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Hepatoprotection of yangonin against hepatic fibrosis in mice via farnesoid X receptor activation

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ABSTRACT

Hepatic fibrosis is a reversible would-healing response following chronic liver injury of different aetiologies and represents a major worldwide health problem. Up to date, there is no satisfactory drugs treated for liver fibrosis. The present study was to investigate hepatoprotection of yangonin against liver fibrosis induced by thioacetamide (TAA) in mice and further to clarify the involvement of farnesoid X receptor (FXR) in vivo and in vitro. Yangonin treatment remarkably ameliorated TAA-induced liver injury by reducing relative liver weight, as well as serum ALT and AST activities. Moreover, yangonin alleviated TAA-induced accumulation of bile acids through increasing the expression of bile acid efflux transporters such as Bsep and Mrp2, and reducing hepatic uptake transporter Ntcp expression, all of these are FXR-target genes. The liver sections stained by H&E indicated that the histopathological change induced by TAA was improved by yangonin. Masson and Sirius red staining indicated the obvious anti-fibrotic effect of yangonin. The mechanism of anti-fibrotic effect of yangonin was that yangonin reduced collagen content by regulating the genes involved in hepatic fibrosis including COL1-α1 and TIMP-1. Besides, yangonin inhibited hepatic stellate cell activation by reducing TGF-β1 and α-SMA expression. In addition, yangonin protected against TAA-induced hepatic inflammation via its inhibition of NF-κB and TNF-α. These hepatoprotective effects of yangonin were abrogated by guggulsterone which is a FXR antagonist. In vitro experiment further demonstrated dose-dependent activation of FXR by yangonin using dual-luciferase reporter assay. In summary, yangonin produces hepatoprotection against TAA-induced liver fibrosis via FXR activation.

1. Introduction

Hepatic fibrosis is a common and devastating manifestation in many chronic liver diseases such as alcoholic liver disease, nonalcoholic fatty liver disease, cholestatic liver disease and viral hepatitis [1–3]. Although removal of the underlying injurious process (e.g., with antiviral therapy) may halt the progression of liver fibrosis, liver transplantation remains the only effective treatment for the endstage of liver fibrosis including advanced fibrosis, liver cirrhosis, and even hepatocellular carcinoma [4,5]. Thus, developing drugs with effective anti-fibrotic activities is urgently needed.

The pathogenesis of hepatic fibrosis is associated with the accumulation of the extracellular matrix [6]. The excessive extracellular matrix is produced by activated mesenchymal cells which resemble myofibroblasts [7]. They derive from quiescent hepatic stellate cells and periportal or perivenular fibroblasts. Except for these cells, other cells such as hepatocytes, Kupffer cells and liver sinusoidal endothelial cells are also involved in the formation and relief of hepatic fibrosis [8]. Nuclear receptors have been demonstrated to modulate a variety of metabolic processes including bile acid homeostasis and liver fibrosis [9]. Farnesiod X receptor (FXR, NR1H4) is a member of the nuclear receptor superfamily highly expressed in liver. FXR agonist was reported to alleviate liver fibrosis by reducing hepatic gene expression of fibrotic markers such as transforming growth factor-β1 (TGF-β1), tissue inhibitor of metalloproteinase-1 (TIMP-1) and α-smooth muscle actin (α-SMA) [10]. Moreover, FXR agonist has been shown to protect

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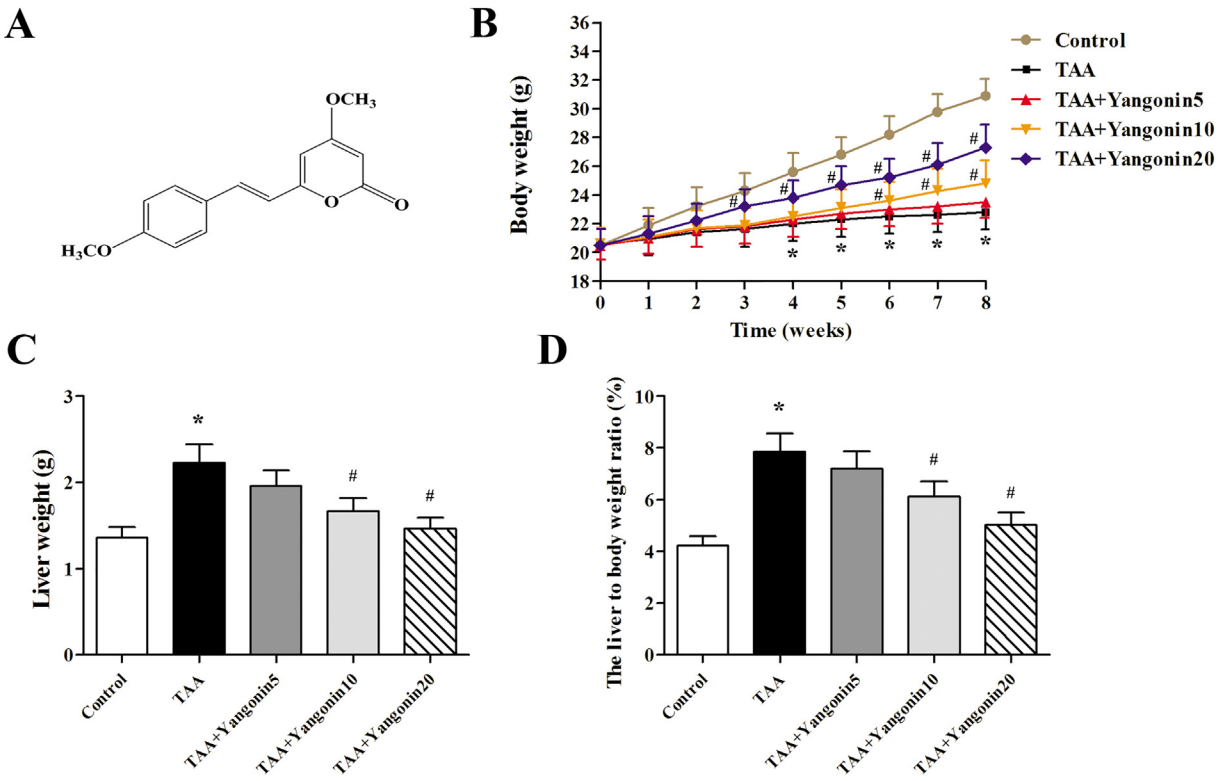

Fig. 1. Yangonin reverses the changes in body weight and the liver to body weight ratios induced by TAA in mice. (A) The chemical structure of yangonin. (B) Yangonin treatment dose-dependently reversed the TAA-induced decrease in body weight gain. (C) Effect of yangonin on liver weight. (D) Yangonin reversed the TAA-induced increase in relative liver weight ratios. Data are presented as mean ± S.D. (n = 6). * P < 0.05 versus Control; # P < 0.05 versus TAA alone.

against fibrosis in cholestatic and non-alcoholic steatohepatitis animal models [11,12]. FXR activation can also inhibit the activation of hepatic stellate cells [13]. In addition, obeticholic acid, a synthetic FXR agonist, has been demonstrated to have protective effect against hepatic inflammation through inhibiting the nuclear factor-κB (NF-κB) [14]. Thus, FXR activation is considered to be a promising therapeutic strategy in fibrotic diseases.

Yangonin (Fig. 1A) is a natural product isolated from Kava that is a perennial tropical shrub widely cultivated in the south pacific island countries [15,16]. Yangonin was found by us to activate FXR by binding to the FXR ligand binding domain [17]. Pharmacological studies have demonstrated that yangonin has anti-oxidant and anti-tumor activities [18,19]. Noteworthly, yangonin has been demonstrated to have hepatoprotective activities [20]. So, an intriguing and important question arises whether yangonin can protect against liver fibrosis. And another further question is that whether the activation of FXR signaling pathway is involved in the hepatoprotection of yangonin against liver fibrosis.

This study is to evaluate the protective effects of yangonin against hepatic fibrosis induced by thioacetamide (TAA), and further to clarify the involvement of FXR in vivo and in vitro.

2. Materials and methods

2.1. Materials

Yangonin and guggulsterone (GS, purity > 98%) were obtained from Sigma-Aldrich (St. Louis, MO). Thioacetamide (TAA) and Masson's Trichrome staining kit were from Solarbio Company (Beijing, China). Primary antibodies against Bsep, Mrp2 and Ntcp were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Animal experiments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Dalian Medical University and were carried out in accordance with the guide for Institutional Animal Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. After one week of acclimatization, thirty-six male C57BL/6J mice (RRID: IMSR_JAX: 000664) weighing 20–25 g (6–8 weeks) were divided into six groups (n = 6 per group) and yangonin (5, 10 or 20 mg/kg) was dissolved in olive oil: (1) Control group in which mice were not administered any treatment but normal saline (i.p.) and intragastric gavage with olive oil; (2) TAA group in which mice were intraperitoneally injected with TAA (100 mg/kg) and intragastrically administered olive oil; (3) TAA + Yangonin5 group in which mice were intraperitoneally injected with TAA (100 mg/kg) and intragastrically administered yangonin (5 mg/kg); (4) TAA + Yangonin10 group in which mice were intraperitoneally injected with TAA (100 mg/kg) and intragastrically administered yangonin (10 mg/kg); (5) TAA + Yangonin20 group in which mice were intraperitoneally injected with TAA (100 mg/kg) and intragastrically administered yangonin (20 mg/kg); (6) TAA + Yangonin + GS group in which mice were intraperitoneally injected with TAA (100 mg/kg) and GS (10 mg/kg, 4 h before vehicle administration) and intragastrically administered yangonin (20 mg/kg). Mice were intraperitoneally injected TAA on Monday, Wednesday and Friday, and orally administered yangonin on Tuesday, Thursday and Saturday for 8 weeks. At 12 h after yangonin administration for the last time, mice were sacrificed under anesthesia (65 mg/kg pentobarbital sodium, i.p.). Blood and liver were collected.

2.3. Serum biochemical analysis

Serum aspartate aminotransferase (AST), alanine aminotransferase

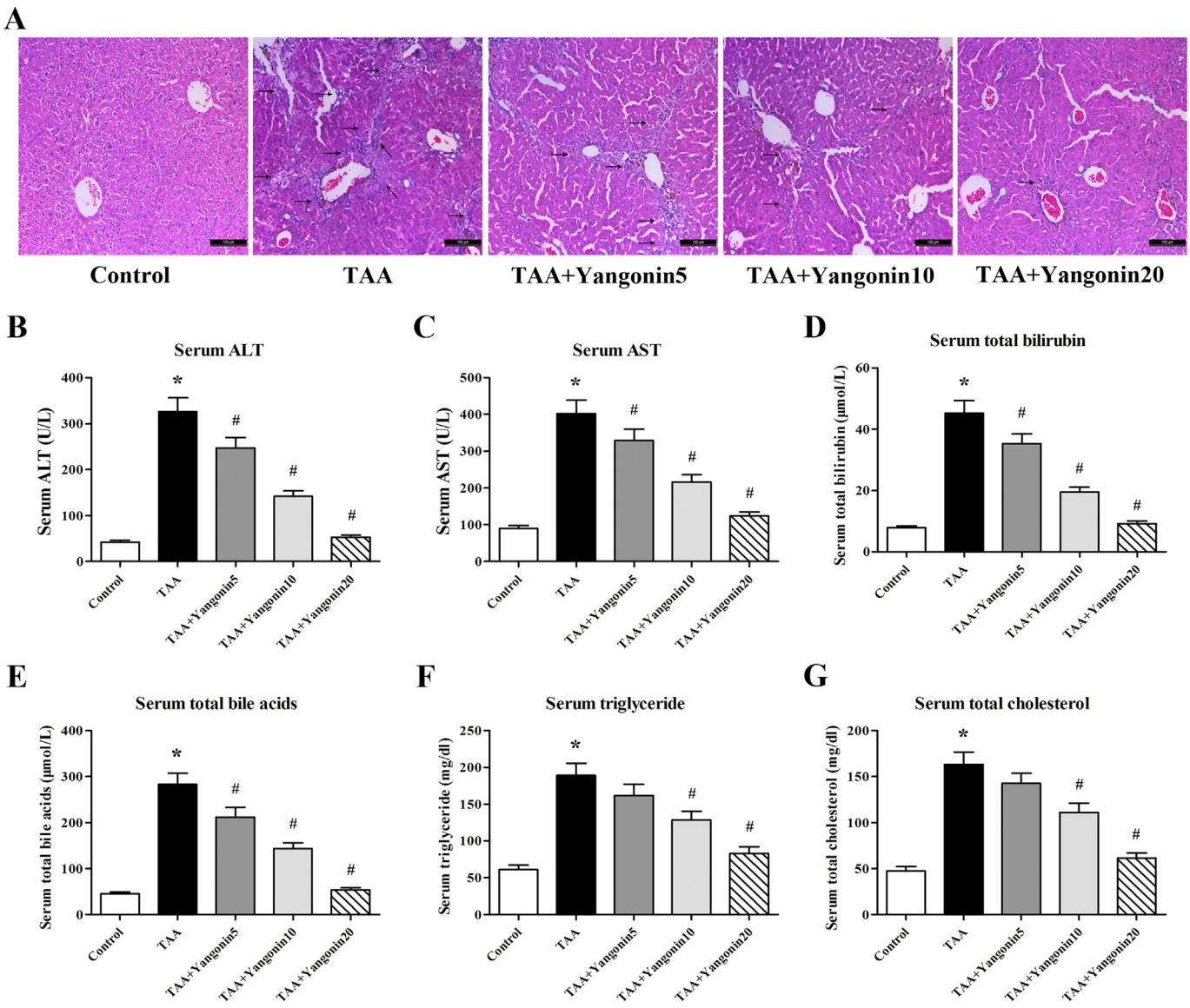

Fig. 2. Yangonin ameliorates TAA–induced liver injury in mice. (A) Representative photomicrographs of liver sections stained by H&E (200 × magnification). Areas of severe liver necrosis and inflammatory cell infiltration were marked by arrows. (B) Serum ALT (B) and AST (C) levels elevated by TAA were significantly reduced by treatment with different doses of yangonin. Likewise, serum total bilirubin (D) and total bile acids (E) were also increased by TAA and were remarkably decreased by yangonin. Effect of yangonin on serum levels of triglyceride (F) and total cholesterol (G). Data are presented as mean ± S.D. ($n = 6$). * $P < 0.05$ versus Control; # $P < 0.05$ versus TAA alone.

(ALT), total bilirubin, total bile acids, triglycerides and total cholesterol were determined by indicators kits purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China).

2.4. Histopathology

Liver tissues were fixed in formalin, embedded in paraffin wax, sectioned and stained for hematoxylin and eosin (H&E). H&E-stained liver sections were determined using an Olympus BX41 microscope. Masson's Trichrome and Sirius Red staining were performed using standard protocols and examined microscopically for structural changes. The degree of liver fibrosis was evaluated and scored as previously reported [21].

2.5. Quantitative real-time PCR

Total RNAs from liver and cells were extracted using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, USA). One μg of total RNA from each sample was reverse-transcribed to cDNA by the PrimeScript RT reagent kit (TaKaRa Biotech, Dalian, China). For each sample, the Ct value of the target genes was normalized to that of β-actin.

2.6. Western blotting

The total protein samples from mouse liver were extracted with RIPA buffer (Beyotime, China). The protein concentration was quantified using a BCA Protein Assay Kit (Beyotime, China). The western blot markers were from Solarbio® Life Sciences (Beijing, China). Protein was loaded on SDS-PAGE (8–12%), transferred to PVDF membranes, blocked with 5% dried skim mile for 2 h and incubated with primary antibodies overnight. The protein in the bands was then incubated with secondary antibodies and was detected on a Bio-Spectrum Gel Imaging System (UVP, USA).

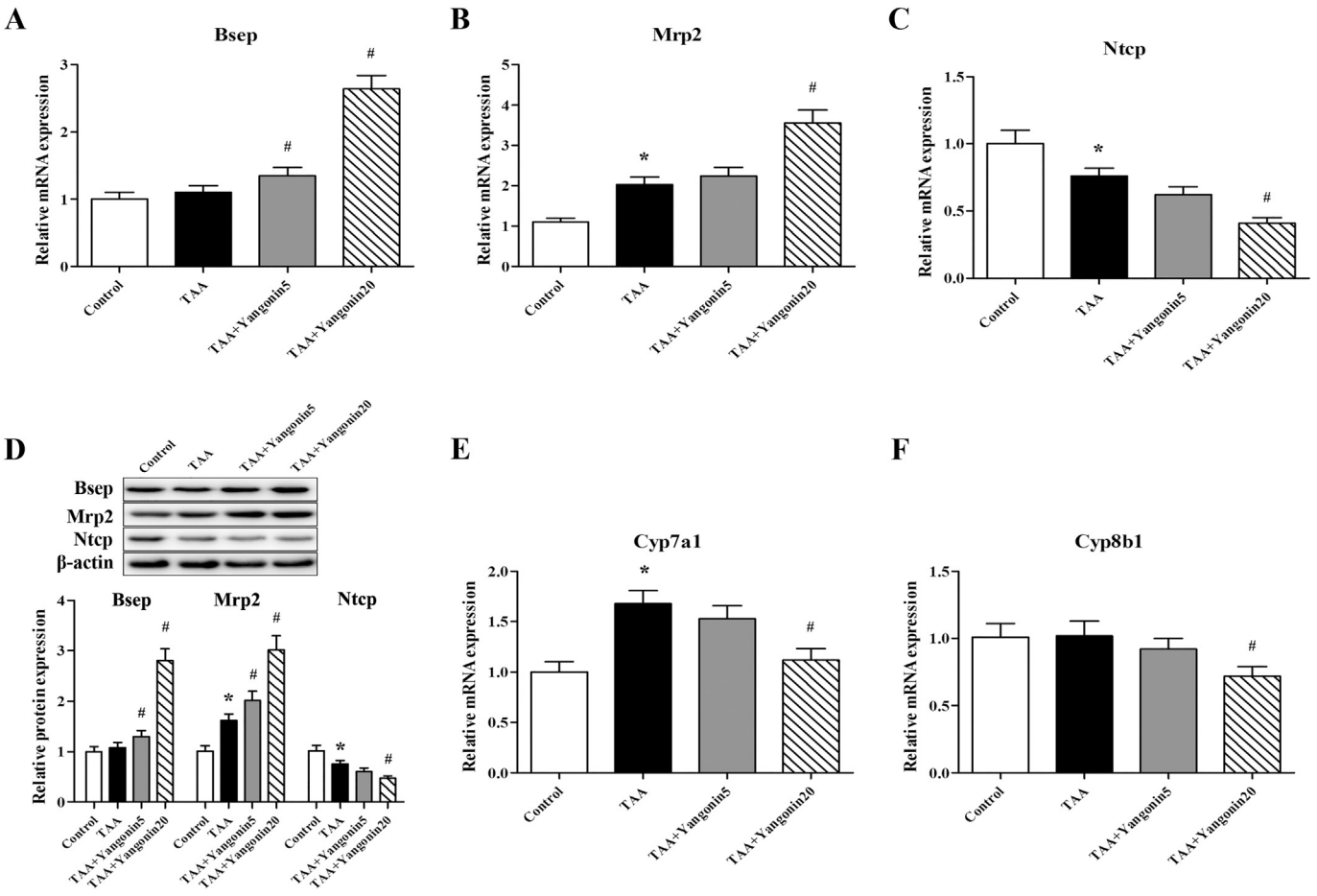

Fig. 3. The expression of genes involved in bile acid homeostasis is regulated by yangonin. The gene expression of (A) Bsep, (B) Mrp2 and (C) Ntcp were determined by quantitative real-time PCR analysis. (D) Western blotting analysis was used to measure Bsep, Mrp2 and Ntcp protein expression. Specific band intensity was quantified, normalized to β-actin. Quantitative real-time PCR analysis was performed to measure the gene expression of bile acid synthetic enzymes including (E) Cyp7a1 and (F) Cyp8b1. Data are presented as mean ± S.D. ($n = 3$). * $P < 0.05$ versus Control; # $P < 0.05$ versus TAA alone.

2.7. Dual-luciferase reporter gene assay

Human hepatoma HepG2 cells were transfected with FXR expression plasmid (100 ng), SHP promoter luciferase reporter vector (100 ng) and the null-Renilla luciferase plasmid (10 ng). Twenty-four hours later, cells were treated with vehicle DMSO (0.1%) and yangonin (0.1, 1 and 10 μM) for 24 h, and then determined the luciferase activity using the Dual-Light Chemiluminescent Reporter Gene Assay System (Berthold, Germany), which was normalized to Renilla luciferase activity.

2.8. Statistical analysis

Data were evaluated as the means ± S.D. Data analysis for multiple group comparisons was performed using a one-way analysis of variance (ANOVA) and comparison between two groups was performed using Student's *t*-tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Yangonin provided remarkable protection against TAA-induced liver injury

To determine whether yangonin has any biological effect on liver fibrosis in vivo, C57BL/6 mice were intraperitoneally injected TAA on Monday, Wednesday and Friday, and then orally administered yangonin (5, 10 and 20 mg/kg) on Tuesday, Thursday and Saturday for 8 weeks. The body weight gain analysis in mice administered TAA and different doses of yangonin indicated significant difference. Compared to control group, the body weight was significantly decreased in TAA mice. Yangonin treatment dose-dependently increased body weight gain (Fig. 1B). Besides, the liver to body weight ratio was increased in TAA mice. Yangonin treatment dose-dependently reduced the liver to body weight ratio (Fig. 1C and D). To further evaluate the histopathological change, H&E staining was performed. As illustrated in Fig. 2A, TAA led to focal necrosis and vacuolisation in some liver cells, as well as inflammatory cell infiltration, suggesting that the model of animal liver injury were successfully established. However, the degree of necrotic foci and inflammatory cell infiltration was significantly attenuated by yangonin treatment. Besides, yangonin treatment dose-dependently reversed TAA-induced increases in serum ALT and AST, the biochemical indicators of hepatic cell damage (Fig. 2B and C). Likewise, the biochemical indicator of cholestasis used to test hepatobiliary function, serum total bilirubin and total bile acids were also increased by TAA and were remarkably decreased by yangonin in a dose-dependent manner (Fig. 2D and E). In addition, compared to control group, serum triglyceride and total cholesterol were increased by TAA. After yangonin treatment, the levels of triglyceride and total cholesterol were remarkably reduced (Fig. 2F and G). Taken together, these results indicated that yangonin can provide remarkable protection against TAA-induced liver injury in a dose-dependent manner.

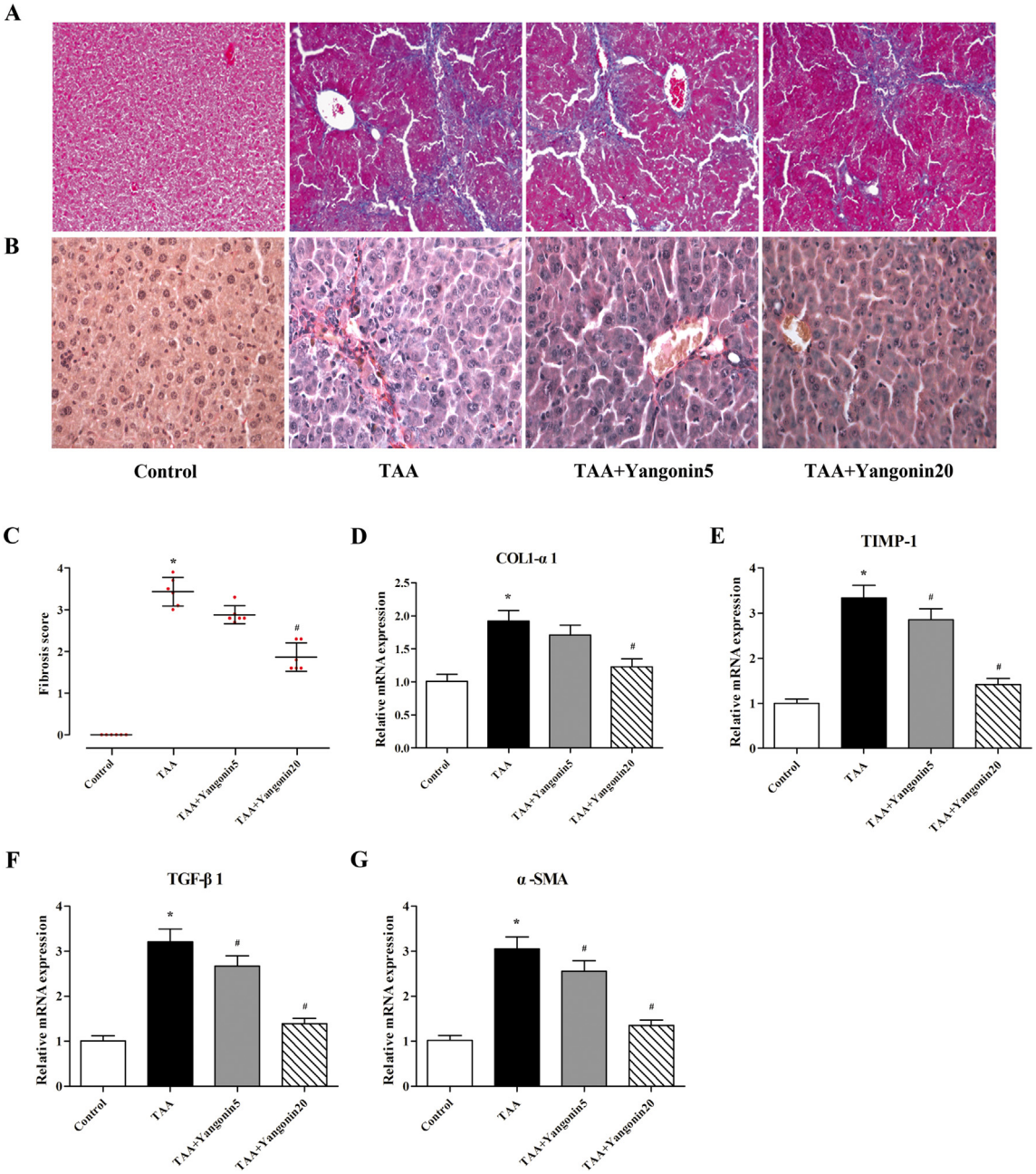

Fig. 4. Yangonin alleviates TAA-induced activation of hepatic stellate cells and liver fibrosis. (A) Effect of yangonin on liver histopathological examination by Masson staining (200 × magnification). (B) Sirius red staining indicated a reduction in liver collagen content in mice with yangonin treatment (400 × magnification). (C) The grade of fibrosis with Masson staining. 0 = no fibrosis; 1+, fibrosis present; 2+, mild fibrosis; 3+, moderate fibrosis; 4+, severe fibrosis. Quantitative real-time PCR analysis was performed to measure the gene expression of COL1-α1 (D) and TIMP-1 (E). Yangonin treatment reversed the TAA-induced increases in the gene levels of TGF-β1 (F) and α-SMA (G). Data are presented as mean ± S.D. ($n = 3$). * $P < 0.05$ versus Control; # $P < 0.05$ versus TAA alone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. The expression of genes involved in bile acid homeostasis is regulated by yangonin

A significant increase in serum total bile acids was observed in TAA mice (Fig. 2D–E), suggesting that TAA-induced hepatotoxicity impaired bile acid homeostasis. To clarify how yangonin regulates bile acid homeostasis, the key genes involved in bile acid homeostasis was examined. Firstly, the expression of transporters involved in hepatic bile acid transport was determined. The expression level of multidrug resistance-related protein 2 (Mrp2), which is responsible for hepatic bile acid transport into bile, was increased by TAA, while the expression of another canalicular efflux transporter bile salt export pump (Bsep) was slightly changed (Fig. 3A and B). Furthermore, TAA reduced the expression of Na^+/taurocholate cotransporting polypeptide (Ntcp) that is a basolateral uptake transporter involved in bile acid uptake into hepatocytes (Fig. 3C). However, the significant increases in the gene levels of Bsep and Mrp2 were observed in mice treated with yangonin. The hepatic expression of Ntcp was further decreased by yangonin. Then, the protein accumulation were further examined using Western blotting analysis to confirm the real-time PCR results. Yangonin caused the increases in Bsep, Mrp2 and a decrease in Ntcp protein levels (Fig. 3D), which was consistent with the gene results. Besides, bile acid synthesis also plays an important role in bile acid homeostasis. As illustrated in Fig. 3E and F, TAA increased the expression of cholesterol

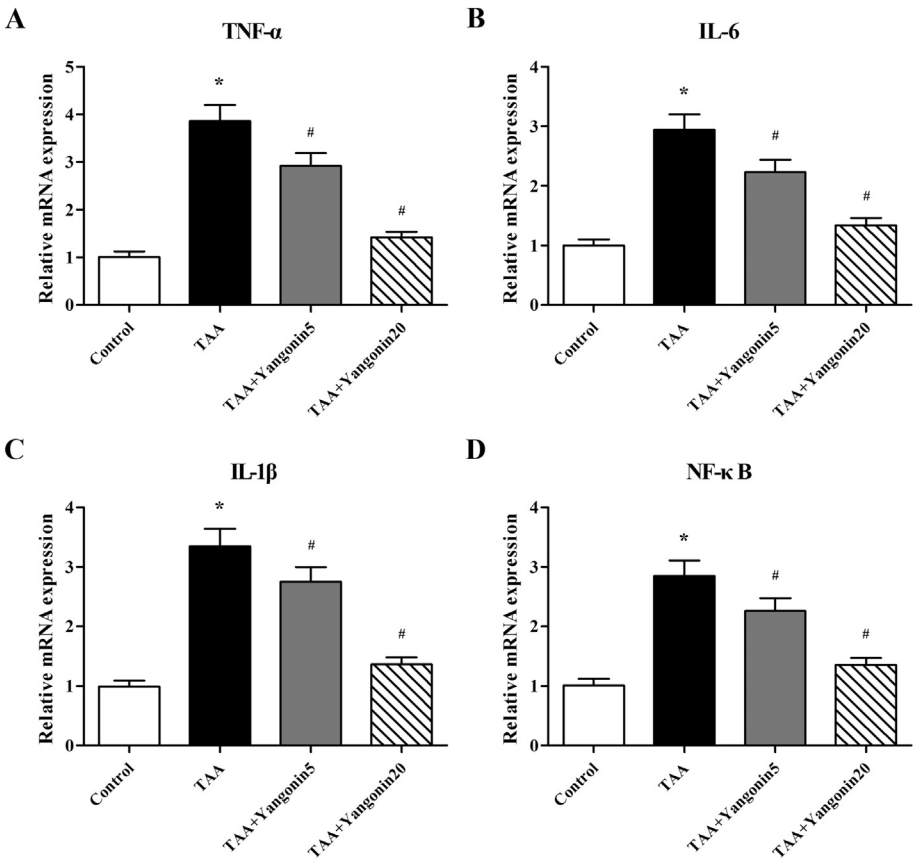

Fig. 5. The effects of yangonin on TAA-induced inflammation. Quantitative real-time PCR analysis was performed to measure the gene expression of inflammatory gene TNF-α (A), IL-6 (B), IL-1β (C) and NF-κB (D). Data are presented as mean ± S.D. (n = 3). * P < 0.05 versus Control; # P < 0.05 versus TAA alone.

7α-hydroxylase (Cyp7a1) that is the rate-limiting enzyme in bile acid synthesis. The significant decreases in Cyp7a1 and sterol-12α-hydroxylase (Cyp8b1), another bile acid synthetic enzyme, were observed in yangonin-treated mice. Together, these results indicated that yangonin increased bile acid efflux and decreased bile acid uptake and synthesis through the induction of Bsep, Mrp2 expression and the inhibition of Ntcp, Cyp7a1, and Cyp8b1 expression.

3.3. Yangonin alleviates TAA-induced activation of hepatic stellate cells and liver fibrosis

To test the effect of yangonin on hepatic fibrosis, the liver sections were stained by Masson's Trichrome and Sirius Red staining. As shown in Fig. 4A and B, TAA caused extensive fibrosis, evidenced by blue collagen staining using the Mason Trichrome staining and red collagen staining using the Sirius Red staining. The beneficial effects of yangonin treatment on hepatic fibrosis were observed in yangonin-treated mice. Yangonin treatment significantly reduced the grade of fibrosis (Fig. 4C). To elucidate the mechanism of anti-fibrotic effect of yangonin, the expression levels of genes involved in hepatic fibrosis and stellate cell activation were determined. The collagen type I α1 (COL1-α1) and TIMP-1, the hepatic fibrosis genes, were increased by TAA and were decreased by yangonin (Fig. 4D and E). The TGF-β1 and α-SMA were also increased by TAA, suggesting significant hepatic stellate cell activation by TAA. However, yangonin treatment reversed the TAA-induced increases in the gene levels of TGF-β1 and α-SMA (Fig. 4F and G). These results indicated that the beneficial effect of yangonin on the development of TAA-induced hepatic fibrosis results from its ability to limit hepatic stellate cell activation and collagen deposition.

3.4. Effects of yangonin on TAA-induced inflammation

Inflammatory responses occur throughout the pathological process in various hepatic disorders. To clarify the mechanism of yangonin protection against TAA-induced hepatic inflammation, related genes involved in inflammation were determined. TAA induced the expression of hepatic tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), while treatment with yangonin significantly reduced these gene levels (Fig. 5A–C). These three inflammatory genes are regulated by NF-κB which is a critical regulator of inflammation. Yangonin markedly reversed TAA-induced increase in NF-κB expession (Fig. 5D). Collectively, these results suggested that yangonin protected against TAA-induced hepatic inflammation via its inhibition of NF-κB.

3.5. Yangonin protects against TAA-induced hepatic fibrosis via FXR activation

To investigate whether the involvement of FXR activation in yangonin hepatoprotection in vivo, FXR was blocked by FXR antagonist GS in mice. As illustrated in Fig. 6A, the hepatoprotection of yangonin was cancelled by GS. Moreover, the results of Masson's Trichrome staining indicated that the yangonin-induced collagen changes in liver as well as fibrosis grade were abrogated by GS, suggesting the involvement of FXR in the hepatoprotection of yangonin against TAA-induced liver fibrosis (Fig. 6B–C). At the same time, the yangonin-induced decreases in the expression of genes involved in hepatic fibrosis and hepatic stellate cell activation including TIMP-1 and α-SMA were abrogated by GS (Fig. 6D). Besides, the regulation of bile acid transporters such as Bsep, Mrp2 and synthetic enzyme Cyp7a1 by yangonin was also abrogated by GS (Fig. 6E). Together, the results indicated that yangonin provided marked hepatoprotection against TAA-induced hepatic fibrosis via FXR

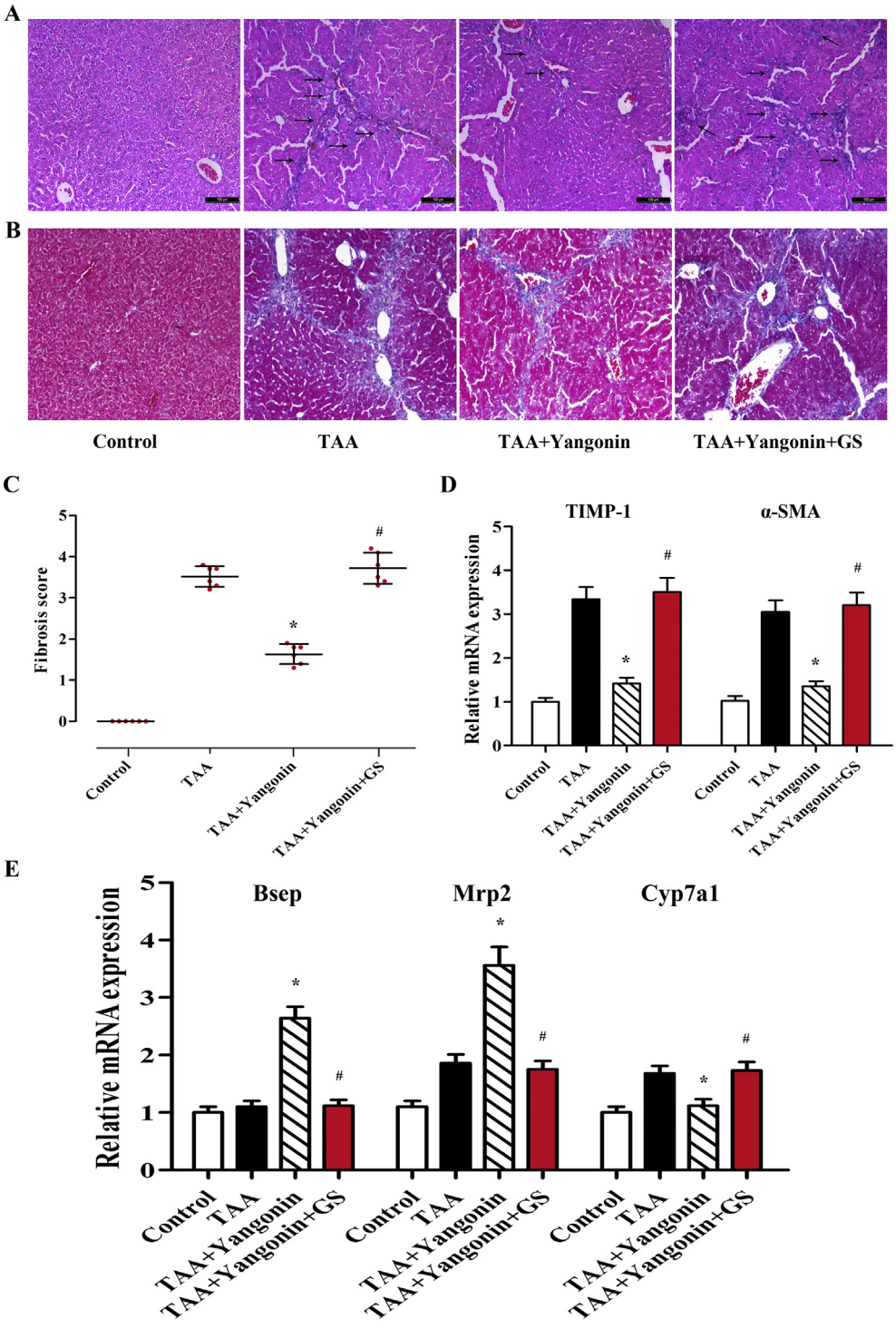

Fig. 6. Yangonin protects against TAA-induced hepatic fibrosis via FXR activation. The images of (A) H&E stained liver sections (200 × magnification, areas of severe liver necrosis and inflammatory cell infiltration were marked by arrows) and (B) Masson staining (200 × magnification) after GS administration were shown. The protective effect of yangonin on the TAA-treated mice were abrogated by GS. (C) The grade of fibrosis after GS administration. 0, no fibrosis; 1+, fibrosis present; 2+, mild fibrosis; 3+, moderate fibrosis; 4+, severe fibrosis. (D) Quantitative real-time PCR analysis was performed to measure the gene expression of TIMP-1 and α-SMA. (E) The regulation of bile acid transporters such as Bsep, Mrp2 and synthetic enzyme Cyp7a1 by yangonin was also abrogated by GS. Data are the mean ± S.D. ($n = 3$). * $P < 0.05$ versus TAA alone; # $P < 0.05$ versus TAA + Yangonin.

activation in vivo.

3.6. Yangonin modulated SHP transactivation via FXR activation in vitro

In in vivo experiments, the regulation of yangonin on the FXR target genes had been demonstrated; however, these effects may not be enough to represent the FXR activation by yangonin. Thus, luciferase reporter assay were performed in HepG2 cells. The cells were co-transfected with FXR expression plasmid and FXR target gene SHP promoter reporter vector. As shown in Fig. 7, yangonin increased the FXR reporter gene activity in a dose-dependent manner. These data indicated that yangonin is a strong FXR activator.

4. Discussion

Hepatic fibrosis is a major worldwide health problem that affects millions of patients with chronic liver diseases and leads to the significant morbidity and mortality [22]. Up to date, there is no curative treatment for hepatic fibrosis, while liver transplantation is the only option for the end-stage hepatic fibrosis [23]. Thus, the development of new drugs for hepatic fibrosis therapy is necessary. In the current study, we demonstrated that yangonin, a natural product isolated from Kava, had remarkable hepatoprotection against hepatic fibrosis induced by TAA.

TAA itself has no toxicity but it can be metabolized into potent toxic

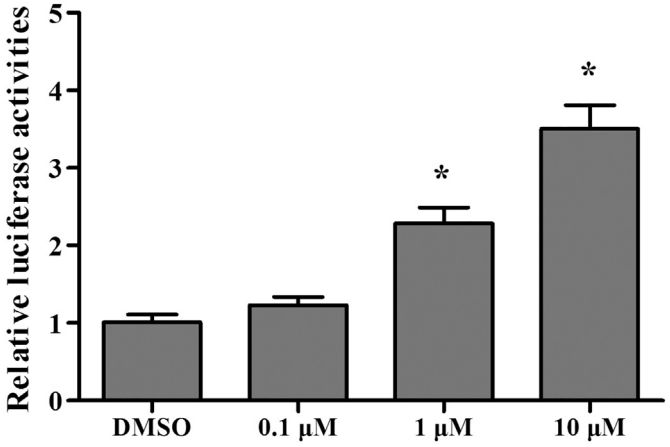

Fig. 7. Yangonin modulated SHP transactivation via FXR activation in vitro. Luciferase reporter gene assay were performed in HepG2 cells. The cells were transiently cotransfected with FXR expression plasmid and FXR target gene SHP promoter reporter vector. Yangonin increased the FXR reporter gene activity in a dose-dependent manner. Data are the mean ± S.D. ($n = 5$). * $P < 0.05$ versus DMSO.

metabolites that can induce liver injury [24]. The form of hepatic damage induced by TAA depends on the dosage and duration of its administration. High doses causes fulminant hepatic failure and early death, while low dose administration over a prolong period of time causes hepatic fibrosis [25]. In this study, low-dose (100 mg/kg) and chronic (8 weeks) TAA administration effectively induced liver dysfunction and caused hepatic fibrosis, as evidenced by the significant increase in serum AST, ALT, total bilirubin and total bile acids, as well as pathological injury in liver. Treatment with yangonin reduced these serum biomarkers of liver function and reduced the degree of hepatic pathological injury. In this study, yangonin had at least three roles to protect against TAA-induced liver injury. Firstly, yangonin attenuated TAA-induced cholestasis through increasing hepatic efflux and decreasing uptake of bile acids. Secondly, yangonin reduced collagen accumulation and fibrosis through inhibiting activation of hepatic stellate cells. Thirdly, yangonin alleviated TAA-induced hepatic inflammation via regulation of hepatic inflammation-related genes.

Bile acids have been demonstrated to be toxic and play a critical role in the pathogenesis of many liver diseases [26]. Accumulation of toxic bile acids causes hepatocyte death and ultimately leads to liver fibrosis [27]. In the present study, TAA administration increased the expression of Mrp2 and decreased Ntcp expression. The decrease in Ntcp may be due to the intrinsic hepatic repair mechanism. Yangonin increased hepatic efflux of bile acids and decreased bile acid uptake as well as synthesis through increasing Bsep, Mrp2 expression and reducing Ntcp, Cyp7a1, Cyp8b1 expression.

Nuclear receptors play primary roles in mediating the pathogenesis and progression of a variety of hepatic diseases. Among nuclear receptors, FXR is a ligand-regulated transcription factor that alters gene transcription by binding DNA sequences in the promoter of downstream target genes. FXR has been demonstrated to modulate a variety of metabolic processes including bile acid homeostasis and liver fibrosis [28]. Furthermore, FXR agonist was reported to alleviate liver fibrosis by reducing hepatic gene expression of fibrotic markers [29]. Thus, FXR activation is considered to be a promising therapeutic strategy in fibrotic diseases. Yangonin was found by us to bind to the ligand binding domain of FXR and activate FXR [17]. In the current study, yangonin through activating FXR increased the expression of FXR downstream genes including Bsep and Mrp2, and reduced the expression of Ntcp, Cyp7a1 and Cyp8b1, which are also downstream genes of FXR, resulting in an increase in bile acid efflux and decreases in bile acid uptake and synthesis. To further investigate whether the involvement of FXR activation in yangonin hepatoprotection in vivo, FXR was blocked by FXR antagonist GS in mice. After GS administration, the hepatoprotection of yangonin was cancelled. Moreover, the regulation of bile acid transporters such as Bsep, Mrp2 and synthetic enzyme Cyp7a1 by yangonin was also abrogated by GS. These results indicated that yangonin provided marked hepatoprotection against TAA-induced hepatic fibrosis via FXR activation in vivo.

Hepatic fibrosis is characterized by a continuous inflammatory response and activation of hepatic stellate cells, which lead to the accumulation of extracellular matrix protein in liver tissue [6]. In rodent models of liver injury, appropriate hepatic repair depends on precise interactions of cell–extracellular matrix and cell–cell [30]. However, chronic liver injury such as TAA-induced liver injury disrupts these interactions, arresting the healing process in a continuous inflammatory phase and activation of hepatic stellate cells leading to the development of liver fibrosis. Therefore, inflammatory response and activation of hepatic stellate cells play critical roles in the pathogenesis of hepatofibrosis. Several genes including TNF-α, IL-6, IL-1β and NF-κB play important roles in the inflammatory response. In the present study, TAA induced significant increases in TNF-α, IL-6 and IL-1β, as well as hepatic NF-κB expression. Through FXR activation, yangonin inhibited the hepatic expression of NF-κB, resulting in the decreases in the gene expression of TNF-α, IL-6 and IL-1β.

The activation of hepatic stellate cells is the key initial event in the pathogenesis of hepatic fibrosis. Activated hepatic stellate cells deposit collagen in the space of Disse, leading to the disruption of the functional structure of hepatic lobules, eventually liver fibrosis [24]. The increases in the gene levels of α-SMA and COL1-α1 are a fundamental step that conveys activation of hepatic stellate cells and liver fibrosis. TIMP-1 is a known inhibitor of metalloproteinases. The decreased TIMP-1 can cause an additional increase in extracellular matrix degradation and further ameliorates the misbalance between accumulation and degradation of collagens of fibrosis. TGF-β1 is another crucial gene in the activation of hepatic stellate cells. In the current study, through FXR activation, yangonin alleviated TAA-induced activation of hepatic stellate cells and liver fibrosis via regulating the expression levels of genes such as TIMP-1 and TGF-β1.

In conclusion, yangonin exerts anti-fibrotic and anti-inflammatory effects against TAA-induced hepatic fibrosis via activating FXR signaling pathway. Yangonin may be an effective pharmacological strategy to protect against liver fibrosis.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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References

[1] E. Moran-Salvador, J. Mann, Epigenetics and liver fibrosis, Cell Mol Gastroenterol Hepatol 4 (2017) 125–134.
[2] H.S. Lee, et al., AMP-activated protein kinase activator, HL156A reduces thioacetamide-induced liver fibrosis in mice and inhibits the activation of cultured hepatic stellate cells and macrophages, Int. J. Oncol. 49 (2016) 1407–1414.
[3] S. Petta, et al., Hepatitis C virus eradication by direct-acting antiviral agents improves carotid atherosclerosis in patients with severe liver fibrosis, J. Hepatol. 69 (2018) 18–24.
[4] B.M. Fung, et al., Cancer risk in primary sclerosing cholangitis: epidemiology, prevention, and surveillance strategies, World J. Gastroenterol. 25 (2019) 659–671.
[5] K.M. Hart, et al., Type 2 immunity is protective in metabolic disease but exacerbates NAFLD collaboratively with TGF-beta, Sci. Transl. Med. 9 (2017) 1–14.
[6] M.E. Wang, et al., Curcumin protects against thioacetamide-induced hepatic fibrosis by attenuating the inflammatory response and inducing apoptosis of damaged hepatocytes, J. Nutr. Biochem. 23 (2012) 1352–1366.

[7] F. Magdaleno, et al., Cartilage oligomeric matrix protein participates in the pathogenesis of liver fibrosis, J. Hepatol. 65 (2016) 963–971.
[8] L. Campana, J.P. Iredale, Regression of liver fibrosis, Semin. Liver Dis. 37 (2017) 1–10.
[9] M.D. Thompson, et al., Beta-catenin regulation of farnesoid X receptor signaling and bile acid metabolism during murine cholestasis, Hepatology 67 (2018) 955–971.
[10] S. Fiorucci, et al., The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis, Gastroenterology 127 (2004) 1497–1512.
[11] Z.M. Ding, et al., Progression and regression of hepatic lesions in a mouse model of NASH induced by dietary intervention and its implications in pharmacotherapy, Front. Pharmacol. 9 (2018) 1–10.
[12] R.F. van Golen, et al., FXR agonist obeticholic acid induces liver growth but exacerbates biliary injury in rats with obstructive cholestasis, Sci. Rep. 8 (2018) 16529.
[13] L. Verbeke, et al., FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis, Sci. Rep. 6 (2016) 33453.
[14] D.G. Zhang, et al., Obeticholic acid protects against carbon tetrachloride-induced acute liver injury and inflammation, Toxicol. Appl. Pharmacol. 314 (2017) 39–47.
[15] A. Ligresti, et al., Kavalactones and the endocannabinoid system: the plant-derived yangonin is a novel CB(1) receptor ligand, Pharmacol. Res. 66 (2012) 163–169.
[16] J. Wang, et al., Kavalactone content and chemotype of kava beverages prepared from roots and rhizomes of Isa and Mahakea varieties and extraction efficiency of kavalactones using different solvents, J. Food Sci. Technol. 52 (2015) 1164–1169.
[17] X. Gao, et al., Yangonin protects against cholestasis and hepatotoxity via activation of farnesoid X receptor in vivo and in vitro, Toxicol. Appl. Pharmacol. 348 (2018) 105–116.
[18] Z. Liu, et al., Kavalactone yangonin induces autophagy and sensitizes bladder cancer cells to flavokawain A and docetaxel via inhibition of the mTOR pathway, J. Biomed. Res. 31 (2017) 408–418.
[19] C.J. Wruck, et al., Kavalactones protect neural cells against amyloid beta peptide-induced neurotoxicity via extracellular signal-regulated kinase 1/2-dependent nuclear factor erythroid 2-related factor 2 activation, Mol. Pharmacol. 73 (2008) 1785–1795.
[20] Y. Kong, et al., Protective effects of yangonin from an edible botanical Kava against lithocholic acid-induced cholestasis and hepatotoxicity, Eur. J. Pharmacol. 824 (2018) 64–71.
[21] X.L. Wei, et al., Protective effects of extracts from pomegranate peels and seeds on liver fibrosis induced by carbon tetrachloride in rats, BMC Complement. Altern. Med. 15 (2015) 389.
[22] V. Sanchez-Valle, et al., Role of oxidative stress and molecular changes in liver fibrosis: a review, Curr. Med. Chem. 19 (2012) 4850–4860.
[23] D. Schuppan, et al., Liver fibrosis: direct antifibrotic agents and targeted therapies, Matrix Biol. 69 (2018) 435–451.
[24] R. Bruck, et al., Prevention of liver cirrhosis in rats by curcumin, Liver Int. 27 (2007) 373–383.
[25] R. Bruck, et al., Pyrrolidine dithiocarbamate protects against thioacetamide-induced fulminant hepatic failure in rats, J. Hepatol. 36 (2002) 370–377.
[26] X. Gao, et al., Hepatoprotection of auraptene from peels of citrus fruits against thioacetamide-induced hepatic fibrosis in mice by activating farnesoid X receptor, Food Funct. 9 (2018) 2684–2694.
[27] M. Wagner, et al., Nuclear receptors in liver disease, Hepatology 53 (2011) 1023–1034.
[28] G.M. Hirschfield, et al., Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid, Gastroenterology 148 (2015) 751–761.
[29] S. Zhang, et al., Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis, J. Hepatol. 51 (2009) 380–388.
[30] G.S. Schultz, A. Wysocki, Interactions between extracellular matrix and growth factors in wound healing, Wound Repair Regen. 17 (2009) 153–162.