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Study on mechanism of coix seed oil intervening hepatic fibrosis in immune injury rats

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Abstract

This study aims to investigate the effect and specific mechanism of Coix Seed Oil (CSO) on immune hepatic fibrosis in immune injury rats. In this study, healthy Wister rats were injected with porcine serum in order to establish hepatic fibrosis model. We choose the compound Sophora flavescens injection as the positive control drug. HE staining was used to observe the morphological and histological changes. HSC-T6 cells were cultured in vitro. MTT and AO/EB staining were used to detect the effect of CSO on the proliferation and apoptosis of HSC-T6 cells. The results shown that Coix seed oil significantly improved hepatic fibrosis of immune injury rats, and the degree of hepatic fibrosis in CSO group was significantly reduced. Intraperitoneal injection of 15 mg/kg, 30 mg/kg and 40 mg/kg significantly reduced the levels of serum ALT, AST, ALP, HA, LN, PIIINP and C-IV in rats ($P < 0.01$).), MTT assay showed that the cells proliferated well. The results of HE staining showed that Coix seed oil had a good therapeutic effect on immune-induced hepatic fibrosis in rats. AO/EB staining showed that 0.5 mg/mL CSO for 24 h and 0.1 mg/mL CSO for 48 h both significantly promoted the apoptosis of HSC-T6 cells. In conclusion, Coix seed oil has a significant therapeutic effect on immune hepatic fibrosis, and its molecular mechanism may be related to antagonizing cytokine TGF-β1, inhibiting the expression of TIMP-1, promoting collagen degradation, inhibiting the activation of hepatocyte and promoting the apoptosis of activated hepatic stellate cells.

Keywords: coix seed oil; immune hepatic fibrosis; hepatic interstitial fibrosis; hepatic stellate cells; apoptosis.

Practical Application: Coix seed oil has a significant therapeutic effect on immune hepatic fibrosis.

1 Introduction

Fibrosis was a pathological process in which cells were stimulated by chronic inflammation for a long time, resulting in the imbalance of extracellular matrix (extracellularmatrix, ECM) metabolism and collagen accumulation in tissues and organs, forming a large number of scar tissue, and then affecting the physiological function of diseased organs (Montesi et al., 2019; Wynn, 2008; Wynn & Ramalingam, 2012). Fibrotic diseases involved many organs of the body, such as lung, kidney, intestine, liver, etc., which posed a serious threat to human health (Wang et al., 2022; Chen et al., 2022). Therefore, slowing down or even blocking fibrosis had great clinical significance. Although relevant fibrosis treatments have been reported internationally, such as FXR agonists, acetyl-CoA carboxylase inhibitors, peroxisome proliferator activator receptor agonists, FGF-19 activators, ASK1 inhibitors. Galectin-3 inhibitors, etc (Tacke & Weiskirchen, 2018; Inagaki et al., 2012; Oakley et al., 2019). Recently, there was still a lack of effective western medicine methods to alleviate the progression of fibrosis.

Traditional Chinese medicine has played a good therapeutic effect in a variety of diseases (Li et al., 2022), and fortunately, the advantages of traditional Chinese medicine in the anti-fibrosis effect are increasingly obvious and also obtained significant progress in the pathogenesis of liver fibrosis. Therefore, traditional Chinese medicine, such as activating blood circulation and removing blood stasis, tonifying qi and invigorating spleen had good effects on hepatic fibrosis. What's more, the anti-fibrosis effect of some effective components or monomer preparations of traditional Chinese medicine has attracted the attention of the researchers (Xie et al., 2003; Huang et al., 2010).

Coix seed, also known as Job's tears, is a dry, mature kernel of the Gramineae plant Coix lacryma-jobi L. var. ma-yuen (Roman) Stapf (Wu et al., 2021). Coix seed is mainly planted in Asia, Africa, and the marginal regions of the Mediterranean, and can be used for soups, porridges, cakes, drinks, tea, and alcoholic beverages (Wu et al., 2021). In addition to being a healthy food, coix seed has also been used as a medicinal ingredient. It consists of a series of biologically active ingredients, such as polysaccharides, proteins, polyphenols, oils, coixenolide, sterols, and others (Ni et al., 2021). In traditional Chinese medicine, coix seed is considered of far superior value than the common grains and is used to treat chronic digestion problems, promote diuresis, and enhance immunity (Wu et al., 2021). Coix seed oil (CSO), a mixture of polyunsaturated fatty acids, is a new type of fat emulsion made from the active ingredient which

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extracted from Coix seed (Bai et al., 2011; Xiao et al., 2011). CSO has various pharmacological activities, including antitumor, anticancer, hypoglycemic, the lowering blood lipids activity and the enhancing immunological activity (Zheng et al., 2021). In particular, CSO has been attempted to treat malignant tumors including lung cancer, liver cancer, gastric cancer and cervical cancer (Zhan et al., 2012; Chen et al., 2018; Zhang et al., 2019). The medicine of CSO for injecting, such as Kanglaite injection, had been approved by FDA and had entered the phase III clinical trial (Zheng et al., 2021). Previous studies have showed that CSO had also strong anti-proliferation effects on non-tumor cells, such as hepatic fibroblasts and glomerular Mesangial cells. Thus, it may have effect on hepatic fibrosis.

In this study, the animal model of hepatic fibrosis in rats was established by injection of porcine serum in order to explore the effect and mechanism of CSO on immune hepatic fibrosis in immune injury rats.

2 Materials and methods

2.1 Experimental animals

In this study, 120 healthy clean grade Wister rats, weighing 80-100 g, were selected as experimental animals. All the experimental animals were purchased from the Experimental Animal Center of Henan Cancer Hospital. The license number of the experimental animal is SCXK(Yu)2015-0047.

2.2 Drugs, reagents and instruments

The main drugs and reagents used in this study included Coix seed oil (Kanglaite injection, Hangzhou Kanglaite Pharmaceutical Co., Ltd.). Compound Sophora flavescens injection (Tianjin biochemical Pharmaceutical Factory), HA, LN, PIII NP, IV-C radioimmunoassay kit (Beijing Huamei Biotechnology Co., Ltd.), TGF-β1, TIMP-1 antibody (Beijing Huamei Biotechnology Co., Ltd.), SP-9001 immunohistochemical kit (Beijing Huamei Biotechnology Co., Ltd.), DAB chromogenic agent (Beijing Huamei Biotechnology Co., Ltd.) and HSC-T6 cells (Cell Center of Henan Cancer Hospital).

The main equipment used in this study included Hitachi 7020 automatic biochemical analyzer, FJ-2003 radioimmunoassay counter (Xi'an 262 Factory), KD-MB biological tissue embedding machine (Zhejiang Jinhua Kodi instrument and equipment Co., Ltd.), RM2255 paraffin slicer (Beijing Boyikang instrument Co., Ltd. 3), HM525 Frozen slicer (Microm, Germany), GL-16G-II high speed freezing centrifuge (Shanghai Luxi Scientific instrument Factory), PT-MR2100 tissue homogenizer (Switzerland Kinematica company) and UV-2010 ultraviolet visible spectrophotometer (Japan Shimadzu company).

2.3 Animal modeling and grouping

In this study, 60 rats were randomly divided into control group (N group, n = 10), model group (M group, n = 10), positive drug group (OM group, n = 10), CSO low dose group (CSO-L group, n = 10), CSO medium dose group (CSO-M, n = 10) and CSO high dose group (CSO-H, n = 10). In this study, the rat model of hepatic fibrosis was established by injection of porcine serum (Zhang et al., 2019). Except for the control group, the other rats were intraperitoneally injected with aseptic porcine serum 0.5 mL, twice a week for 8 weeks. Medication intervention was started at the ninth week.

2.4 Intervention

In this study, medication intervention was started at the ninth week and once a day for 8 weeks. The rats in OM group, CSO-L group, CSO-M group and CSO-H group were treated with OM 45 mg/kg, CSO 15 mg/kg, 30 mg/kg and 40 mg/kg, respectively. The rats in N group and M group received intraperitoneal injection of sodium chloride injection 10 mg/kg as control. The rats in each group were weighed once a week, the dosage was adjusted according to the change of body weight. In addition, we will observe the difference of weight change in each group every week (Figure 1).

2.5 Pathological observation

Blood samples were extracted from the inner canthus at the end of 24 hours after the last administration at the end of the 16th week, 4000 rpm centrifugation for 5 minutes in order to obtain the serum for later use. After that, the rats were killed by neck breaking method, and the 4th lobe of liver was dissected and fixed in 10% formaldehyde, then embedded in paraffin and sectioned.

2.6 Culture and subculture of HSC-T6 cells

We prepared DMEM medium containing 10% FBS, 2 mmol/L glutamine, 100 IU/mL penicillin and 100 μg/mL streptomycin. HSC-T6 cells were inoculated into the culture flask of 100 mL

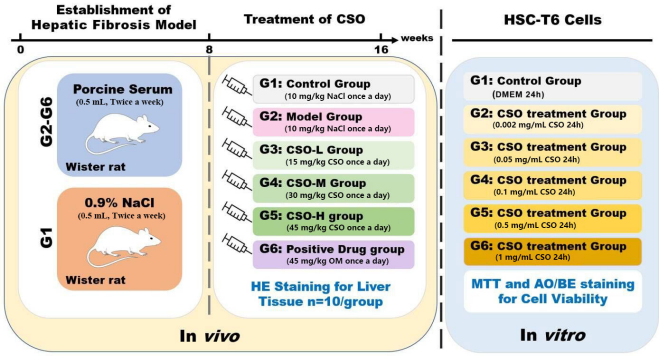

Figure 1. Experimental design. In vivo study, Group G1 and groups G2-6 received injection of NaCl 0.9% or aseptic porcine serum twice a week from 0-8 week, respectively. Medication intervention was started at the ninth week. G1: control group (10 mg/kg NaCl, n = 10); G2: model group (10 mg/kg NaCl, n = 10); G3: CSO low dose group (CSO-L group, 15 mg/kg CSO, n = 10); G4: CSO medium dose group (CSO-M group, 30 mg/kg CSO, n = 10); G5: CSO high dose group (CSO-H group, 45 mg/kg CSO, n = 10); G6: positive drug group (OM group, 45 mg/kg CSO, n = 10). Mice were euthanized at the end of week 16. In vitro study, MTT and AO/EB staining were used to detect the effect of dose concentrations CSO on the proliferation and apoptosis of HSC-T6 cells. CSO: Coix Seed Oil; OM: compound Sophora flavescens injection; HE: hematoxylin and eosin

at the concentration of 1 x 10^4/mL, and then placed in the 5% CO_2 incubator at 37 °C. We changed the culture medium every 4 days. After the cells grow into a monolayer, we discarded the medium and use 0.25% trypsin to digest cells for 2-3 minutes, then absorb trypsin, add 3 mL DMEM to gently blow the culture bottle. At the end, we collected cell suspension, 3000 rpm centrifugation for 5 minutes, discard the supernatant, add DMEM suspension, and then inoculate in 100 mL medium at the concentration of 3-6 x 10^4/mL. We used 0.4% trypan blue staining before each experiment, count dead cells, and identify more than 95% living cells before starting the experiment.

2.7 MTT detected the effect of Coix seed oil on the proliferation of HSC-T6 cells

In this study, the cell suspension was prepared with DMEM medium without phenol red. The suspension was inoculated into 96-well plate and 100 μL/well at a concentration of 1 x 10^5/mL. After 24 hours, the culture medium was absorbed, and then different concentrations of DMEM were added to CSO,100 μL/well (the final concentration was 0.002 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL). There were six kinds of balance holes for each concentration. After 24 hours of culture, each well medium was absorbed, and then 90 μL and MTT (5 mg/mL) 10 μL of aseptic serum DMEM solution were added, and the culture continued for 4-6 hours. The culture medium was discarded, dimethyl sulfoxide ((DMSO)) 100 μL was added to each well. Then, the oscillator was shaken at a uniform speed for 5 minutes. The absorbance of 570 nm in each well was detected by DG-302 enzyme-linked immunosorbent assay.

2.8 AO/EB double staining detected the apoptosis of HSC-T6 cells

In this study, the cells were diluted by DMEM to form suspension and inoculated in 96-well microplate at a concentration of 1 x 10^5/mL, 100 μL/ well. After 24 hours of culture, the medium was absorbed from the well. Then add different concentrations of CSO, 100 μL/well diluted with DMEM (the final concentration was 0.1 mg/mL, 0.5 mg/mL, 1.0 mg/mL), set 6 balance holes for each concentration, incubate for 24 hours respectively. We prepared the same amount of AO/EB 1 mL mixture at a concentration of 1 mg/mL and avoid light. Then, we took out the culture plate, add 10 μL mixture to each hole, immediately observe and take pictures under the inverted microscope.

2.9 Statistical analysis

We used the software program SPSS 15.0 to conduct the statistical analysis. The continuous variables of normal distribution were expressed as mean ± standard deviation, the continuous variables of non-normal distribution were expressed as median (interquartile range [IQR]), the categorical variables were expressed as frequency (percentage [%]). For multiple comparisons, each value was compared by one way ANOVA following Dunnett test when each datum conformed to normal distribution, while the non-normally distributed continuous data were compared using non-parametric tests. The counting data were tested by chi-square test. A value of $P < 0.05$ was considered statistically significant.

3 Results

3.1 Effect of Coix seed oil on liver histopathology

HE staining showed that the liver cells of normal rats arranged radially around the central vein without pathological changes. In the M group, inflammatory cells infiltrated in the lobules and interlobules, the portal area expanded due to fibrous tissue hyperplasia, collagen fibers extended from the portal area to the interlobular area, and the liver tissue was segmented to form a network of fibrous septum, with false lobules forming. The degree of liver fibrosis in CSO-L group was improved in varying degrees, connective tissue hyperplasia was significantly decreased, fiber septum was narrowed, CSO-M group and CSO-H group were significantly improved, fibrous connective tissue in portal area was significantly reduced, inflammatory infiltration was significantly reduced (Figure 2).

3.2 The apoptosis of HSC-T6 cells induced by Coix seed oil by double staining

According to the AO/EB double staining, cells could be divided into normal cell group, early apoptotic group and late apoptotic group. The normal group was stained dark green, mainly AO staining. In early apoptotic group, AO staining was obvious, EB staining was light. In late apoptotic group, AO red staining was enhanced, dead cell group EB staining was enhanced, almost no AO staining. The results showed that 0.5 mg/mL CSO for 24 h and 0.1 mg/mL CSO for 48 h could both significantly promoted the apoptosis of HSC-T6 (Figure 3).

3.3 The effect of CSO on the proliferation of HSC-T6 cells

The results showed that the activity of succinate dehydrogenase decreased and the ability of reducing exogenous MTT decreased in the late stage of apoptosis and necrotic cells. Therefore, the number of living cells growing well could be detected by MTT method, which indirectly reflected the effect of CSO on the proliferation of HSC-T6. The experimental results showed that 0.1 mg/mL could significantly inhibited the proliferation of HSC-T6 after 24 hours. The inhibitory effect of CSO on the proliferation of HSC-T6 showed a good dose correlation (Table 1).

3.4 Effect of CSO on serum biochemical indexes

The results showed that intraperitoneal injection of porcine serum increased the levels of ALT, AST and ALP in rats. CSO 15 mg/kg, 30 mg/kg and 40 mg/kg significantly reduced the levels of ALT, AST and ALP in the serum of rats with hepatic fibrosis. Ater continuous intraperitoneal injection of porcine serum, the levels of ALT, AST and ALP in rats with hepatic fibrosis were significantly decreased by intraperitoneal injection of porcine serum. The level of HA, LN, PIIINP and C-IV in rats were significantly increased (Tables 2 and 3).

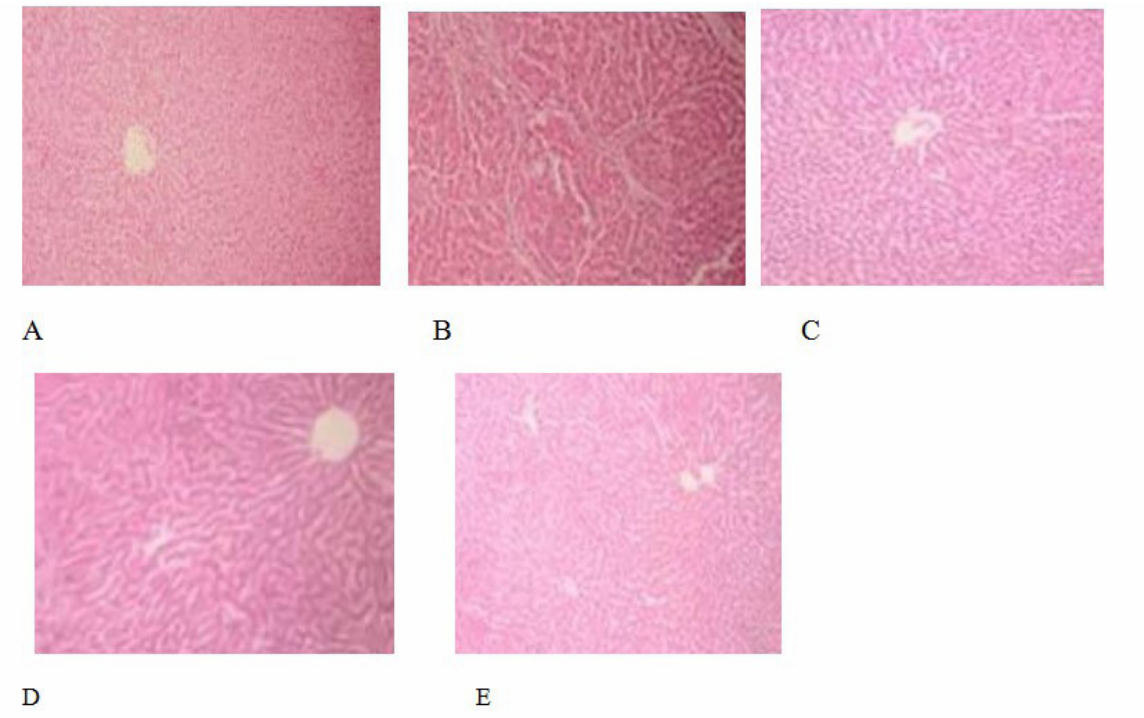

Figure 2. The effects of CSO on the liver pathology of treatment group. A: Normal group, B: Model group, C: CSO-L Dose group, D:CSO-M Dose group, E:CSO-H Dose group.

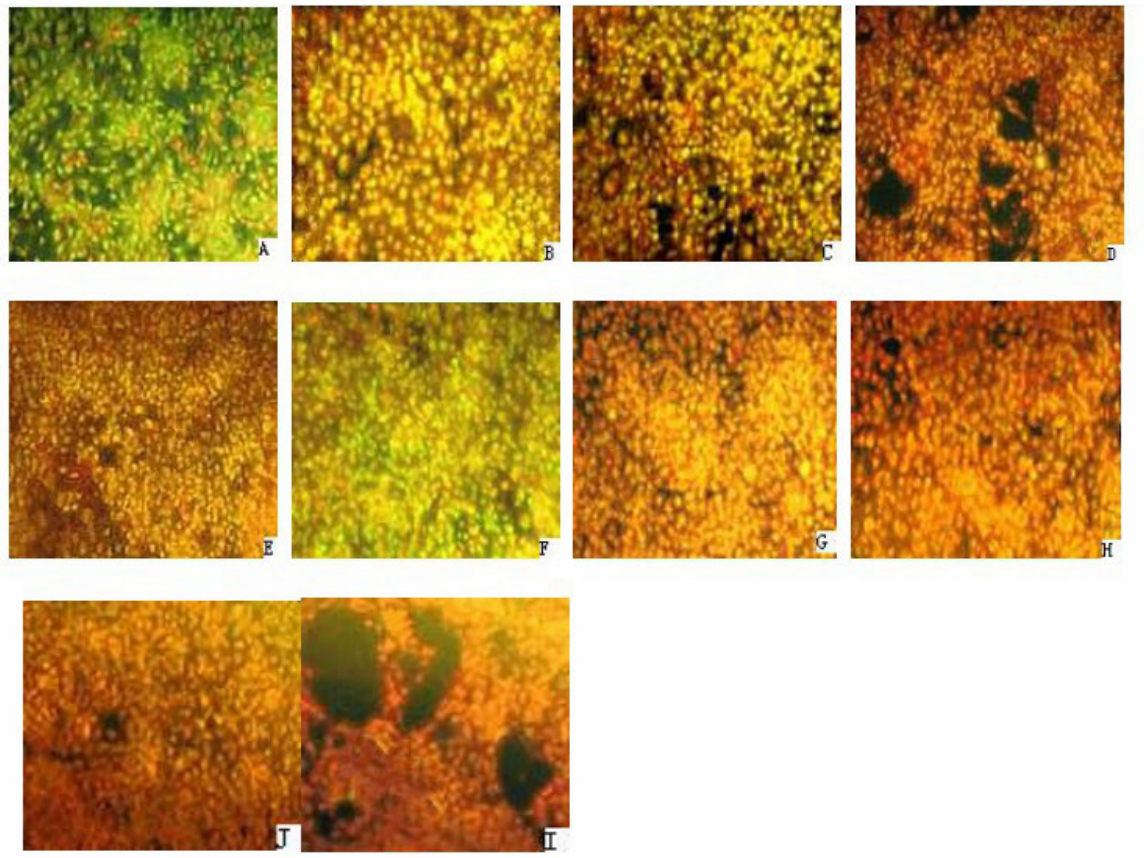

Figure 3. The fluorescent dye photos of AO/EB. A: Blank control group 24h. B: 0.5 mg/mL OM 24h. C: 0.1 mg/mL CSO 24h. D: 0.5 mg/mL CSO 24h. E: 1.0 mg/mL CSO 24h. F: Blank control group 24 h. G: 0.5 mg/mL OM 48h. H: 0.1 mg/mL CSO 48h. I: 0.5 mg/mL CSO 48 h. J: 1.0 mg/mL CSO 48 h.

Table 1. The influence of CSO on the proliferation of HSC-T6.

Dose	concentration (mg/mL)	ABS (24 h)	ABS (48 h)
Blank group	--	0.96 ± 0.05	0.88 ± 0.03
Positive control group	0.50	0.90 ± 0.06	0.77 ± 0.06**
Cso-0.002 mg/mL	0.002	0.96 ± 0.04	0.87 ± 0.05
Cso-0.01 mg/mL	0.01	0.95 ± 0.03	0.78 ± 0.02**
Cso-0.005 mg/mL	0.05	0.90 ± 0.05*	0.76 ± 0.04**
Cso-0.10 mg/mL	0.10	0.86 ± 0.06**	0.77 ± 0.03**
Cso-0.50 mg/mL	0.50	0.78 ± 0.05**	0.73 ± 0.06**
Cso-1.00 mg/mL	1.00	0.75 ± 0.03**	0.68 ± 0.03**

Note: compared with the blank control group. *P < 0.01. **P < 0.01.

Table 2. The influence of CSO on the ALT,AST,ALP of rat serum.

Group	n	Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)
N	8	--	32.18 ± 5.66	128.8 ± 29.00	92.6 ± 23.20
M	9	--	80.60 ± 11.40*	308.88 ± 63.30*	349.30 ± 41.20*
OM	8	45	41.50 ± 16.33△	154.90 ± 39.96△	147.50 ± 69.90△
CSO-L	8	15	44.00 ± 15.50△	185.70 ± 50.95△	156.80 ± 60.30△
CSO-M	9	30	36.56 ± 11.15△	159.56 ± 62.25△	159.66 ± 60.23△
CSO-H	8	40	35.12 ± 7.28△	146.52 ± 32.41△	144.21 ± 43.20△

Note: Compared with group N. *P < 0.01. △P < 0.01.

Table 3. The influence of CSO on the HA,LN, PIIINP, C-IV of rat serum.

Group	n	Dose (mg/kg)	HA (ng/mL)	LN (ng/mL)	PIIINP (ng/mL)	C-IV (ng/mL)
N	8	--	103.39 ± 14.29	126.25 ± 17.01	66.49 ± 8.07	75.89 ± 9.37
M	9	--	207.04 ± 35.86*	218.63 ± 28.56*	101.37 ± 15.66*	106.12 ± 8.29*
OM	8	45	129.24 ± 49.57△	145.22 ± 30.63△	74.58 ± 15.56△	81.26 ± 15.77△
CSO-L	8	15	149.00 ± 35.23△	162.99 ± 18.20△	97.25 ± 12.56	91.45 ± 11.51
CSO-M	9	30	136.53 ± 41.99△	156.78 ± 42.44△	91.62 ± 8.55	87.58 ± 12.52△
CSO-H	8	40	137.23 ± 32.51△	147.33 ± 19.59△	83.89 ± 13.86△	86.25 ± 16.22△

Note: Compared with group N. *P < 0.01. △P < 0.01.

4 Discussion

The essence of liver fibrosis is the imbalance between the synthesis and degradation of extracellular matrix (ECM) (Jiao et al., 2009; Fan et al., 2020), which leads to the excessive deposition of ECM in the liver. ECM included collagen, non-collagen glycoprotein and proteoglycan. The four serum markers of liver fibrosis, HA, LN, PC-III and IV-C, mainly used to measure the activity of inflammation and the degree of fibrosis (Wang, 2001). HA was the most sensitive index in all clinical serum indexes of liver fibrosis, which was of great significance in judging the degree of liver fibrosis and the efficacy of drugs (Karsdal et al., 2020). LN was a kind of non-collagen glycoprotein, and its level was closely related to the degree of liver fibrosis and inflammation. IV-C may be the earliest fiber to proliferate, and its conversion rate was fast. It deposits heavily in the liver and forms a complete basement membrane with LN. At present, IV-C was considered to be an index reflecting the production of collagen rather than degradation. The connective tissue of the liver was mainly composed of type I and type III collagen. When the liver was damaged, the connective tissue proliferated rapidly, and the level of PC-III increased accordingly. The level of PC-III could reflect the transformation of normal liver tissue to connective tissue (Tsuji et al., 2020; Tao et al., 2003; Meurer et al., 2020).

In this experiment, the therapeutic effect of Coix seed oil on immune liver injury in rats was studied for the first time. The pathological results showed that liver injury in CSO-M group and CSO-H group significantly improved, fibrous connective tissue decreased significantly, inflammatory infiltration decreased significantly. AO/EB staining results showed that 0.5 mg/mL CSO treatment for 24 hours and 0.1 mg/mL CSO treatment for 48 hours significantly promoted HSC-T6 apoptosis. The results also showed that Coix seed oil significantly promoted the recovery of immune-induced liver fibrosis and was valuable for treatment of the disease. It has been reported that the mechanism of Coix seed oil in the treatment of hepatic fibrosis may be related to the antagonism of cytokine TGF-β1, inhibition of the expression of TIMP-1, promotion of collagen degradation, inhibition of hepatocyte activation and promotion of apoptosis of activated hepatic stellate cells (HSC-T6 cells). Thus, inhibiting the proliferation of activated HST-T6 cells and inducing their

apoptosis was one of the mechanisms of CSO in the prevention of liver fibrosis.

There were still several limitations in this study. Firstly, although this study suggested that the specific molecular mechanism of Coix seed oil on immune hepatic fibrosis may be related to the antagonism of cytokine TGF-β1 and inhibition of TIMP-1 expression, the expression levels of TGF-β1 and TIMP-1 have not been deeply studied in this study. Therefore, the effect of Coix seed oil on the expression of TGF-β1 and TIMP-1 is still worthy of further study. Secondly, the results of this study showed that the level of HA, LN, PIIINP and C-IV in serum of rats were significantly increased after continuous intraperitoneal injection of porcine serum, but the standard levels of HA, LN, PIIINP and C-IV in liver tissue have not been detected and need to be further studied.

5 Conclusion

Coix seed oil has a significant therapeutic effect on immune hepatic fibrosis, and its molecular mechanism may be related to antagonizing cytokine TGF-β1, inhibiting the expression of TIMP-1, promoting collagen degradation, inhibiting the activation of hepatocyte and promoting the apoptosis of activated hepatic stellate cells.

Abbreviations

CSO: Coix seed oil. DMSO: dimethyl sulfoxide. ECM: extracellularmatrix.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Huaihe Hospital of Henan University.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Availability of data and material

The datasets used and analyzed during the current study are avaliable from the corresponding author on reasonable request.

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Author contributions

Conception and design of the research: Zhang YZ, Wang BQ. Acquisition of data: Wang BQ, Meng T, Ping J. Analysis and interpretation of the data: Zhou LM, Shen ZH. Statistical analysis: Wang BQ, Shi ZH, Meng T, Ping J, Zhou LM, Shen ZH, Zhang YZ. Obtaining financing: None. Writing of the manuscript: Wang BQ. Critical revision of the manuscript for intellectual content: Zhang YZ, Wang BQ.

References

Bai, C., Peng, H., Xiong, H., Liu, Y., Zhao, L., & Xiao, X. (2011). Carboxymethylchitosan-coated proliposomes containing coix seed oil: characterisation, stability and in vitro release evaluation. *Food Chemistry*, 129(4), 1695-1702. http://dx.doi.org/10.1016/j.foodchem.2011.06.033.

Chen, X. M., Zhang, J. Y., Yang, Y. L., Wang, Y. W., Yu, Y. Y., & Xu, H. H. (2022). Pristimerin improve renal fibrosis by regulating miRNA-145-5p in vitro and vivo study. *Food Science and Technology*, 42, e79021. http://dx.doi.org/10.1590/fst.79021.

Chen, Y., Qu, D., Fu, R. P., Guo, M. F., Qin, Y., Guo, J., & Chen, Y. (2018). A Tf-modified tripterine-loaded coix seed oil microemulsion enhances anti-cervical cancer treatment. *International Journal of Nanomedicine*, 13, 7275-7287. http://dx.doi.org/10.2147/IJN.S182475. PMid:30510417.

Fan, Q. Q., Zhang, C. L., Qiao, J. B., Cui, P. F., Xing, L., Oh, Y. K., & Jiang, H. L. (2020). Extracellular matrix-penetrating nanodrill micelles for liver fibrosis therapy. *Biomaterials*, 230, 119616. http://dx.doi.org/10.1016/j.biomaterials.2019.119616. PMid:31837823.

Huang, C., Ma, T. T., Meng, X. M., Lv, X. W., Zhang, L., Wang, J. Q., & Li, J. (2010). Potential protective effects of a traditional Chinese herb, Litsea coreana Levl. on liver fibrosis in rats. *The Journal of Pharmacy and Pharmacology*, 62(2), 223-230. http://dx.doi.org/10.1211/jpp.62.02.0010. PMid:20487202.

Inagaki, Y., Higashiyama, R., & Higashi, K. (2012). Novel anti-fibrotic modalities for liver fibrosis: molecular targeting and regenerative medicine in fibrosis therapy. *Journal of Gastroenterology and Hepatology*, 27(Suppl. 2), 85-88. http://dx.doi.org/10.1111/j.1440-1746.2011.07006.x. PMid:22320923.

Jiao, J., Friedman, S. L., & Aloman, C. (2009). Hepatic fibrosis. *Current Opinion in Gastroenterology*, 25(3), 223-229. http://dx.doi.org/10.1097/MOG.0b013e3283279668. PMid:19396960.

Karsdal, M. A., Daniels, S. J., Nielsen, S. H., Bager, C., Rasmussen, D. G. K., Loomba, R., Surabattula, R., Villesen, I. F., Luo, Y., Shevell, D., Gudmann, N. S., Nielsen, M. J., George, J., Christian, R., Leeming, D. J., & Schuppan, D. (2020). Collagen biology and non-nvasive biomarkers of liver fibrosis. *Liver International*, 40(4), 736-750. http://dx.doi.org/10.1111/liv.14390. PMid:31997561.

Li, H. B., Nie, D. Q., Wang, S. Q., Li, D., & Liu, C. (2022). Clinical value of turbidity-elimination gout soup combined with external application of traditional chinese medicine to improve the pain and the volume of tophi in patients with gout. *Food Science and Technology*, 42, e37420. http://dx.doi.org/10.1590/fst.37420.

Meurer, S. K., Karsdal, M. A., & Weiskirchen, R. (2020). Advances in the clinical use of collagen as biomarker of liver fibrosis. *Expert Review of Molecular Diagnostics*, 20(9), 947-969. http://dx.doi.org/10.1080/14737159.2020.1814746. PMid:32865433.

Montesi, S. B., Désogère, P., Fuchs, B. C., & Caravan, P. (2019). Molecular imaging of fibrosis: recent advances and future directions. *The Journal of Clinical Investigation*, 129(1), 24-33. http://dx.doi.org/10.1172/JCI122132. PMid:30601139.

Ni, C., Li, B., Ding, Y., Wu, Y., Wang, Q., Wang, J., & Cheng, J. (2021). Anti-cancer properties of coix seed oil against HT-29 colon cells through regulation of the PI3K/AKT signaling pathway. *Foods*, 10(11), 2833. http://dx.doi.org/10.3390/foods10112833. PMid:34829119.

Oakley, F., Gee, L. M., Sheerin, N. S., & Borthwick, L. A. (2019). Implementation of pre-clinical methodologies to study fibrosis and

test anti-fibrotic therapy. *Current Opinion in Pharmacology*, 49, 95-101. http://dx.doi.org/10.1016/j.coph.2019.10.004. PMid:31731225.

Tacke, F., & Weiskirchen, R. (2018). An update on the recent advances in antifibrotic therapy. *Expert Review of Gastroenterology & Hepatology*, 12(11), 1143-1152. http://dx.doi.org/10.1080/17474124.2018.1530110. PMid:30261763.

Tao, J., Peng, H. Q., Cai, W. M., Dong, F. Q., Weng, H. L., & Liu, R. H. (2003). Influence factors of serum fibrosis markers in liver fibrosis. *World Journal of Gastroenterology*, 9(11), 2497-2500. http://dx.doi.org/10.3748/wjg.v9.i11.2497. PMid:14606083.

Tsuji, Y., Namisaki, T., Kaji, K., Takaya, H., Nakanishi, K., Sato, S., Saikawa, S., Sawada, Y., Kitagawa, K., Shimozato, N., Kawaratani, H., Moriya, K., Noguchi, R., Akahane, T., Mitoro, A., & Yoshiji, H. (2020). Comparison of serum fibrosis biomarkers for diagnosing significant liver fibrosis in patients with chronic hepatitis B. *Experimental and Therapeutic Medicine*, 20(2), 985-995. http://dx.doi.org/10.3892/etm.2020.8798. PMid:32765655.

Wang, J. Y. (2001). The study on diagnostic value of co-detection of serum IV-C HA and LN in liver fibrosis patients. *Journal Medical Theory and Practice*, 14(2), 106-108.

Wang, S. L., Shang, S. N., Lv, J., & Hou, D. D. (2022). Effects of quercetin on renal autophagy and interstitial fibrosis in diabetes mellitus. *Food Science and Technology*, 42, e122821. http://dx.doi.org/10.1590/fst.122821.

Wu, Y., Ye, J., Xuan, Z., Li, L., Wang, H., Wang, S., Liu, H., & Wang, S. (2021). Development and validation of a rapid and efficient method for simultaneous determination of mycotoxins in coix seed using one-step extraction and UHPLC-HRMS. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 38(1), 148-159. http://dx.doi.org/10.1080/19440049.2020.1833089. PMid:33166220.

Wynn, T. A. (2008). Cellular and molecular mechanisms of fibrosis. *The Journal of Pathology*, 214(2), 199-210. http://dx.doi.org/10.1002/path.2277. PMid:18161745.

Wynn, T. A., & Ramalingam, T. R. (2012). Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nature Medicine*, 18(7), 1028-1040. http://dx.doi.org/10.1038/nm.2807. PMid:22772564.

Xiao, X. N., Yi, H. B., Yi, X., & Xiong, H. (2011). A simple and convenient method for simultaneous determination of oleic and linoleic acid in coix seed oil. *Advanced Materials Research*, 233-235, 2629-2632. http://dx.doi.org/10.4028/www.scientific.net/AMR.233-235.2629.

Xie, Y. M., Nie, Q. H., Zhou, Y. X., Huang, C. X., Kang, W. Z., Zhang, Y., Hao, C. Q., Wang, J. P., & Zhu, X. H. (2003). Effect of Chinese herb Shuangjiawuling on expression of TIMPs in rat with liver fibrosis. *World Chinese Journal of Digestology*, 11(2), 199-203. http://dx.doi.org/10.11569/wcjd.v11.i2.199.

Zhan, Y. P., Huang, X. E., Cao, J., Lu, Y. Y., Wu, X. Y., Liu, J., Xu, X., Xiang, J., & Ye, L. H. (2012). Clinical safety and efficacy of Kanglaite (Coix Seed Oil) injection combined with chemotherapy in treating patients with gastric cancer. *Asian Pacific Journal of Cancer Prevention*, 13(10), 5319-5321. http://dx.doi.org/10.7314/APJCP.2012.13.10.5319. PMid:23244156.

Zhang, P., Meng, X. Y., Tang, X. H., Ren, L., & Liang, J. (2019). The effect of a coix seed oil injection on cancer pain relief. *Supportive Care in Cancer*, 27(2), 461-465. http://dx.doi.org/10.1007/s00520-018-4313-z. PMid:29971522.

Zheng, W., Chen, Z. P., Yang, Y. H., Yang, R., Yang, T. D., Lai, P. L., Chen, T. L., Qiu, S. L., Wang, S. Y., & Liao, L. (2021). Improved stabilization of coix seed oil in a nanocage-coating framework based on gliadin-carboxymethyl chitosan-Ca2. *Carbohydrate Polymers*, 257, 117557. http://dx.doi.org/10.1016/j.carbpol.2020.117557. PMid:33541626.