF-κB probe were: 5’- AGT TGA GGG GAC TTT CCC AGG C -3’, and 3’- TCA ACT CCC CTG AAA GGG TCC G-5’.

**Statistical analysis**
Data analysis were performed by SPSS Version 21.0 (SPSS Inc., Chicago, IL, USA). \( P < 0.05 \) was regarded as significant.

**Results**

**DHZCP alleviated the liver fibrosis in rats**

hepatic lobule structures was clear and hepatic cell cords were arranged radically around the central veins in NC group; severe macrosteatosis, numerous complete pseudo lobules and fibrous septa were observed in MC group. In comparison, few pseudo lobules and fibrous tissue were found in DHZCP group (Figure 1). The hepatic fibrosis in NC group were all in S0 phase which had a significant difference with MC group (\( P < 0.05 \)). The hepatic fibrosis phases of DHZCP group mainly distributed in the S1-S2 phases, were better than the MC group in the S3 phase (\( P < 0.05 \), Table 1).

**DHZHP inhibited primary HSCs activation in rats**

The main characteristics of HSCs activation are proliferation and myofibroblastic transformation. The marker of activated HSCs is \( \alpha \)-SMA, positive staining of \( \alpha \)-SMA was visible in each group, but much deeper positive staining of \( \alpha \)-SMA was observed in MC group. However, weaker staining was observed in the DHZCP group compared with MC group (Figures 2). The average optical density of \( \alpha \)-SMA in each group was obtained by image analysis and statistical analysis. The average optical density of \( \alpha \)-SMA in NC group, MC group and DHZCP group were 29.69 ± 8.23, 57.73 ± 25.48, 35.37± 16.08. The average optical density of \( \alpha \)-SMA in MC group was higher than that of NC group (\( P < 0.05 \)), the average optical density of \( \alpha \)-SMA in DHZCP group was lower than that MC group (\( P < 0.05 \)).

**The effect of DHZCP on associated molecular of NF-\( \kappa \)B activation in primary HSCs**

Primary HSCs were isolated from liver tissue of rats in different groups, to investigate the effect of DHZCP on associated molecular of NF-\( \kappa \)B activation in primary HSCs, signal molecules expression were detected by Western blot and EMSA. The protein expression of TLR4 and MyD88 were up-regulated (\( P < 0.05 \), Figure 3A and 3B), and BAMBI was down-regulated in the MC group compared with NC group (\( P < 0.05 \); the TLR4 and MyD88 were down-regulated, and BAMBI was up-regulated significantly in the DHZCP group than the MC group (\( P < 0.05 \)). The activity of NF-\( \kappa \)B increased significant in MC group compared with another two groups (\( P \) was both < 0.001), but decreased in DHZCP group compared with MC group (\( P < 0.001 \), Figure 3C and 3D).

**DHZCP inhibited the expression of TLR4/MYD88, BAMBI stimulated by LPS in HSC-T6 cells.**

Immunohistochemical analysis were used in this part study. Compared with the DHZCP group, the expression of TLR4 and MyD88 cells were higher, but the expression of BAMBI was lower in the LPS group; compared with the LPS group, TLR4 and MyD88 was lower, BAMBI was increased (Figure 4A). The semi-quantitative scores of TLR4 and MyD88 were increased (\( P \) was both < 0.05), BAMBI was decreased in LPS group compared with NC group (\( P < 0.05 \); TLR4 and MyD88 were decreased and BAMBI was
increased in DHZCP+LPS group compared with LPS group ($P$ was both $< 0.05$, Figure 4 B). TLR4, MyD88 and BAMBI showed similar results with Immunohistochemical analysis by Western blot in HSCs-T6 cells (Figure 5A and 5B).

**DHZCP inhibited the activity of NF-κB induced by LPS in HSC-T6 cells.**

The activity of NF-κB was significantly higher in LPS group compared to other groups ($P$ was all $< 0.001$); after DHZCP containing serum intervention, NF-κB was decreased significantly ($P < 0.001$, Figure 6).

**Discussion**

BAMBI was a TGF-β pseudoreceptor, and down-regulation of BAMBI made HSCs become more sensitive to TGF-β[18], that was the main mechanism of endotoxin-induced hepatic fibrosis [19, 20]. HSCs was the naïve cells that responds to LPS by TLR4[21], BAMBI was down-regulated after TLR4-MyD88-NF-κB pathway was activated in HSCs[22].

DHZCP is a TCM ancient formula from “Jin Kui Yao Lue”, authorized by Zhang Zhongjing, The components in DHZCP had the effect of promoting blood circulation to removing blood stasis and strengthening the body resistance, which in accordance with the clinical pathogenesis of hepatic fibrosis[23, 24]. In our previous study, we used CCl4 to induced liver fibrosis model and found that DHZCP could alleviate liver fibrosis and improve the level of serum markers of liver function (alanine aminotransferase, aspartate aminotransferase, TGF-β1 and LPS)[25]. In this study, the effects of DHZCP on the histological changes in liver also revealed that DHZCP had significant effects on improving the liver tissue morphology and ameliorated hepatic fibrosis in rats.

Some studys have showed DHZCP regulated the level of inflammatory factors, reduced the deposit of collagen I, and further inhibited the apoptosis of hepatocytes [26]; DHZCP regulated the expression of metalloproteinases1 mRNA and increased the expression of matrixmetalloproteinase1 mRNA in rats with liver fibrosis [27]. However, whether the DHZCP interfered with LPS-activated NF-κB signal pathway was not clear.

HSCs were very essential to the generate and development of hepatic fibrosis. Persistent inflammatory stimulation resulted in the transition of HSCs into a myofibroblast, the expression of α-SMA is one of the main characteristics of its activation[28]. As shown in Fig. 2, the expression of α-SMA was smaller in liver slices of normal rats, but increased in model group. Administration of DHZCP resulted in decrease in α-SMA expression.

Activated HSCs expresses TLR4 and had a higher response to LPS[21]. LPS was a definitive ligand of TLR4, it activated HSCs through the MyD88-NF-κB pathway. In our study, the expression of TLR4 and MyD88 in HSCs was increased, the expression of BAMBI was decreased in rats with hepatic fibrosis, and the activity of NF-κB was significantly higher. Further more TLR4 and MyD88 and the activity of NF-κB was inhibited, and the BAMBI was promoted after DHZCP treat.
We then performed experiment on HSC-T6 cells by use of DHZCP drug-containing serum. This method reflected the actual effect of drugs in vivo and was be used widely[29]. In our study, we found that there was no significant difference in the protein expression of TLR4, MyD88, NF-κB and BAMBI between the NC group and DHZCP group, suggesting that DHZCP may not directly target at HSCs. BAMBI was a TGF-β pseudoreceptor that silences TGF-β signaling[30], only one LPS-related gene significantly down-regulated in 121 gene in HSCs regulated by LPS[3]. In the present study, LPS induced HSC-T6 cells to express TLR4, activated the inflammatory signal pathway, enhanced the activity of NF-κB, and decreased the expression of BAMBI. While LPS-induced HSC-T6 cells were treated with DHZCP containing serum, the protein expression of TLR4 and MyD88 decreased, the activity of NF-κB protein decreased, and the expression of BAMBI increased.

**Conclusion**

Chronic hepatitis eventually develops into liver fibrosis, cirrhosis, and even liver cancer. Therefore, it is very important to intervene the pathological procession of liver fibrosis. With multilevel, multichannel, multitarget pharmacological effects, TCM has been selected as an alternative treatment for liver fibrosis. DHZCP has been well recorded therapeutic ecacies since thousands of years in the past, and has been applied in the clinical treatment of liver fibrosis. This study provides a new point to clarify the mechanism for the application of DHZCP in liver fibrosis. DHZCP inhibited NF-κB activation induced by LPS, and upregulated BAMBI expression in HSCs. This was one of important mechanisms of DHZCP in improving fibrosis.

**Abbreviations**

DHZCP: DaHuangZheChong pill; BAMBI: bone morphogenic protein and activin membrane-bound inhibitor; HSCs: hepatic stellate cells; TLR4: Toll-like receptor 4; MyD88: myeloid differentiation factor 88; EMSA: electrophoretic mobility shift assay; ECM: extracellular matrix; TGF-β: Transforming growth factor-β; LPS: Lipopolysaccharide; NF-κB: nuclear factor-κB; TCM: traditional Chinese medicine; H&E: hematoxylineosin; NPC: Non-parenchymal cell; PBS: phosphate buffered saline.

**Declarations**

**Ethics approval and consent to participate**

The experimental protocol was established according to the ethical guidelines and was approved by the author's Ethical Committee.

**Consent for publication**

The manuscript is approved by all authors for publication.

**Availability of data and materials**
The data and materials generated or analyzed during this study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

LXD and TYM conceived and designed the experiments. LP, ZZZ and XXJ performed the experiments. TYF and ZZX analyzed the data. Wrote the paper: LXD. All authors read and approved the final manuscript.

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**References**

1. Parola M, Pinzani M. Liver fibrosis: Pathophysiology, pathogenetic targets and clinical issues. Molecular aspects of medicine. 2019 Feb;65:37–55.

2. Lin M, Chang Y, Xie F, Shi Y, Pang L, Chen D. ASPP2 Inhibits the Profibrotic Effects of Transforming Growth Factor-β1 in Hepatic Stellate Cells by Reducing Autophagy. Digestive Diseases Sciences. 2018;63(1):146–54.

3. Chen M, Liu J, Yang W, Ling W. Lipopolysaccharide mediates hepatic stellate cell activation by regulating autophagy and retinoic acid signaling. Autophagy. 2017;13(11):1–15.

4. Tao L, Xue D, Shen D, Ma W, Zhang J, Wang X, et al. MicroRNA-942 mediates hepatic stellate cell activation by regulating BAMBI expression in human liver fibrosis. Archives of Toxicology. 2018.

5. Campana L, Iredale JP. Regression of Liver Fibrosis. Semin Liver Dis. 2017;58(01):001–10.

6. Cornel B, Alina C, Hildegard H, Marcel R, Maria BO, Anca H. Dose-Dependent Antifibrotic Effect of Chrysin on Regression of Liver Fibrosis: The Role in Extracellular Matrix Remodeling. Dose-Response. 2018;16(3):155932581878983-.

7. Nwaezeigwe MD, Kiat C, Corbett S, Crosbie O. Mo1426 - Regression of Liver Fibrosis in Patients with Chronic Hepatitis C Treated with Direct Anti-Viral Drugs. Gastroenterology. 2018;154(6):–1203-.

8. Sood V, Lal BB, Rastogi A, Khanna R, Rawat D, Alam S. Regression of fibrosis in pediatric liver diseases. Indian Journal of Gastroenterology. 2018:1–5.
9. Wang RQ, Mi HM, Li H, Zhao SX, Jia YH, Nan YM. Modulation of IKKbeta/NF-kappaB and TGF-beta1/Smad via Fuzheng Huayu recipe involves in prevention of nutritional steatohepatitis and fibrosis in mice. Iranian journal of basic medical sciences. 2015 Apr;18(4):404–11.

10. Li XM, Peng JH, Sun ZL, Tian HJ, Duan XH, Liu L, et al. Chinese medicine CGA formula ameliorates DMN-induced liver fibrosis in rats via inhibiting MMP2/9, TIMP1/2 and the TGF-beta/Smad signaling pathways. Acta pharmacologica Sinica. 2016 Jun;37(6):783–93.

11. Chinese Association of Integrative Medicine LDC. Guideline for the diagnosis and treatment of liver fibrosis with integrative medicine. Chinese Journal of Hepatology. 2006;14(11):866–70.

12. Chen C, Yao X, Xu Y, Zhang Q, Wang H, Zhao L, et al. Dahuang Zhechong Pill suppresses colorectal cancer liver metastasis via ameliorating exosomal CCL2 primed pre-metastatic niche. Journal of ethnomedicine. 2019.

13. Gong Z, Lin J, Zheng J, Wei L, Liu L, Peng Y, et al. Dahuang Zhechong pill attenuates CCl4-induced rat liver fibrosis via the PI3K-Akt signaling pathway. J Cell Biochem. 2020;121(2):1431–40.

14. Wei F, Lang Y, Gong D, Fan Y. Effect of Dahuang zhechong formula on liver fibrosis in patients with chronic hepatitis B: A meta-analysis. Complementary Therapies in Medicine. 2015;23(1):129–38.

15. Shrestha N, Chand L, Han MK, Lee SO, Kim CY, Jeong YJ. Glutamine inhibits CCl4 induced liver fibrosis in mice and TGF-β1 mediated epithelial–mesenchymal transition in mouse hepatocytes. Food Chem Toxicol. 2016;93:129–37.

16. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology. 2010;1(5):431–5.

17. Lv Q, Rong N, Liu LJ, Xu XL, Liu JT, Jin FX, et al. Antitumoral Activity of (20R)- and (20S)-Ginsenoside Rh2 on Transplanted Hepatocellular Carcinoma in Mice. Planta Med. 2016;82(08):705–11.

18. Hernandez H, Millar JC, Curry SM, Clark AF, Mcdowell CM. BMP and Activin Membrane Bound Inhibitor Regulates the Extracellular Matrix in the Trabecular Meshwork. Invest Ophthalmol Vis Sci. 2018;59(5):2154–66.

19. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nature medicine. 2007 Nov;13(11):1324–32.

20. Friedman S. A deer in the headlights: BAMBI meets liver fibrosis. Nature medicine. 2007;13(11):1281.

21. Schoeniger A, Fuhrmann H, Schumann J. LPS- or Pseudomonas aeruginosa-mediated activation of the macrophage TLR4 signaling cascade depends on membrane lipid composition. Peerj. 2016;4(6):e1663.

22. Zou Y, Cai Y, Lu D, Zhou Y, Yao Q, Zhang S. MicroRNA-146a-5p attenuates liver fibrosis by suppressing profibrogenic effects of TGFβ1 and lipopolysaccharide. Cell Signal. 2017;39:1.

23. Cai HB, Sun XG, Liu Z, Liu YW, Tang J, Liu Q, et al. Effects of dahuangzhechong pills on cytokines and mitogen activated protein kinase activation in rats with hepatic fibrosis. J Ethnopharmacol. 2010;132(1):157–64.
24. Xing XY, Zhao YL, Jia L, Kong WJ, Zhong YW, Wang JB, et al. Evaluation of the liver protection and toxicity of Da-Huang-Zhe-Chong pill in rats. Pharm Biol. 2012;50(3):344–50.
25. Liu XD, Zhao XF, Tang YF, Xu XJ. Effect of Dahuang Chong Pill on Endotoxemia in Rats with Hepatic Fibrosis. Lishizhen Med Mater Med Res. 2017(09):2091–3.
26. Z WC, Z CY, Lv LG, Huang ZP. SH. Protective effects of Dahuang Zhechong Pills on mice with alcohol-induced liver fibrosis. Chinese Traditional Patent Medicine. 2017(12):2475–80.
27. Li WX, Chen NC, Wang JG, Du YQ, Liu Y, Si Y, et al. Effect of Dahuangzhechong pill plus Radix Astragali and Radix Paeonias on Antagonizing Liver Fibrosis in Rats. China Journal of Traditional Chinese Medicine and Pharmacy. 2016(12):5320–2.
28. Mehta KJ, Coombes JD, Brionesorta M, Manka PP, Williams R, Patel VB, et al. Iron Enhances Hepatic Fibrogenesis and Activates Transforming Growth Factor-β Signaling in Murine Hepatic Stellate Cells. Am J Med Sci. 2018;355(2):183.
29. Zeng YY, Li KY. Effects of Jiawei Shaoyao-Gancao Decoction and Its Drug-Containing Serum on Proliferation, Apoptosis, and Ultrastructure of Human Adenomyosis Foci Cells. Evidence-Based Complementary Alternative Medicine. 2017;2017:1–8.
30. Raykhel I, Moafi F, Myllymäki SM, Greciano PG, Matlin KS, Moyano JV, et al. BAMBI is a novel HIF1-dependent modulator of TGFβ-mediated disruption of cell polarity in hypoxia. Journal of Cell Science. 2018:jcs.210906.

### Table

| Group         | n | S0 | S1 | S2 | S3 | Mann-Whitney test |
|---------------|---|----|----|----|----|-------------------|
| NC group      | 6 | 6  | 0  | 0  | 0  | #P < 0.05         |
| MC group      | 6 | 0  | 0  | 2  | 4  | *P < 0.05         |
| DHZCP group   | 6 | 0  | 2  | 3  | 1  | #P < 0.05         |

NC group: normal control group, MC group: model control group.; #P < 0.05 vs. MC group; *P < 0.05 vs. DHZCP group.
Figure 1

DHZCP alleviated the liver fibrosis in rats. H&E staining was performed to detect morphological structure of each group (× 100).
Figure 1

DHZCP alleviated the liver fibrosis in rats. H&E staining was performed to detect morphological structure of each group (×100).

Figure 2

Effects of DHZCP on α-SMA expression in each group, protein expression of α-SMA in primary HSCs were detected by immunohistology (×100).
Effects of DHZCP on α-SMA expression in each group, protein expression of α-SMA in primary HSCs were detected by immunohistology (×100).
Figure 3

Effects of DHZCP on associated molecular of NF-κB activation in primary HSCs, signal molecules expression in the NF-κB signal pathway were detected by Western blot (A and B) and EMSA (C and D). #P < 0.05 vs. NC group; *P < 0.05 vs. MC group; ##P < 0.001 vs. NC group; **P < 0.001 vs. MC group.
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Protein expression of TLR4/MYD88, BAMBI induced by LPS in HSC-T6 cells was detected by immunohistochemistry (A × 400) and semi-quantitatively scores (B). #P < 0.05 vs. NC group; *P < 0.05 vs. LPS group.

Figure 4
Protein expression of TLR4/MYD88, BAMBI induced by LPS in HSC-T6 cells was detected by immunohistochemistry (A × 400) and semi-quantitatively scores (B). #P < 0.05 vs. NC group; *P < 0.05 vs. LPS group.

Figure 4
Protein expression of TLR4/MYD88, BAMBI induced by LPS in HSC-T6 cells was detected by immunohistochemistry (A × 400) and semi-quantitatively scores (B). #P < 0.05 vs. NC group; *P < 0.05 vs. LPS group.
Figure 5

Protein expression of TLR4/MYD88,BAMBI induced by LPS in HSC-T6 cells was detected by Western blot. 
#P < 0.05 vs. NC group; *P < 0.05 vs. LPS group.
Figure 5

Protein expression of TLR4/MYD88,BAMBI induced by LPS in HSC-T6 cells was detected by Western blot. #P < 0.05 vs. NC group; *P < 0.05 vs. LPS group.
Figure 5

Protein expression of TLR4/MYD88,BAMBI induced by LPS in HSC-T6 cells was detected by Western blot. #P < 0.05 vs. NC group; *P < 0.05 vs. LPS group.
Figure 6

The activity of NF-κB induced by LPS in HSC-T6 cells was detected by EMSA. Images and expression levels for each detected protein. ##P < 0.001 vs. NC group; **P < 0.001 vs. LPS group.
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