ACTA ABP BIOCHIMICA POLONICA

Vol. 69, No 2/2022
363–370
https://doi.org/10.18388/abp.2020_5802

Regular paper

6-Shogaol alleviates CCl_4-induced liver fibrosis by attenuating inflammatory response in mice through the NF-κB pathway

Jian-li Qiu[1], Yu-na Chai[2], Feng-yang Duan[1], Hui-juan Zhang[1], Xiao-yan Han[3], Ling-yan Chen[4]✉ and Fei Duan[3*]✉

[1]Department of Pediatrics, The First Affiliated Hospital of Henan University of Chinese Medicine, Zhengzhou, Henan, China; [2]Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China; [3]Henan University of Chinese Medicine, Zhengzhou, Henan, China; [4]Department of Rehabilitation, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, China

Liver fibrosis is a global health problem caused by a number of diseases related to liver damage. 6-Shogaol is a biologically active substance derived from the rhizome of *Zingiber officinale Roscoe* with anti-tumor, anti-inflammatory, and antioxidant properties. To explore the effects of 6-Shogaol on liver fibrosis, we used a mouse model of the condition in which mice were injected intraperitoneally with carbon tetrachloride (CCl_4) at a dose of 2 mL/kg three times per week for a period of 4 weeks. 6-Shogaol was administered orally at two different doses (5 mg/kg, 20 mg/kg) 30 min before CCl_4 injection. CCl_4 induced severe liver injury and fibrosis, as indicated by significant inflammatory cell infiltration, disordered liver structure, increased activities of aspartate aminotransferase and alanine aminotransferase (liver damage markers) in serum, elevated collagen deposition, and overexpressed alpha-smooth muscle actin (α-SMA, marker of hepatic stellate cells activation) in liver tissues, whereas 6-Shogaol administration rescued those alterations dose-dependently. We found that 6-Shogaol suppressed CCl_4-induced inflammatory response by inhibiting macrophage recruitment, release of pro-inflammatory factors, and activation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome in liver tissues. Additionally, we demonstrated that 6-Shogaol blocked CCl_4-induced activation of the nuclear factor-kappa B (NF-κB) pathway, which is a vital transcriptional regulator of the inflammatory response. Altogether, this study demonstrates that 6-Shogaol can prevent CCl_4-induced liver fibrosis by suppressing inflammatory response through the NF-κB pathway and suggests that 6-Shogaol can be used for liver fibrosis prevention.

Keywords: inflammatory response; liver fibrosis; NF-κB pathway; 6-Shogaol

Received: 27 July, 2021; revised: 18 October, 2021; accepted: 26 October, 2021; available on-line: 28 April, 2022

✉e-mail: qiuduan001@126.com (FD); 125505219@qq.com (L-yC)
Acknowledgements of Financial Support: This research was funded by the Special Research Project of Traditional Chinese Medicine in Henan Province (2019ZY2017) and the National Natural Science Foundation of China (81804142).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCl_4, carbon tetrachloride; α-SMA, alpha-smooth muscle actin; NLRP3, NOD-like receptor family pyrin domain containing 3; NF-κB, nuclear factor-kappaB; PMSF, phenylmethanesulfonyl fluoride; RIPA, radioimmunoprecipitation assay; TGF-β1, transforming growth factor beta1; TLR4, toll-like receptor 4

INTRODUCTION

Liver fibrosis is a common health problem, usually induced by multiple factors, including alcohol consumption, non-alcoholic fatty liver disease, viral hepatitis, and cholestatic liver diseases (Aydin & Akcali, 2018). If not treated, it may result in advanced cirrhosis, liver failure, or hepatocellular carcinoma (HCC), and eventually death (Bataller & Brenner, 2005). Liver fibrosis is reversible in the early stage but becomes either irreversible or very difficult to reverse in the advanced stage (Ismail & Pinzani, 2009). The early stage of liver fibrosis is usually asymptomatic and often ignored by patients and their families. At present, liver transplantation is the only treatment for patients with advanced liver fibrosis (Manns, 2013). However, limited liver donations and high cost of the procedure hinders the treatment of liver fibrosis (Manns, 2013). Therefore, a search for drugs with therapeutic potential against liver fibrosis is much needed and may provide a basis for adjunct clinical treatment in a number of diseases.

6-Shogaol is a biologically active substance derived from the rhizomes of ginger (*Zingiber officinale Roscoe*) that gives the ginger its pungent taste (Semwal *et al*., 2015). 6-Shogaol exerts a variety of biological activities, such as anti-cancer, antioxidative, antibacterial, anti-inflammatory, and anti-allergic (Dugasani *et al*., 2010; Semwal *et al*., 2015). 6-Shogaol also has a hepatoprotective effect (Kim *et al*., 2014; Zhang *et al*., 2019; Zhuang *et al*., 2015). In addition, 6-Shogaol was reported to inhibit transforming growth factor beta1 (TGF-β1)-induced collagen type I alpha1 (COL1A1) expression and Smad2 phosphorylation in intestinal fibroblasts (Hiraishi *et al*., 2018). COL1A1 contributes to excessive tissue deposition of extracellular matrix (ECM) proteins and Smad2 is a vital factor for myofibroblast transformation (Xu *et al*., 2016). Therefore, 6-Shogaol may potentially prevent liver fibrosis as well.

The progression of liver fibrosis is dynamic and is characterized by excessive ECM production and activation of the hepatic stellate cells (Puche *et al*., 2013; Yin *et al*., 2013). Inflammation is an important contributor to liver fibrosis. A large number of inflammatory factors are produced during the process of liver fibrosis, which further activates hepatic stellate cells (Koyama & Brenner, 2017; Sanchez-Valle *et al*., 2012). Targeted suppression of inflammatory response is reported to alleviate liver fibrosis. For instance, progranulin attenuated carbon tetrachloride (CCl_4)-induced liver fibrosis by downregulating the inflammatory response (Yoo *et al*., 2019). Macrophage Sphingosine 1-Phosphate Receptor 2 blockade attenuated bile duct ligation-induced liver inflammation and fibrosis by inhibiting the activation of NLR family

pyrin domain containing 3 (NLRP3) inflammasome (Hou *et al.*, 2020). Therefore, inflammation is an important therapeutic target in alleviating liver fibrosis. 6-Shogaol is reported to play an anti-inflammatory role in a variety of pathological processes, including asthma (Yocum *et al.*, 2020), multiple sclerosis (Sapkota *et al.*, 2019), and endometriosis (Wang *et al.*, 2018). Based on the above, this study aimed to explore the effects of 6-Shogaol on liver fibrosis and we speculated that 6-Shogaol might prevent liver fibrosis by inhibiting the inflammatory response.

MATERIALS AND METHODS

CCl_4-induced liver fibrosis model

Healthy male C57BL/6 mice (20–25 g) at age of 8 weeks were obtained from Liaoning Changsheng Biotechnology Co., Ltd. (China). All mice were adaptively fed for one week, had free access to diet and water, and were kept in a house with a 12 hours dark-light cycle. All the animal experiments were conducted following the National Institutes of Health Guide for the care and use of laboratory animals and approved by the ethic committee of First Affiliated Hospital of Henan University of Chinese Medicine.

As described earlier (Ki *et al.*, 2013), liver fibrosis was induced in a murine model by a well-established method using CCl_4. Mice were randomly divided into four groups: control group, which received intraperitoneal (i.p.) injections of olive oil (10%) (2 mL/kg) three times per week for 4 weeks; CCl_4 group, in which mice received CCl_4 solution injections (CCl_4 dissolved in 10% olive oil) at a dose of 2 mL/kg i.p. 3 times a week for 4 weeks; CCl_4+6-Shogaol (5 mg/kg or 20 mg/kg) groups, in which 6-Shogaol was administered orally 30 min before each CCl_4 injection. On day 28 mice were sacrificed and serum samples and liver tissues were collected.

Serum enzyme activities

Liver damage markers: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were measured with commercial AST and ALT detection kits (Wanleibio, Shenyang, China) according to the manufacturer's instructions.

Histological Examinations

After fixed by 4% paraformaldehyde, the liver tissues were rinsed under running water for 4 hours. Tissues were then dehydrated with increasing concentration of ethanol, cleared with xylene, and embedded in liquid paraffin. The paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin to observe liver tissue structure and inflammation. Sections were stained with Sirius Red to assess collagen deposition in liver tissues. An Olympus BX53 microscope was used to obtain the images (Tokyo, Japan).

Immunofluorescence assay

Immunofluorescence staining of liver sections was conducted to evaluate alpha-smooth muscle actin (α-SMA), NLRP3, and p65 expression, and the number of CD68-positive cells in liver tissues. In short, sections were dewaxed using xylene. By using gradient ethanol series, sections were gradually hydrated. After antigen retrieval, sections were blocked with goat serum (Solarbio, Beijing, China) for 15 minutes. The slides were then incubated with primary antibody (1:100 dilution) against α-SMA, NLRP3, p65, or CD68 overnight at 4ºC. Rabbit (host) anti-CD68 was purchased from ABclonal (https://abclonal.com.cn/, Wuhan, China) and rabbit anti-α-SMA, rabbit anti-NLRP3, and rabbit anti-p65 were obtained from Affinity (http://www.affbiotech.cn/, Cincinnati, OH, USA). After washing with PBS, the sections were incubated with Cy3-labeled goat anti-rabbit IgG (1:200 dilution, Beyotime, Shanghai, China) for 1 hour at room temperature. After washing, sections were stained with 4',6-diamidino-2-phenylindole (DAPI, Aladdin, Shanghai, China). A fluorescence microscope (Olympus BX53) was used to obtain images.

Western blot

Total protein was collected from liver tissues using commercial radioimmunoprecipitation assay (RIPA) buffer (Beyotime) supplemented with phenylmethanesulfonyl fluoride (PMSF, Beyotime). Nuclear protein extraction was performed using a nuclear protein extraction kit (Beyotime) according to the manufacturer's instructions. A BCA protein assay kit (Beyotime) was used to determine the protein concentration. Proteins were separated on an SDS-PAGE gel and then transferred onto PVDF membranes. After blocking with TBST buffer containing 5% bovine serum albumin, the membranes were incubated with primary antibody against TGF-β (1:1000 dilution), COL1A1 (1:1000 dilution), type III collagen (COL3A1, 1:1000 dilution), fibronectin (1:1000 dilution), α-SMA (1:1000 dilution), NLRP3 (1:1000 dilution), cleaved caspase-1 (p20, 1:1000 dilution), cleaved interleukin-1beta (IL-1β) (p17, 1:1000 dilution), I-κBα (1:1000 dilution), p-I-κBα (Ser32, 1:1000 dilution), p65 (1:1000 dilution), p-p65 (Ser536, 1:1000 dilution), histone H3 (1:500 dilution) or β-actin (1:2000 dilution) overnight at 4ºC. All the primary antibodies were purchased from Affinity except for anti-histone H3 (Proteintech, Wuhan, China) and anti-β-actin (Proteintech, Wuhan, China). After washing with TBST, the membranes were blotted with goat anti-mouse secondary antibodies (1:10000 dilution; Proteintech). The protein bands were visualized using ECL chemiluminescence solution (7 Sea biotech, Shanghai, China). Histone H3 and β-actin served as internal controls.

Detection of proinflammatory cytokines

The homogenized tissues and blood samples were centrifuged to obtain tissue supernatant and serum, respectively. The levels of cytokines were measured using commercial mouse ELISA kits (LianKe Biotech, Hangzhou, China) according to the manufacturer's protocols.

Statistical analysis

Data are presented as mean ±S.D. Comparisons between groups were performed with one-way ANOVA with Tukey's test. GraphPad Prism software version 8.0 was used for data analysis. A p-value <0.05 was considered significant. At least 6 mice in each group were used for index detection.

RESULTS

6-Shogaol administration improves CCl_4-induced liver damage in mice.

The effect of 6-Shogaol on CCl_4-induced liver damage in mice was first examined. After CCl_4 injection,

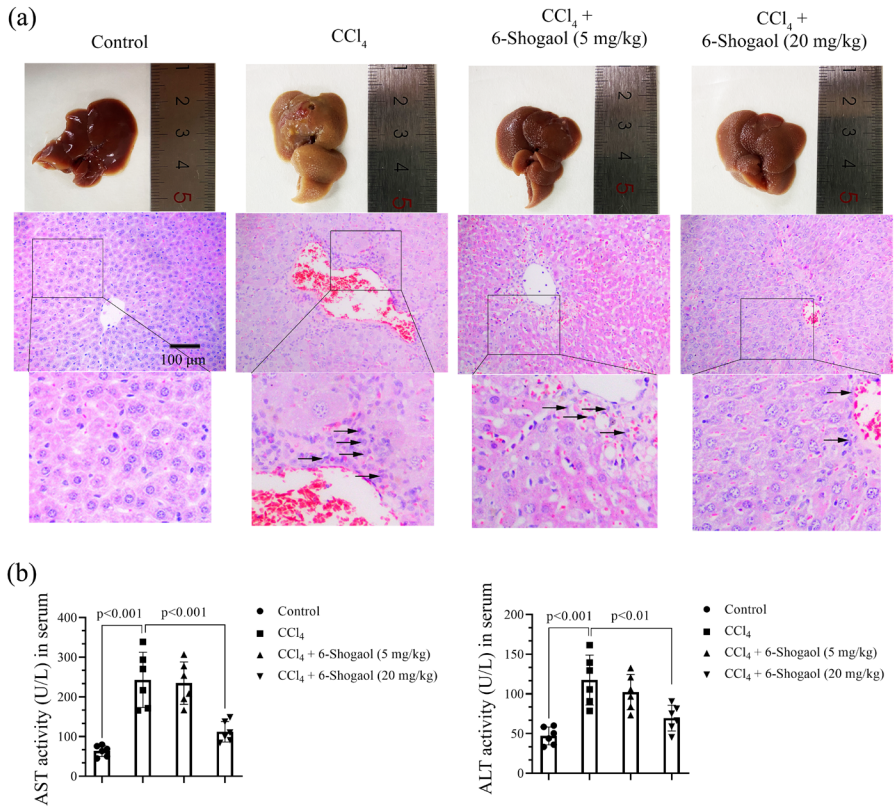

Figure 1. 6-Shogaol treatment prevents CCl_4-induced liver damage in mice.
(**a**) The images of liver and hepatic tissue H&E staining. The arrows indicate inflammatory cell infiltration. (**b**) The activities of AST and ALT in serum. Values were expressed as mean ± standard deviation. H&E, hematoxylin and eosin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

mice exhibited significant inflammatory cell infiltration and disordered liver structure (Fig. 1a). Moreover, the enzyme activities of liver damage markers ALT and AST in serum were markedly increased in this group (Fig. 1b). The data indicated that CCl_4 induced severe liver injury. However, 6-Shogaol administration ameliorated liver damage induced by CCl_4 in a dose-dependent manner, as indicated by decreased inflammatory cell infiltration and ALT and AST enzyme activities (Fig. 1a–b).

6-Shogaol administration attenuates CCl_4-induced liver fibrosis in mice

Sirius Red staining of liver tissue was performed to observe the collagen disposition. The results showed that hepatic collagen disposition in mice was significantly increased by CCl_4 injection, but this elevation was attenuated by 6-Shogaol dose-dependently (Fig. 2a). α-SMA, a marker of hepatic stellate cells (HSCs) activation, was reported to play a vital role in liver fibrosis progression (Puche *et al.*, 2013). Data in Fig. 2b indicated that CCl_4 injection induced α-SMA expression, which was inhibited by 6-Shogaol in a dose-dependent manner. Furthermore, the protein levels of COL1A1 (encoding collagen-I), COL3A1 (encoding collagen-III), fibronectin, and α-SMA were markedly upregulated in CCl_4-treated mice but their levels dropped in 6-Shogaol (5 mg/kg and 20 mg/kg)-treated mice (Fig. 2c). Overall, these results suggested that 6-Shogaol might alleviate CCl_4-induced liver fibrosis.

6-Shogaol administration reduces CCl_4-induced inflammatory response in liver tissues of mice

Given that inflammatory response plays a key role in liver fibrosis, we explored the role of 6-Shogaol in CCl_4-induced inflammatory response. As presented in Fig. 3a, a number of CD68 (macrophage marker)-positive cells was increased remarkably in CCl_4-treated mice; this phenomenon was inhibited by 6-Shogaol in a dose-dependent manner. Meanwhile, the levels of pro-inflammatory factors (IL-6, TNF-α, and MCP-1) in liver tissues and serum were significantly increased by CCl_4, whereas 6-Shogaol administration suppressed the production of inflammatory factors (Fig. 3b–3c). All the data indicated that 6-Shogaol relieved CCl_4-induced inflammatory response in mice.

6-Shogaol administration inhibits CCl_4-induced NLRP3 inflammasome activation in liver tissues of mice

The NLRP3 inflammasome regulates the cleavage of IL-1β and IL-18 precursors into mature forms to mediate the inflammatory response. Results of immunofluorescence assay showed that NLRP3 expression was significantly upregulated in CCl_4-treated mice but decreased back by 6-Shogaol in a dose-dependent manner (Fig. 4a). Furthermore, changes in expression of NLRP3 inflammasome-related factors were confirmed by Western blot: NLRP3, cleaved caspase-1, and cleaved IL-1β protein levels were elevated by CCl_4 and rescued by 6-Shogaol (Fig. 4b). Similarly, levels of IL-1β and IL-18 detected with ELISA showed that the induction of IL-1β and IL-18 by CCl_4 was reversed by 6-Shogaol (Fig. 4c). Collec-

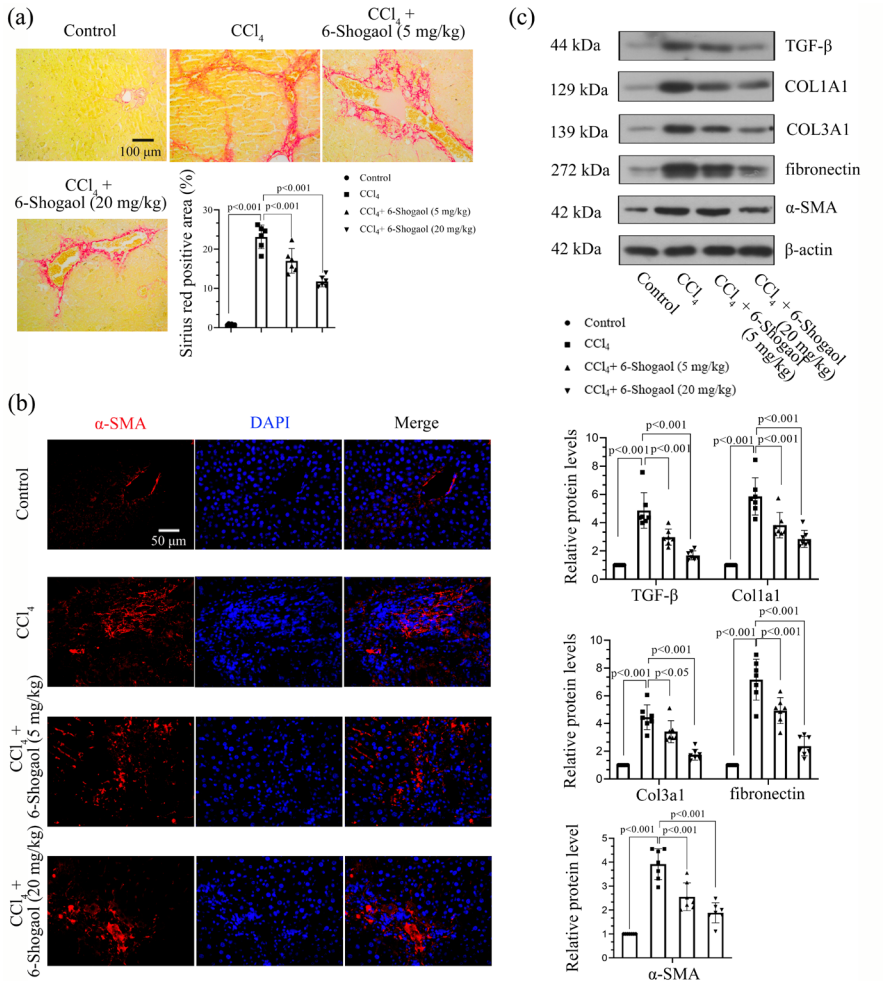

Figure 2. 6-Shogaol treatment prevents CCl_4-induced liver fibrosis in mice.
(**a**) Sirius Red staining of liver sections and its quantification (**b**) Immunofluorescence staining of liver sections for α-SMA. (**c**) Relative protein expression of TGF-β, COL1A1, COL3A1, fibronectin, and α-SMA in liver tissues. Values were expressed as mean ± standard deviation. α-SMA, alpha-smooth muscle actin; TGF-β, transforming growth factor-beta.

tively, the results indicated that 6-Shogaol could suppress CCl_4-induced NLRP3 inflammasome activation.

6-Shogaol administration blocks CCl_4-induced NF-κB pathway activation in liver tissues of mice

The NF-κB pathway is considered a prototypical pro-inflammatory signaling pathway. We thus investigated whether 6-Shogaol regulated the NF-κB pathway. Nuclear translocation of p65 in hepatocytes was first measured in each group. Liver tissue immunofluorescent staining presented in Fig. 5a showed that CCl_4 induced nuclear translocation of p65, which was inhibited by 6-Shogaol in a dose-dependent manner. Furthermore, the phosphorylation of I-κBα (Ser32) and p65 (Ser536) as well as p65 protein level in the nucleus was increased in CCl_4-treated mice, whereas 6-Shogaol administration reduced this increase (Fig. 4b). Conversely, the I-κBα protein level in CCl_4-treated mice was decreased but increased by 6-Shogaol (Fig. 4b). The data suggested that 6-Shogaol inhibited CCl_4-induced NF-κB pathway activation in mice.

DISCUSSION

The current study demonstrated that 6-Shogaol administration had beneficial effects in a hepatic fibrosis mouse model. 6-Shogaol administration decreased CCl_4-induced inflammatory cell infiltration, the release of pro-inflammatory factors, and NLRP3 inflammasome activation, which led to reduced hepatic fibrosis. The underlying mechanisms responsible for the improvement of hepatic inflammation in CCl_4-treated mice were further explored. 6-Shogaol administration was proved to inhibit CCl_4-induced NF-κB pathway activation in mice. NF-κB is a vital transcriptional regulator of the inflammatory response and exerts an important role in the regulation of inflammatory signaling in the liver (Luedde & Schwabe, 2011). Altogether, the results suggest that 6-Shogaol may relieve CCl_4-induced liver fibrosis *in vivo*.

As the main cell type in the liver, hepatocytes play an important role in the maintenance of liver homeostasis (Qian *et al.*, 2015). Liver cell injury leads to increased inflammatory cell infiltration and the release of pro-inflammatory cytokines. Therefore, liver cell injury is the initial inducer of liver fibrosis. By evaluating the levels of liver inflammation and liver injury enzyme markers (AST and ALT), we found that 6-Shogaol alleviated CCl_4-induced liver inflammation and injury. Excessive accumulation of extracellular matrix (ECM) components (collagen is the main structural component) and the activation of hepatic stellate cells (HSCs) caused by chronic injury are the main features of liver fibrosis (Puche

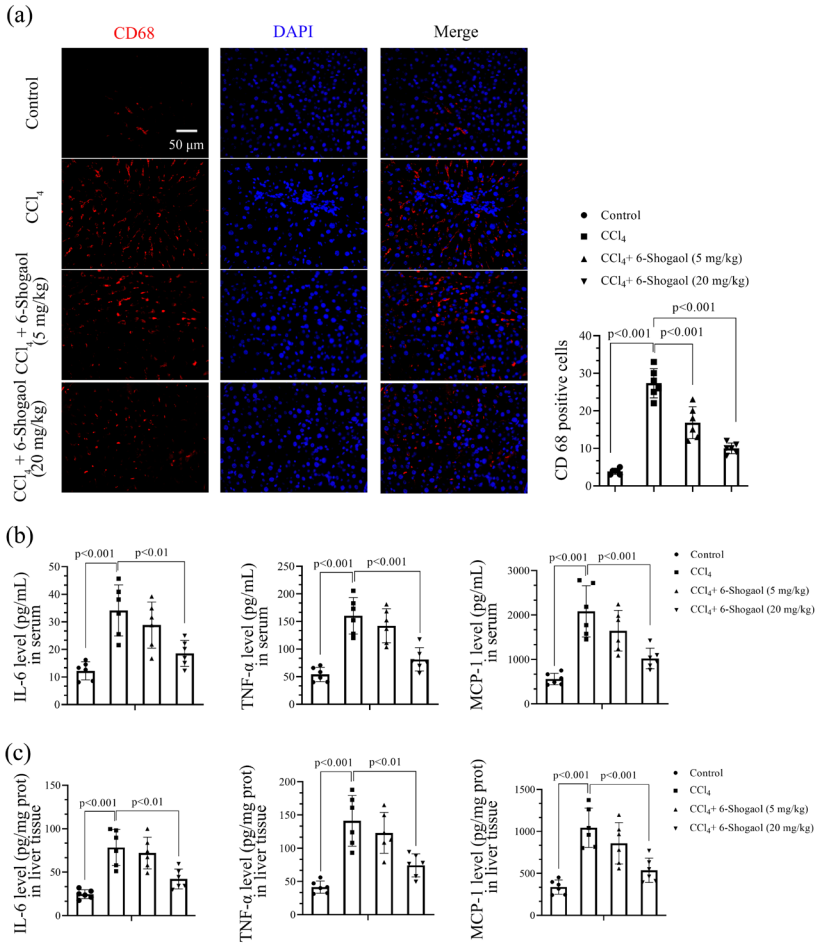

Figure 3. 6-Shogaol treatment ameliorates CCl_4-induced inflammatory response in the liver tissues of mice.
(**a**) CD68 immunofluorescence staining of the liver and quantification of CD68-positive cells. (**b & c**) Levels of pro-inflammatory cytokines (IL-6, TNF-α, and MCP-1) in serum and liver tissues. Values were expressed as mean ± standard deviation. IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1

et al., 2013; Yin *et al.*, 2013). The gradual accumulation of the ECM leads to excessive collagen deposition (Iredale, 2007). Inflammation activates HSCs, which convert into collagen-producing myofibroblasts (Elpek, 2014). Excessive deposition of collagen and the increase of myofibroblasts lead to the occurrence of liver fibrosis and the disorder of liver physiological function. In this study, we proved that 6-Shogaol alleviated CCl_4-induced collagen deposition. Furthermore, by evaluating α-SMA we found that 6-Shogaol inhibited CCl_4-induced expression of this marker of HSC activation. This suggests that 6-Shogaol may suppress the activation of HSCs. In general, 6-Shogaol may protect against development of liver fibrosis.

Inflammation is considered to be the main inducer of liver fibrosis (Koyama & Brenner, 2017). Kupffer cells, located mainly in the sinusoidal cavity and accounting for about 30% of the sinusoidal cells, play a major role in liver inflammation (Bouwens *et al.*, 1986). In response to various injury stimuli, macrophages are recruited to the injury site and secrete large amounts of pro-inflammatory factors (Cohn *et al.*, 1989). Previous studies have shown that 6-Shogaol has anti-inflammatory effects in a variety of pathological processes. Specifically, 6-Shogaol attenuated the inflammatory response of microglia induced by lipopolysaccharide (LPS) (Han *et al.*, 2017). Moreover, 6-Shogaol suppressed lung inflammation in asthmatic mice (Yocum *et al.*, 2020) and reduced kidney inflammation in ischemic kidney injury (Han *et al.*, 2019). The present study showed that 6-Shogaol inhibited the recruitment of hepatic macrophages and the release of pro-inflammatory factors in liver fibrosis mouse model. The activation of NLRP3 inflammasome has been reported to be another important factor in inducing inflammation. NLRP3 inflammasome activation mediates the activation of caspase-1, leading to the maturation of effector pro-inflammatory cytokines, such as pro-IL-1β and pro-IL-18 (Strowig *et al.*, 2012). Previous studies found that the activation of liver caspase-1 occurred in both marrow-derived Kupfer cells (macrophages) and hepatocytes, and this activation appeared to be mediated by NLRP3 inflammasomes (Dixon *et al.*, 2012). Additionally, activation of NLRP3 inflammasome led to severe liver inflammation and collagen deposition (Wree *et al.*, 2014). Our results showed that 6-Shogaol inhibited NLRP3 inflammasome activation in CCl_4-treated mice, as evidenced by decreased cleaved caspase-1, IL-1β, and IL-18 levels in liver tissues. These results corroborate that 6-Shogaol plays an anti-inflammatory role in liver fibrosis. It is worth noting that 6-Shogaol also has an antioxidant effect (Na *et al.*, 2016). Oxidative stress is one of the causes of liver fibrosis (Luangmonkong *et al.*, 2018). Therefore, 6-Shogaol may exert the protective effects of liver fibrosis in many ways, including anti-inflammatory and antioxidant.

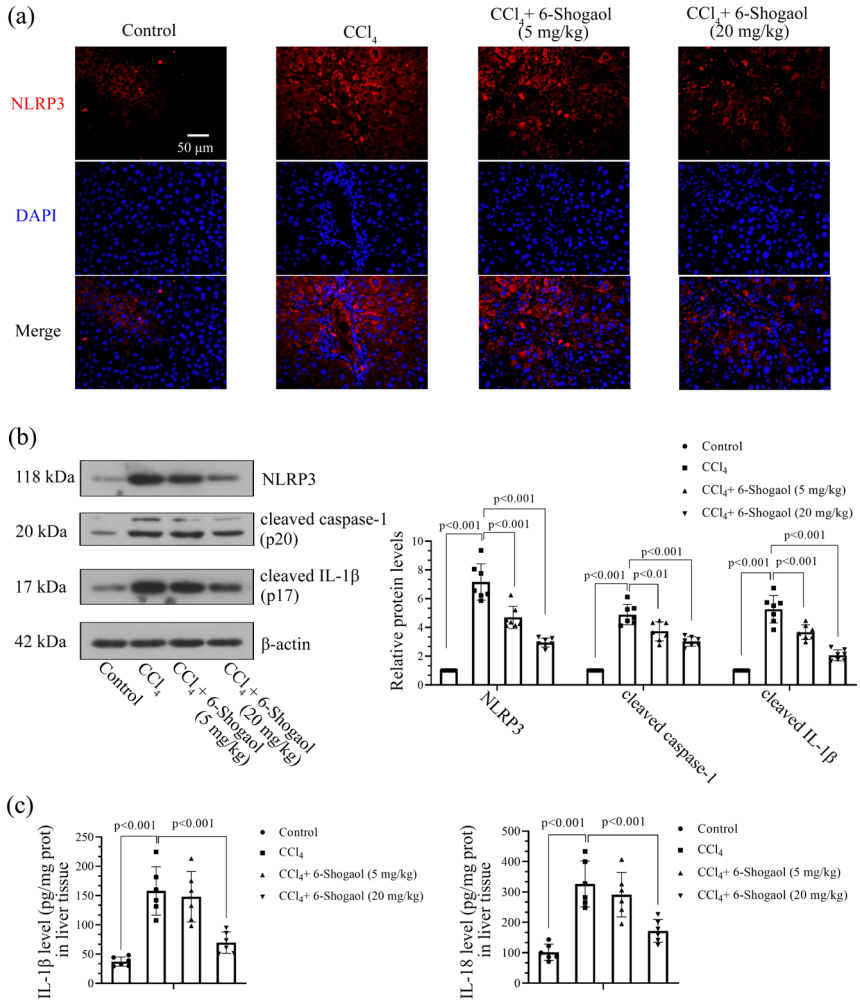

Figure 4. 6-Shogaol treatment inhibits CCl4-induced NLRP3 inflammasome activation in liver tissues of mice.
(**a**) NLRP3 immunofluorescence staining of the liver. (**b**) Relative protein expression of NLRP3-cleaved caspase-1 (p20), and cleaved IL-1β (p17) in liver tissues. (**c**) Levels of IL-1β and IL-18 in liver tissues. Values were expressed as mean ± standard deviation. NLRP3, NOD-like receptor family pyrin domain containing 3; IL-1β, interleukin-1beta; IL-18, Interleukin-18

We further explored the mechanism of 6-Shogaol anti-inflammatory effect in liver fibrosis. The NF-κB pathway is considered to be a typical positive regulatory pathway of inflammatory response, including regulating the activation of NLRP3 inflammasome (Afonina *et al.*, 2017). The NF-κB pathway is activated in a variety of chronic liver diseases, including alcoholic liver disease, non-alcoholic fatty liver, viral hepatitis, and biliary liver disease (Boya *et al.*, 2001; Kosters & Karpen, 2010; Mandrekar & Szabo, 2009; Videla *et al.*, 2009). Meanwhile, the NF-κB pathway was reported to be activated in liver fibrosis as well, and NF-κB pathway inhibition attenuated the induction of liver fibrosis by CCl_4 (Wei *et al.*, 2019; Zheng *et al.*, 2019). Previous studies reported that in an ischemia-reperfusion induced renal injury model 6-Shogaol alleviated renal tissue inflammation by inhibiting the activation of the NF-κB pathway (Han *et al.*, 2019). Similar to previous studies, we demonstrated that 6-Shogaol inhibited the activation of the NF-κB pathway in CCl_4-induced liver fibrosis. Therefore, the ameliorating effect of 6-Shogaol on CCl_4-induced liver fibrosis may be achieved through regulating the NF-κB pathway. Furthermore, in pancreatic cancer, toll-like receptor 4 (TLR4) mediated the activation of the NF-κB pathway and 6-Shogaol inhibited TLR4 expression (Zhou *et al.*, 2014). The question whether in liver fibrosis 6-Shogaol affects the NF-κB pathway directly or is mediated by other factors, such as TLR4, needs further investigation. Furthermore, it was reported that 6-Shogaol protected melanocytes against oxidative stress through activation of the Nrf2 pathway (Yang *et al.*, 2020) and this pathway plays a protective role in liver fibrosis (Shi *et al.*, 2020). The finding suggests that 6-Shogaol may play a protective role in liver fibrosis through multiple signaling pathways. In the present study, the 6-Shogaol/NF-κB pathway/inflammatory response axis has been identified as a possible way in which 6-Shogaol protects the liver.. However, hepatotoxic activity of CCl_4 is mainly due to lipid covalent binding and peroxidation (Boll *et al.*, 2001) and cannot fully model clinical conditions. Although covalent binding and lipid peroxidation induce liver fibrosis, the inflammation, mitochondrial dysfunction, and ROS accumulation can also induce the liver fibrosis process (Scholten *et al.*, 2015). The present study shows a protective effect of 6-Shogaol in CCl_4-induced liver fibrosis, but it does not determine whether it results from its anti-inflammatory potential or other properties.

In conclusion, the results of this study indicated that 6-Shogaol might play an anti-inflammatory role in CCl_4-induced liver fibrosis by inhibiting the recruitment of

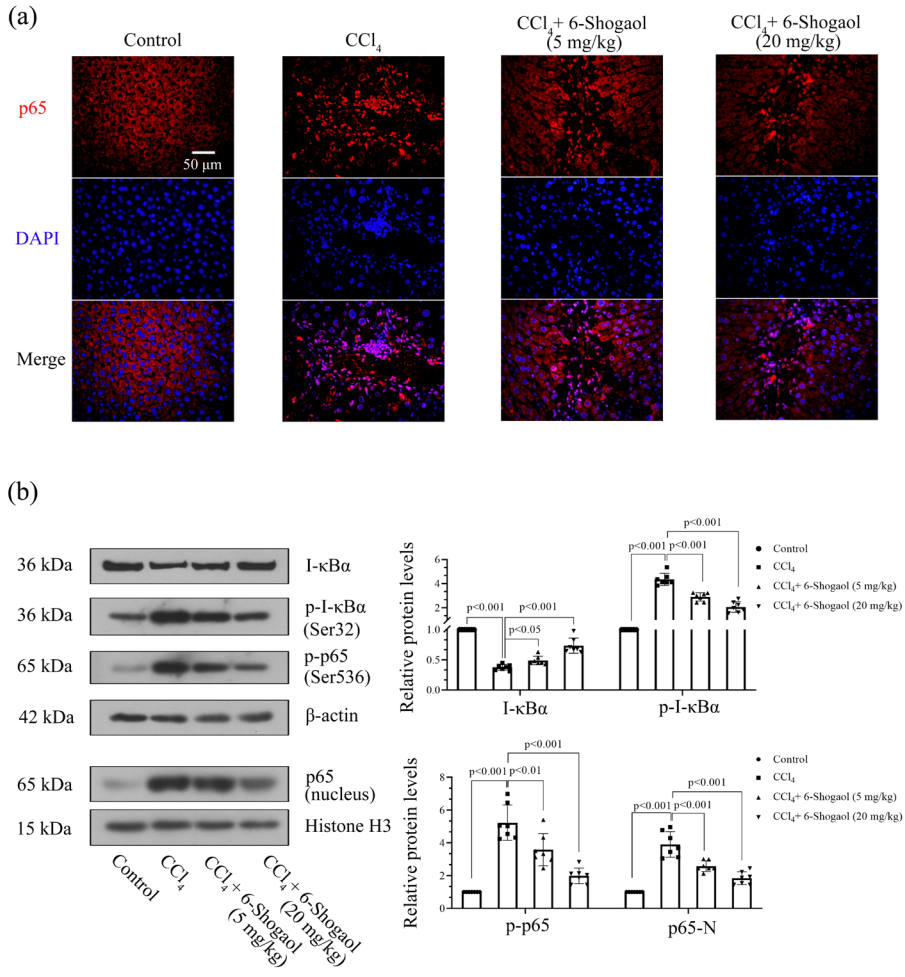

Figure 5. 6-Shogaol treatment blocks CCl4-induced NF-κB pathway activation in liver tissues of mice.
(**a**) Liver p65 immunofluorescence staining. (**b**) Relative protein expression of I-κBα, p-I-κBα (Ser32), p-p65 (Ser536), and p65 (nucleus) in liver tissues. Values were expressed as mean ± standard deviation.

macrophages, the release of pro-inflammatory factors, and the activation of NLRP3 inflammasome. Moreover, the observed inhibitory effect of 6-Shogaol on the inflammatory response could be explained by suppression of the NF-κB pathway. Our findings may provide new ideas for the clinical treatment of liver fibrosis.

Declaration

The authors declare that there is no conflict of interests.

Data availability statement

The data will be made available from the corresponding author on reasonable request.

Acknowledgements

Not applicable

Author contribution

Jian-li Qiu designed the experiments and wrote the paper. Yu-na Chai and Feng-yang Duan performed the experiments and analyzed the data. Hui-juan Zhang and Xiao-yan Han contributed reagents/materials/analysis tools. Ling-yan Chen and Fei Duan were responsible for supervision, writing-reviewing, and editing.

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