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Original Research Article

Saikosaponin A alleviates rat liver fibrosis by inhibiting Hedgehog signaling pathway-mediated autophagy and NLRP3 inflammasome

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Abstract

Purpose: *To investigate the effect of saikosaponin A (SSa) on liver fibrosis, and the underlying mechanism of action.*
Methods: *Sprague-Dawley (SD) rats were treated with carbon tetrachloride (CCl_4) in an in vivo model of liver fibrosis. The effect of SSa on liver fibrosis was determined by spectrophotometry, Sirius staining, and western blotting. The mechanism of SSa in autophagy and NOD-like receptors containing pyrin domain 3 (NLRP3) inflammasome were examined by western blotting.*
Results: *Saikosaponin A treatment reduced CCl_4-induced serum aminotransferase (ALT) and aspartate aminotransferase (AST) levels, hydroxyproline (HYP) content, relative protein expression of alpha-smooth muscle actin (α-SMA), as well as Collagen 1 and deposition of collagenous fiber in liver tissues ($p < 0.05$). Saikosaponin A administration also downregulated the CCl_4-induced protein levels of Hedgehog signaling pathway-related proteins that mediate autophagy and the NLRP3 inflammasome in hepatic tissues ($p < 0.05$).*
Conclusion: *Saikosaponin A mitigates liver fibrosis by suppressing Hedgehog signaling pathway-mediated autophagy and NLRP3 inflammasome in CCl_4-induced rats. Thus, SSa is a potential drug for the management of liver fibrosis.*

Keywords: *Liver fibrosis, Saikosaponin A, Autophagy, NLRP3 inflammasome, Hedgehog*

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INTRODUCTION

Liver fibrosis, a representative pathological feature of chronic liver diseases, primarily results from alcoholism, viral hepatitis, steatohepatitis, and metabolic disorders developed from chronic liver injury [1]. Without appropriate attention and treatment, liver fibrosis advance to cirrhosis, hepatoma, and end-stage liver syndrome, among which cirrhosis and liver cancer account for 3.5 % of all deaths worldwide [2]. Numerous reports have demonstrated that hepatic fibrosis is reversible [3,4]. The inversion generally occurs too infrequently or slowly to avert life-threatening

complications, particularly in advanced fibrosis [3]. Therefore, anti-fibrosis agents are needed.

Multiple mechanisms are involved in liver fibrosis, including autophagy and NOD-like receptors containing the pyrin domain 3 (NLRP3) inflammasome [4]. Autophagy and inflammasome mediate the activation of hepatic stellate cells (HSCs) [4]. Autophagy plays a dual role in the progression of liver fibrosis. On the one hand, increased autophagy activates HSCs to accelerate fibrosis development. On the other hand, excessive autophagy can suppress the fibrosis process. NLRP3 inflammasome is strongly involved in the progression of sterile inflammation, a pivotal factor for fibrosis [5]. Therefore, agents that target autophagy and the NLRP3 inflammasome may be candidates for liver fibrosis treatment.

Saikosaponin A (SSa), a triterpenoid glycoside with the formula, $C_{42}H_{68}O_{13}$, is the primary active component of *Radix bupleuri* (Chinese name: Chai Hu) that has exhibited multiple pharmacological characteristics, such as anti-inflammation, anti-oxidation, anti-tumor, and neuromodulation [6]. Wu *et al* [7] reported that SSa ameliorates hepatic antioxidant activity and prevents CCl_4-induced hepatic injury. In addition, SSa and/or curcumin attenuate hepatic inflammation and fibrosis in carbon tetrachloride (CCl_4)-treated rats [8]. Furthermore, saikosaponin D (SSd) alleviates liver fibrosis by regulating autophagy and the NLRP3 inflammasome [9,10]. However, the effect and mechanism of SSa on liver fibrosis remain unknown. In the present study, Sprague-Dawley (SD) rats were treated with CCl_4 to construct an *in vivo* liver fibrosis model. The effect and potential mechanism of SSa on liver fibrosis were investigated in CCl_4-treated rats.

EXPERIMENTAL

Animals and ethical considerations

Adult male SD rats (8 weeks, 200 - 250 g) were provided by Junke Biological Co., LTD. (Nanjing, China) and fed to standard laboratory conditions for seven days before experiments. The rats were offered rodent chow and water freely and were fed at 22 ^oC with 40 - 60 % relative humidity and a 12-h/12-h light-dark cycle. All the procedures were strictly performed following the Guide for the Care and Use of Laboratory Animals [11]. The Animal Ethical Care Committee of Qiqihar Medical University approved the study (approval no. QMU-AECC-2021-239).

Model and treatment

Fifty rats were randomly divided into five diverse groups (n = 10), including control, CCl_4, CCl_4+SSa (5 mg/kg/day), CCl_4+SSa (10 mg/kg/day) and CCl_4+SSa (20 mg/kg/day). Rats in the CCl_4, CCl_4+SSa (5 mg/kg/day), CCl_4+SSa (10 mg/kg/day) and CCl_4+SSa (20 mg/kg/day) groups were first intraperitoneally administered with CCl_4 with a dose of 0.3 ml/kg twice a week for eight consecutive weeks. Carbon tetrachloride (1601168; Sigma, St. Louis, MO, USA) was dissolved in olive oil (1:3, v/v) (IO9000; Solarbio, Beijing, China) before use. Next, rats in the CCl_4 + SSa (5, 10, and 20 mg/kg/day) group were intragastrically administered 5, 10, and 20 mg/kg body weight per day of SSa (CAS:20736-09-8; IS0500; Solarbio), while rats in the CCl_4 group were intragastrically administered the same dose of 0.9 % normal saline.

Rats in the control group were first intraperitoneally injected with 0.3 ml/kg of olive oil and then intragastrically administered with 0.9 % normal saline. Twenty-four hours after the last administration, all the rats were sacrificed by intraperitoneal injection of sodium pentobarbital sodium (100 mg/kg). Blood from the abdominal aorta, and liver tissues were collected for subsequent assays.

Biochemical determinations

The serum level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the content of hydroxyproline (HYP) in liver tissues, was examined using glutamic-pyruvic transaminase (GPT) activity assay kit (BC1550, Solarbio), glutamic-oxalacetic transaminase (GOT) activity assay kit (BC1560, Solarbio), and hydroxyproline (HYP) content assay kit (BC0250, Solarbio). The absorbance of each sample was read at 505 nm (for both ALT and AST) and 560 nm (for HYP) using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Sirius staining

Liver tissue samples were immobilized into 10 % neutral buffered formalin (G2161; Solarbio) overnight and then underwent dehydration, clearance, embedding, and excision. Next, 5-µm sections were stained with Sirius staining, and then images were obtained using light microscopy (Olympus, Tokyo, Japan) and analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, USA).

Western blotting

Total proteins were extracted from liver tissues using RIPA buffer (R0010; Solarbio), and the protein concentration was determined using the BCA Protein Assay Kit (PC0020; Solarbio). Next, 20-µg protein samples were dissolved with sodium dodecyl sulfate poly(acrylamide) gel electrophoresis (SDS-PAGE) and electrically transferred instantly. Following blocking with 5 % skim milk (Anchor, Switzerland) for 1 h at room temperature, the membranes were treated with antibodies targeting alpha-smooth muscle actin (α-SMA; 1:50000; ab124964; Abcam, Cambridge, UK), Collagen 1 (1:1000; ab255809; Abcam), Beclin-1 (1:1000; ab62557; Abcam), p62 (1:1000; ab91526; Abcam), microtubule-associated light chain 3 (LC3) Ⅱ/Ⅰ (1:1000; ab128025; Abcam), NLRP3 (1:1000; ab263899; Abcam), pro-caspase-1 (1:500; AF5418; Affinity, Jiangsu, China), cleaved caspase-1 (1:500; AF4005; Affinity), apoptosis-associated speck-like protein containing a C-terminal caspase activation and recruitment domain (ASC; 1:2000; ab180799; Abcam), patched1 (PTCH1; 1:1000; ab90438; Abcam), glioma-associated oncogene homolod-1 (GLI1; 1:2000; ab273018; Abcam), Hedgehog-interacting protein (HIP1; 1:10000; ab181238; Abcam), histone deacetylase 1 (HDAC1; 1:100; ab19845; Abcam) and GAPDH (1:10000; ab181602; Abcam) overnight at 4 °C. Subsequently, the membranes were incubated with goat Anti-Rabbit IgG H&L (HRP) (1:20000; Abcam) for 2 h at room temperature and visualized using the ECL Western Blotting Substrate (PE0010; Solarbio). The gray values were analyzed using QUANTITY ONE software (Bio Rad, Hercules, CA, USA).

Statistical analysis

Data are reported as mean ± standard deviation (SD), and differences were tested using one-way analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test, using SPSS 26.0 software (IBM, Armonk, New York, USA). $P < 0.05$ was defined as statistically significant.

RESULTS

SSa alleviated CCl_4-induced liver fibrosis

To investigate the effect of SSa on liver fibrosis, rats were treated with CCl_4 to simulate hepatic fibrosis. After injection with CCl_4, the serum levels of ALT and AST as well as the HYP content in liver tissues were significantly increased. These promotions were neutralized following treatment with SSa in a dose-dependent manner (Figure 1a). Consistently, SSa treatment also markedly decreased the CCl_4-induced relative protein expression of α-SMA and Collagen 1 in hepatic tissues in a dose-dependent manner (Figure 1 b). In addition, CCl_4 induced the production of collagenous fiber, which was notably suppressed with the administration of SSa (Figure 1 c). Thus, SSa mitigated CCl_4-induced liver fibrosis in rats.

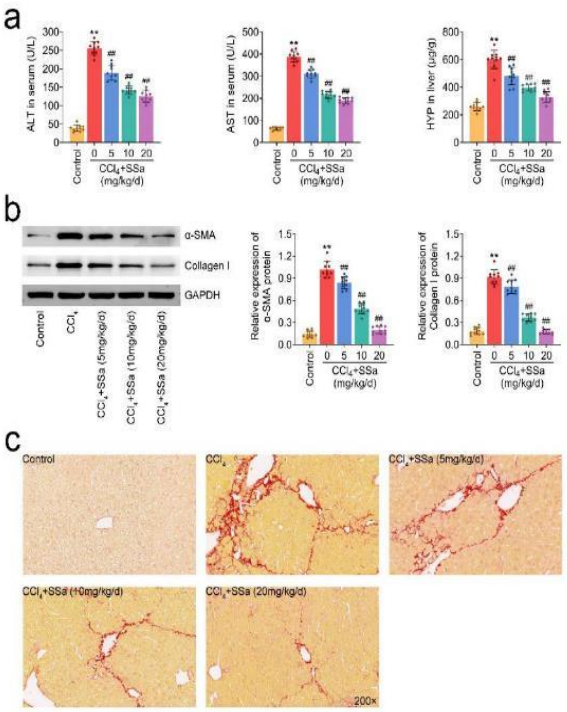

Figure 1: SSa relieved CCl_4-induced liver fibrosis in rats. Rats were first intraperitoneally administered with 0.3 ml/kg CCl_4 twice a week for eight consecutive weeks, and then intragastrically injected with 5, 10, and 20 mg/kg/day SSa. (a) The serum levels of ALT and AST as well as the HYP content in liver tissues as measured using commercial kits and standard spectrophotometric procedures. (b) The relative protein expression of α-SMA and Collagen 1 in hepatic tissues as examined by western blotting. The data were normalized to GAPDH expression. (c) The collagenous fiber of liver tissues was determined by Sirius staining. $^{**}P < 0.01$ *vs.* control; $^{\#\#}P < 0.01$ *vs.* CCl_4

SSa inhibited CCl_4-induced autophagy

The effect of SSa on autophagy was addressed in CCl_4-treated rats. Carbon tetrachloride injection markedly enhanced the relative protein expression of Beclin-1 and LC3 Ⅰ Ⅰ/LC3 Ⅰ but reduced the relative protein expression of p62 in hepatic tissues. These changes were reversed in a dose-dependent way following the introduction of SSa (Figure 2). Therefore, SSa suppressed autophagy in CCl_4-treated rats.

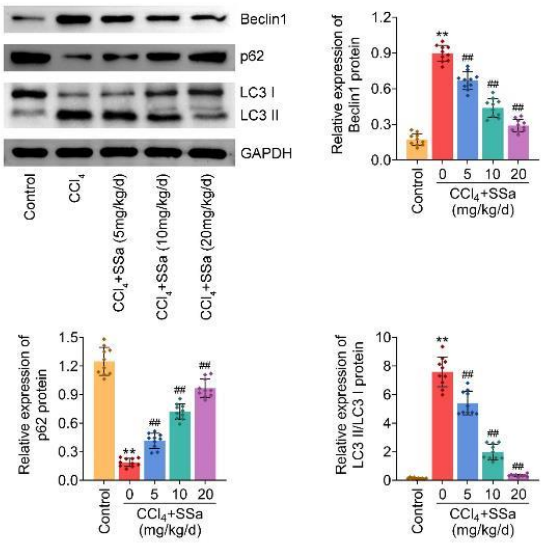

Figure 2: SSa repressed autophagy in CCl_4-treated rats. The relative protein expression of Beclin-1, p62, and LC3 Ⅰ/LC3 Ⅰ in liver tissues was examined using western blotting. The data were normalized to GAPDH expression. $^{**}P < 0.01$ *vs.* control; $^{\#\#}p < 0.01$ *vs.* CCl_4

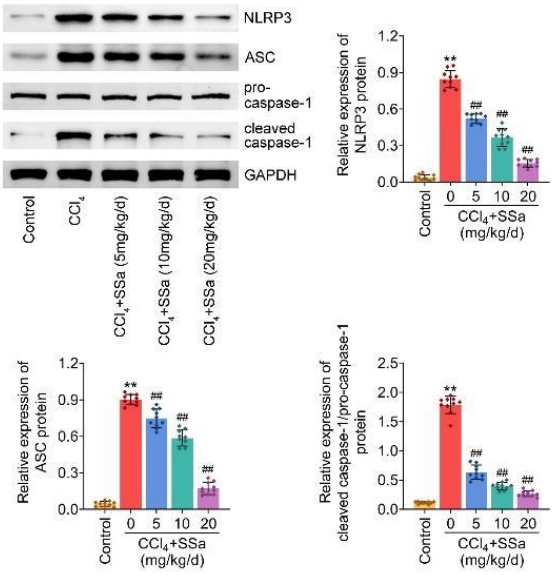

Figure 3: SSa reversed CCl_4-induced activation of the NLRP3 inflammasome. The relative protein expression levels of NLRP3, ASC, cleaved caspase-1, and pro-caspase-1 in liver tissues were determined using western blotting. The data were normalized to GAPDH expression. $^{**}P < 0.01$ *vs.* control; $^{\#\#}p < 0.01$ *vs.* CCl_4

SSa suppressed CCl_4-induced activation of NLRP3 inflammasome

In addition, the role of SSa in NLRP3 inflammasome was investigated in CCl_4-treated rats. The administration of SSa significantly diminished the relative protein levels of NLRP3 ($p < 0.05$), ASC, and cleaved caspase-1/pro-caspase-1 in liver tissues from CCl_4-treated rats in a dose-dependent manner (Figure 3). Hence, SSa attenuated CCl_4-induced activation of the NLRP3 inflammasome.

SSa repressed CCl_4-induced expression of Hedgehog signaling pathway

The underlying mechanism was further resolved by determining the expressions of Hedgehog signaling-related proteins. The relative protein expression of PTCH1, GLI1, HIP1, and HDAC1 was significantly upregulated in liver tissues after rats were treated with CCl_4 (Figure 4). However, SSa treatment significantly attenuated these effects ($p < 0.05$). Thus, SSa reduced CCl_4-induced expression of Hedgehog signaling pathway.

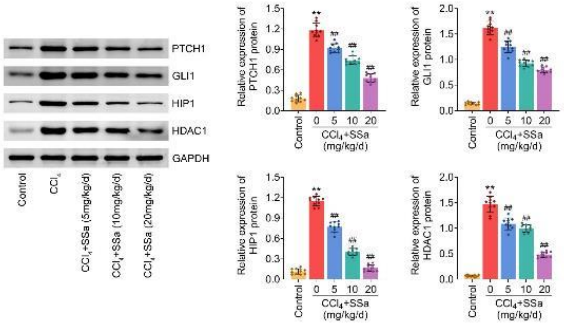

Figure 4: SSa decreased CCl_4-induced expression of the Hedgehog signaling pathway. The relative protein expression of PTCH1, GLI1, HIP1, and HDAC1 was examined by western blotting. The data were normalized to GAPDH expression. $^{**}P < 0.01$ *vs.* control; $^{\#\#}p < 0.01$ *vs.* CCl_4

DISCUSSION

Autophagy and the NLRP3 inflammasome are significant molecular mechanisms of liver fibrosis that activate HSCs [4]. Saikosaponin A and/or curcumin attenuate hepatic inflammation and fibrosis in CCl_4-treated rats, but whether SSa relieves liver fibrosis by modulating autophagy and NLRP3 inflammasome remains unknown. In the present study, an *in vivo* model of liver fibrosis was established by induction with CCl_4. Saikosaponin A alleviated liver fibrosis and inhibited autophagy and NLRP3 inflammasome activation in CCl_4-induced rats. Mechanistically, SSa treatment attenuated the CCl_4-induced expression of Hedgehog signaling pathway-related proteins. Thus, SSa alleviates rat liver fibrosis by inhibiting autophagy and the NLRP3 inflammasome through the Hedgehog signaling pathway.

Carbon tetrachloride stimulates HSC activation, resulting in liver injury and the induction of liver

fibrosis. In the current study, CCl_4 injection markedly increased the serum ALT and AST levels, HYP content, relative protein expression of α-SMA and Collagen 1, and deposition of collagenous fiber in liver tissues. During the conversion of HSCs from the quiescent state to myofibroblasts, the major components of extracellular matrix (ECM), such as α-SMA and Collagen 1 are produced in large quantities. Thus, the accumulation of Collagen 1 and α-SMA is a common feature of fibrosis in different organs, including the liver [12]. Serum ALT and AST are crucial markers of liver function, and HYP is a primary constituent of collagen proteins. The upregulation of these indicators indicates aggravating liver fibrosis. In the present study, Sirius staining showed the deposition of collagenous fiber in liver tissues. Thus, these results revealed that CCl_4 induced liver fibrosis in rats, consistent with the previous studies [13,14]. Saikosaponin A treatment reduced these promotions, suggesting that SSa alleviated CCl_4-induced liver fibrosis.

Autophagy activates HSCs, promotes ECM production, and intensifies fibrosis progression. In addition, a review has generalized that the anti-liver fibrosis activity of natural compounds occurs by regulating the autophagy of HSCs [15]. Saikosaponin A represses AMPK/mTOR pathway-mediated autophagy that attenuates the activation of pancreatic stellate cells, thereby mitigating pancreatic fibrosis [16]. Furthermore, SSd, the paralog of SSa, alleviates liver fibrosis by inhibiting autophagy [9]. In the present study, CCl_4 injection increased the relative protein expression of Beclin-1 and LC3 Ⅱ/LC3 Ⅰ and reduced the relative protein expression of p62 in hepatic tissues, effects that were reversed with the introduction of SSa. Beclin-1, LC3 Ⅱ/LC3 Ⅰ, and p62 are strongly involved in the initiation of autophagy and formation of the autophagosome. Thus, these proteins have been established as typical markers of autophagy. Therefore, these results indicate that SSa restrains autophagy in CCl_4-treated rats.

The NLRP3 inflammasome comprises the NOD-like receptor, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and cysteine protease caspase 1. All components of the NLRP3 inflammasome have been identified in HSCs and were found to correlate with HSC activation and modulate various functions of HSCs [17]. Thus, NLRP3 inflammasome is activated during liver fibrosis. Consistent with these reports, CCl_4 induced the upregulation of the relative protein levels of NLRP3, ASC, and cleaved caspase-1/pro-caspase-1 in liver tissues.

A series of active ingredients isolated from medicinal plants are known to have exhibited anti-liver fibrosis by suppressing activation of NLRP3 inflammasome. Consistently, SSa inhibits NLRP3 inflammasome and autophagy via AMPK/mTOR pathway, which induces the inactivation of pancreatic stellate cells, thereby improving pancreatic fibrosis [16]. Saikosaponin D can also suppress NLRP3 inflammasome activation to relieve liver fibrosis [10,18]. The present study showed that SSa diminished the CCl_4-induced relative levels of markers of the NLRP3 inflammasome in liver tissues, indicating that SSa suppressed CCl_4-induced activation of the NLRP3 inflammasome.

The conserved Hedgehog signaling pathway plays a significant role in mediating survival factors, cell specification and proliferation, and tissue patterning formation during embryonic development. The smoothened receptor (Smo) is released following bond formation between patched receptor PTCH1 and the Hh ligand. Active Smo evokes the release of GliFL from cytoplasmic retention, which eventually transforms into the active form GliA (Gli1 and Gli2) and moves to the nucleus to activate the target genes [19]. In the current study, CCl_4 treatment increased the relative protein expression of PTCH1, GLI1, HIP1, and HDAC1 in liver tissues, suggesting the Hedgehog signaling pathway was activated in CCl_4-induced liver fibrosis, in accordance with previous studies [20,21]. However, SSa treatment markedly attenuated these effects. Saikosaponin B1 and Saikosaponin D inhibit the medulloblastoma via suppressing Hedgehog signaling pathway [22]. Furthermore, SSb2 also exhibits an ameliorative effect on kidney fibrosis by repressing the Hedgehog signaling pathway [23]. Together, these data indicate that SSa reduced CCl_4-induced activation of tHedgehog signaling pathway.

Limitations of the study

Multiple mechanisms are associated with the progression of liver fibrosis, thus the effect of SSa on other mechanisms, such as inflammation and oxidative stress, need to be explored in future studies. In addition, the direct role of Hedgehog signaling pathway should be verified by applying pharmacological blockers or other effective interventions.

CONCLUSION

The results demonstrate that SSa alleviate liver fibrosis and inhibits autophagy and NLRP3

inflammasome activation in CCl_4-induced rats. These are the activities involved in Hedgehog signaling pathway. Thus, SSa can potentially be developed as a therapeutic agent for the management of liver fibrosis.

DECLARATIONS

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Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xinping Wang and Meiling Tang designed and performed the study. Xinping Wang, Yue Ming, Qiang Li, Guiling Li, and Chunmiao Xu supervised the data collection, analyzed the data, and interpreted the data. Xinping Wang and Meiling Tang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

REFERENCES

1. *Aydın MM, Akçalı KC. Liver fibrosis. Turk J Gastroenterol 2018; 29(1): 14-21.*
2. *Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019; 70(1): 151-171.*
3. *Roehlen N, Crouchet E, Baumert TF. Liver Fibrosis: Mechanistic concepts and Therapeutic Perspectives. Cells 2020; 9(4).*
4. *Tao Y, Wang N, Qiu T, Sun X. The role of autophagy and NLRP3 inflammasome in liver fibrosis. Biomed Res Int 2020; 2020: 7269150.*
5. *Kubes P, Mehal WZ. Sterile inflammation in the liver. Gastroenterol 2012; 143(5): 1158-1172.*
6. *Li XQ, Song YN, Wang SJ, Rahman K, Zhu JY, Zhang H. Saikosaponins: a review of pharmacological effects. J Asian Nat Prod Res 2018; 20(5): 399-411.*
7. *Wu SJ, Lin YH, Chu CC, Tsai YH, Chao JC. Curcumin or saikosaponin a improves hepatic antioxidant capacity and protects against CCl4-induced liver injury in rats. J Med Food 2008; 11(2): 224-229.*
8. *Wu SJ, Tam KW, Tsai YH, Chang CC, Chao JC. Curcumin and saikosaponin a inhibit chemical-induced liver inflammation and fibrosis in rats. Am J Chin Med 2010; 38(1): 99-111.*
9. *Chen Y, Que R, Zhang N, Lin L, Zhou M, Li Y. Saikosaponin-d alleviates hepatic fibrosis through regulating GPER1/autophagy signaling. Mol Biol Rep 2021; 48(12): 7853-7863.*
10. *Xu Q, Li J, Chen Z, Mao Y, Tao C. Protective effects of β-eudesmol against septic liver injury via inhibition of NF-κB signaling. Trop J Pharm Res 2022; 21(6).*
11. *National Research Council Committee for the update of the guide for the care and use of laboratory animals: The National Academies Collection: Reports funded by National Institutes of Health. In: Guide for the care and use of laboratory animals. Washington (DC): National Academies Press (US) Copyright © 2011, National Academy of Sciences. 2011.*
12. *Inagaki Y, Okazaki I. Emerging insights into Transforming growth factor beta Smad signal in hepatic fibrogenesis. Gut 2007; 56(2): 284-292.*
13. *Wang R, Song F, Li S, Wu B, Gu Y, Yuan Y. Salvianolic acid A attenuates CCl(4)-induced liver fibrosis by regulating the PI3K/AKT/mTOR, Bcl-2/Bax and caspase-3/cleaved caspase-3 signaling pathways. Drug Des Devel Ther 2019; 13: 1889-1900.*
14. *Chen Y, Liao W, Zhu Z, Chen J, Yang Q, Zheng Y, Zhang X, Limsila B, Lu M, Fu S et al. Essential oil from the raw and vinegar-processed Rhizoma Curcumae ameliorate CCl(4)-induced liver fibrosis: integrating network pharmacology and molecular mechanism evaluation. Food Funct 2021; 12(9): 4199-4220.*

15. Shu Y, Liu X, Huang H, Wen Q, Shu J. Research progress of natural compounds in anti-liver fibrosis by affecting autophagy of hepatic stellate cells. Mol Biol Rep 2021; 48(2): 1915-1924.
16. Cui L, Li C, Zhuo Y, Yang L, Cui N, Li Y, Zhang S. Saikosaponin A inhibits the activation of pancreatic stellate cells by suppressing autophagy and the NLRP3 inflammasome via the AMPK/mTOR pathway. Biomed Pharmacother 2020; 128(110216.
17. Watanabe A, Sohail MA, Gomes DA, Hashmi A, Nagata J, Sutterwala FS, Mahmood S, Jhandier MN, Shi Y, Flavell RA et al. Inflammasome-mediated regulation of hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2009; 296(6): G1248-1257.
18. Lin L, Zhou M, Que R, Chen Y, Liu X, Zhang K, Shi Z, Li Y. Saikosaponin-d protects against liver fibrosis by regulating the estrogen receptor-β/NLRP3 inflammasome pathway. Biochem Cell Biol 2021; 99(5): 666-674.
19. Effendi WI, Nagano T. The Hedgehog signaling pathway in idiopathic pulmonary fibrosis: resurrection time. Int J Mol Sci 2021; 23(1).
20. Jiayuan S, Junyan Y, Xiangzhen W, Zuping L, Jian N, Baowei H, Lifang J. Gant61 ameliorates CCl4-induced liver fibrosis by inhibition of Hedgehog signaling activity. Toxicol Appl Pharmacol 2020; 387(114853.
21. Wei A, Zhao F, Hao A, Liu B, Liu Z. N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) mitigates the liver fibrosis via WTAP/m6A/Ptch1 axis through Hedgehog pathway. Gene 2022; 813: 146125.
22. Luo J, Wang J, Yang J, Huang W, Liu J, Tan W, Xin H. Saikosaponin B1 and Saikosaponin D inhibit tumor growth in medulloblastoma allograft mice via inhibiting the Hedgehog signaling pathway. J Nat Med 2022; 76(3): 584-593.
23. Ren D, Luo J, Li Y, Zhang J, Yang J, Liu J, Zhang X, Cheng N, Xin H. Saikosaponin B2 attenuates kidney fibrosis via inhibiting the Hedgehog Pathway. Phytomed 2020; 67: 153163.