

# *Schisandra chinensis* inhibiting TGF $\beta$ -induced activation of hepatic stellate cells

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**Abstract** Hepatic fibrosis is one of the critical steps contained in the pathogenesis of liver cirrhosis. Excessive deposition of collagen contributes to the development of fibrosis in chronic liver injury. Activation of hepatic stellate cells (HSCs) plays an important role in fibrogenesis and is accountable for providing extracellular matrix components. The berry of *Schisandra chinensis* has been known to exert hepatoprotective properties. However, its effect on HSCs is not completely understood. Therefore, in this study, we investigated the inhibitory effect of its ethanolic extract (SBE) on hepatic fibrogenesis. We found that SBE treatment effectively reduced the serum levels of alanine aminotransferase and aspartate aminotransferase as well as collagen deposition in the hepatic parenchyma in a thioacetamide-induced hepatic fibrosis mouse model. Moreover, SBE inhibited transforming growth factor  $\beta$  (TGF $\beta$ )-induced mRNA expression of  $\alpha$ -smooth muscle actin ( $\alpha$ SMA) and collagen type 1  $\alpha$ 1 (COL1A1) in HSCs, suggesting that SBE exerts anti-fibrotic activity by attenuating TGF $\beta$ -induced HSC activation. To identify the active components of SBE accountable for HSC inhibition, SBE was further partitioned based on the hydrophobicity of the solvents such as water, *n*-butanol, ethyl acetate, chloroform, and *n*-hexane. The *n*-hexane fraction was selected

and further separated using analytical high-performance liquid chromatography. We found that six lignans contained in the *n*-hexane fraction strongly reduced TGF $\beta$ -induced expression of both  $\alpha$ SMA and COL1A1 mRNA. These data suggest that at least six lignans contained in SBE have the strong potential to prevent TGF $\beta$ -induced HSC activation.

**Keywords** Alpha-smooth muscle actin · Collagen type 1  $\alpha$ 1 · Hepatic fibrosis · Hepatic stellate cells · Schisandra · Transforming growth factor  $\beta$

## Introduction

The liver is the largest single organ in our body, is widely distributed over the right upper abdomen and left upper abdomen, and is known to be the most commonly damaged organ in both blunt and penetrating injuries. The liver is largely divided into the left liver and the right liver and is divided into 8 regions according to the running of the veins, arteries, and bile ducts. The liver is divided into the arterial and portal vein. The blood vessels are very abundant in the receiving organs, and a large amount of bleeding may occur when they are damaged. In addition, secondary damage of the bile ducts in the liver may be accompanied by stomach, duodenum, pancreas, and adrenal glands surrounding the liver. The liver is an important organ that plays a pivotal role in the detoxification of metabolism and various endogenous and exogenous harmful substances. However, hepatocytes are destroyed, liver function is less than half, and special symptoms do not appear. Because many drugs are metabolized through the liver, if we take the drug over a liver function, we may

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develop a herd that can cause liver dysfunction. Hepatocyte damage refers to necrosis of hepatocytes, fatty hepatitis or cognitive vaginosis. It can be caused by various drugs including tetracycline, ritonavir, risperidone, phenytoin, valproate, nevirapine, ketoconazole, pyrazinamide, rifampin, trazodone, halothane, aspirin, diclofenac, and acetaminophen. If hepatocyte damage persists for a long time, hepatic fibrosis accumulates in the liver. Hepatic fibrosis is an inflammatory response to liver injury, characterized by excessive accumulation of extracellular matrix (ECM) proteins in the hepatic parenchyma [1]. When hepatocytes are damaged by various stimuli, such as inflammation, alcohol poisoning, or toxic drugs, infiltrating inflammatory lymphocytes and resident liver macrophages (Kupffer cells) release various growth factors, including transforming growth factor- $\beta$  (TGF $\beta$ ), platelet-derived growth factor, and tumor necrosis factor- $\alpha$ . Hepatic stellate cells (HSCs), also known as Ito cells or lipocytes, are star-like-shaped cells located between hepatocytes and sinusoidal endothelial cells. HSCs represent 5–8% of all liver cells and play a role in the uptake and storage of vitamin A [2]. HSCs are activated by these growth factors and play a critical role in promoting tissue regeneration by producing collagen-rich ECM proteins [3]. However, when hepatocyte destruction and regeneration occur repeatedly, massively accumulated collagens are transformed from fine fibrils to thick fibers, leading to the development of hepatic fibrosis [4]. Advanced hepatic fibrosis disrupts the hepatic architecture and eventually causes liver cirrhosis and dysfunction [1]. As fibrosis is a reversible reaction [5], it has been suggested that the prevention or treatment of hepatic fibrosis can be achieved by blocking the synthesis of collagen fibers. Therefore, it is important to inhibit the activity of HSCs to prevent the progression of hepatic fibrosis [3]. Unfortunately, there is no good way to treat hepatic fibrosis.

*Schisandra chinensis* is a medicinal herb that belongs to the Schisandraceae family, whose growth range extends from northeast China, Korea, and Japan to the most eastern part of Russia [6, 7]. Because it tastes sour, sweet, spicy, bitter, and salty, it is called omiza in Korea. The berry of *S. chinensis* is widely used in traditional Chinese, Japanese, Russian, and Korean medicine [7]. One of Korean traditional medical books, Donguibogam reports the berry of *S. chinensis* can restore fatigue, strengthen heart function, improve blood circulation, and prevent memory loss or slowing of thinking ability. The berry of *S. chinensis* contains various active compounds and exerts a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anti-allergic, anti-tumor, anti-angiogenic, antiseptic, and anti-hepatotoxic effects, and protective effects against cardiovascular disease and on pancreatic  $\beta$ -cells in diabetic rats and on oxidative stress and cytotoxic activities. [7]. Many studies have reported

that dibenzocyclooctadiene lignans, such as schisandrin A (deoxyschisandrin), schisandrin B (gomicin N), gomisin A (schisandrol B), and gomisin J, have protective effects against liver injury [8–12]. In addition, more lignans including schisandrol A, gomisin D, micrantherin A, pre-gomisin, angeloylgomisin H, benzoylgomisin H, angeloylgomisin Q, gomisin G, gomisin F, gomisin K1, gomisin K2, schisantherin A, tigloylgomisin P, schisanhenol, gomisin L1, schisantherin C, gomisin M1, gomisin M2, epigomisin O,  $\gamma$ -schisandrin, benzoylisogomisin O, angeloylisogomisin O, schisandrin C, and angeloylgomisin O, and polyphenols including 5-O-caffeoylquinic acid, quercetin-3-O-rutinoside, quercetin-3-O-glucoside, quercetin, kaempferol, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 4-O-p-coumaroylquinic acid, 3-O-p-coumaroylquinic acid, trans- and cis-5-O-p-coumaroylquinic acid, quercetin-3-O-glucoside-7-O-rhamnoside, quercetin-3-O-galactoside, quercetin-3-O-xyloside, kaempferol-3-O-glucoside-7-O-rhamnoside, kaempferol-3-O-glucoside, kaempferol-3-O-arabinose, 6''-O-(4-hydroxycinnamoyl)-astragalins have been identified as the components of *S. chinensis*.

However, the effect of the berry of *S. chinensis* on inhibiting HSCs has not been well characterized. In this study, we aimed to determine the anti-HSC effect of the ethanolic extracts of the berry of *S. chinensis* (named as SBE) against TGF $\beta$ -stimulated HSCs and identify the bioactive ingredient in SBE responsible for inhibiting TGF $\beta$ -induced  $\alpha$ -smooth muscle actin ( $\alpha$ SMA) and collagen type 1  $\alpha$ 1 (COL1A1) expression.

## Materials and methods

### Chemicals and reagents

Thioacetamide was obtained from Sigma-Aldrich (St. Louis, MO, USA). TGF $\beta$  was purchased from R&D Systems (Minneapolis, MN, USA). Lignans including gomisin D, gomisin G, gomisin H, gomisin J, gomisin N, gomisin O, angeloylgomisin H, angeloylgomisin O, and benzoylgomisin O were purchased from Biopurify (Chengdu, Sichuan, China). Other chemicals were purchased from Sigma-Aldrich.

### Preparation of *Schisandra* extracts

The berry of *S. chinensis* was collected from Mungyeong Miso farm (Gyeongbuk, Korea) in September 2016, and a voucher herbarium specimen was deposited at Konkuk University, Korea. The berry was dried under a shaded lot, and the dried berry (9000 g) was extracted three times with ethanol for three days. The ethanolic extract (2747.51 g,

30.53%) was subjected to sequential separation with solvents of different polarities. Five fractions were obtained: *n*-hexane (3.35%), chloroform (0.67%), ethyl acetate (11.12%), *n*-butanol (59.37%), and aqueous fraction (22.99%). Whole fractions were freeze-dried and stored at -20 °C until further experiments. Analytical high-performance liquid chromatography (HPLC) was performed to analyze the components in SBE. The method of the analytical HPLC for the identification of the components followed the procedure provided by the manufacture.

### Induction of hepatic fibrosis with thioacetamide in Balb/c mice

Balb/c mice were purchased from Youngbio (Seongnam, Gyeonggi-do, Korea). Mice were divided into 3 groups ( $n = 3/\text{group}$ ): control, TAA only, and TAA with SBE (Fig. 1A). Control group mice were intraperitoneally administered phosphate-buffered saline (PBS) solution. Hepatic fibrosis was induced by intraperitoneal injection of TAA (100 mg/kg) three times per week for a total of 8 weeks. SBE (300 mg/kg) was orally administered once per day for 10 days before TAA treatment, and then TAA and SBE were administered on the same schedule (Fig. 1A). After 8 weeks of TAA treatment, liver and blood samples were collected. Animal experiments were approved by the Committee of Laboratory Animals of Konkuk University (IACUC No. KU16187).

### Analysis of mouse liver function

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by measuring enzyme activities with a commercial assay kit using a Mindray BS400 automated spectrophotometer according to the manufacturer's instructions (Mindray Medical International Ltd., Shenzhen, China).

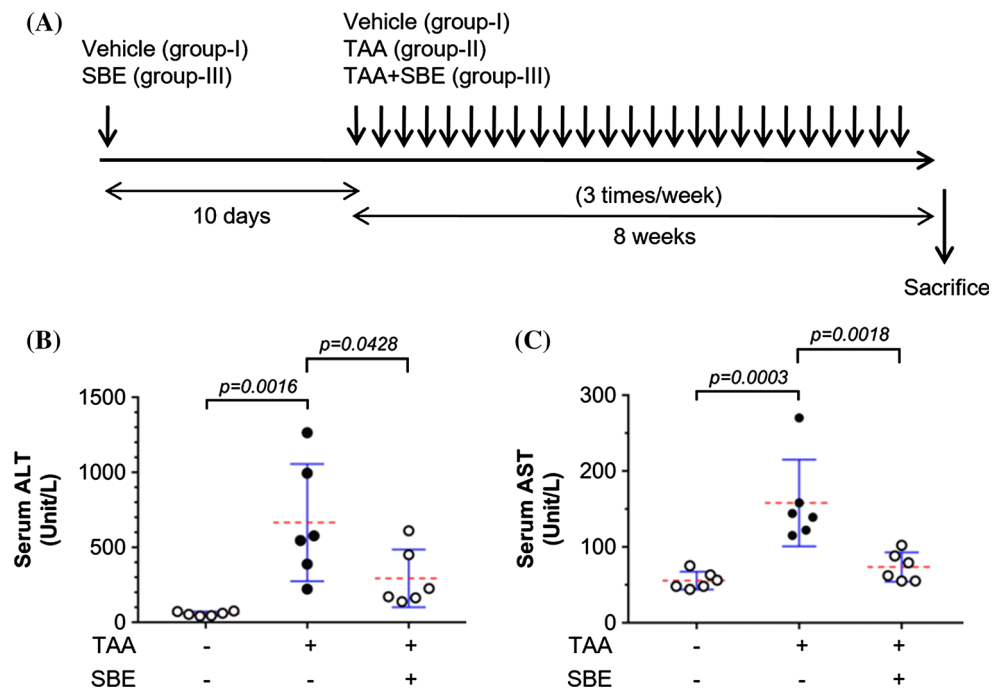
### Collagen staining in the mouse liver tissues

Mouse liver tissues were fixed with 10% neutral formalin and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin (H&E). Histological visualization of collagenous connective tissues was stained with a Trichrome Staining kit (ab150686 Connective Tissue stain; Abcam, Cambridge, UK) and collagen I and III fibers were stained with a Picro Sirius Red Staining Kit (ab150681 Connective Tissue stain; Abcam) according to the manufacturer's instructions.

### Cell line and cell culture

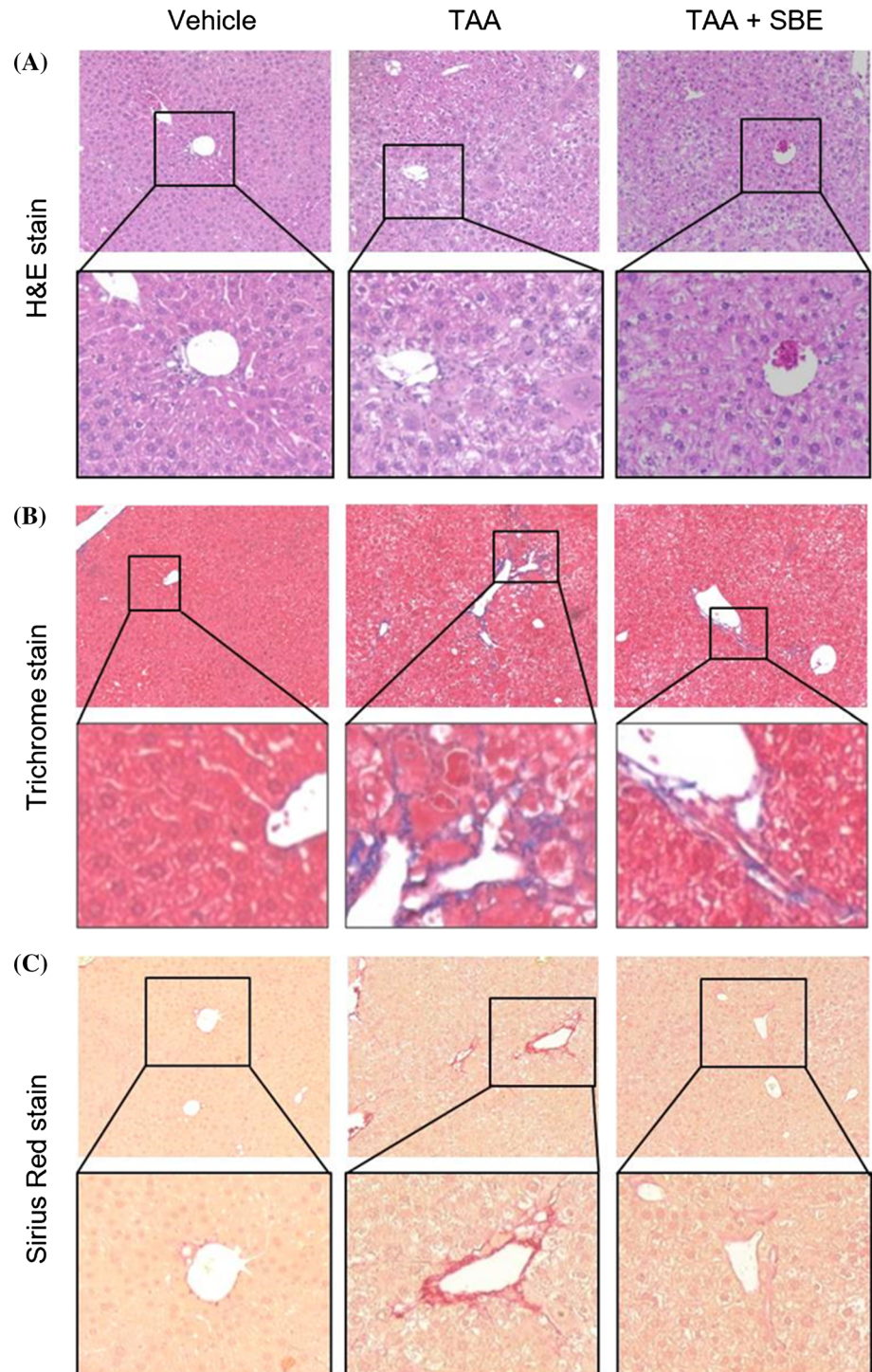
HSCs were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in DMEM containing 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA, USA) and antibiotic–anti-mycotic solution (Invitrogen Life Technologies) at 37 °C in a 5% CO<sub>2</sub> incubator.

**Fig. 1** Inhibitory effect of *Schisandra* berry ethanolic extract (SBE) on TAA-induced liver damage. (A) Schematic illustration of the experimental design. Mice were treated with PBS, 100 mg/kg TAA alone or TAA with 150 mg/kg SBE. (B, C) Effect of TAA alone or TAA with SBE on the serum levels of ALT (B) and AST (C). Data are presented as the mean  $\pm$  SD ( $n = 6$ ). The *P* value was analyzed using ANOVA followed by Sidak's multiple comparisons test





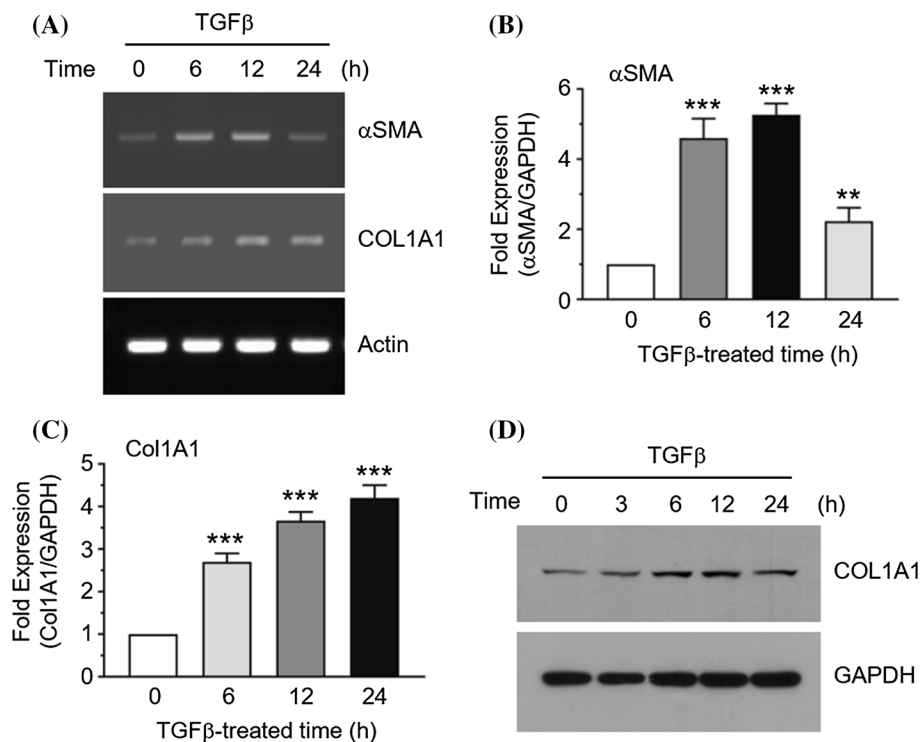
**Fig. 2** Effect of *Schisandra* berry ethanolic extract (SBE) on TAA-induced collagen deposition in mouse liver. Liver specimens were fixed in 10% formalin and stained with H&E (A cytoplasm, pink; nucleus, purple), modified Masson's trichrome (B collagen, blue), and Picro Sirius Red (C collagen fibers, red)



#### Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNAs were isolated using Isol-RNA lysis reagent (NucleoZOL; Clontech, Mountain View, CA, USA). Double-stranded cDNAs were synthesized using an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions. The primer

sequences were synthesized by Macrogen (Seoul, Korea) as follows: actin forward, 5'-TGGAATCCTGTGGC ATC CATGAAAC-3'; actin reverse, 5'-TAAAACGCAGCT CAGTAACAGTCCG-3';  $\alpha$ SMA forward, 5'-CTGAGCGT GGCTATTCCTT-3';  $\alpha$ SMA reverse, 5'-CTTCTGCATCC TGTCAGCAA-3'; COL1A1 forward, 5'-GACGCCATCA AGGTCTACTG-3'; COL1A1 reverse, 5'-ACGGGAATCC ATCGGTCA-3'.



**Fig. 3** Effect of TGFβ on the expression of αSMA and COL1A1 mRNAs. HSCs were treated with 10 ng/mL TGFβ for different time periods. **(A)** Total RNA was isolated, and αSMA and COL1A1 mRNA expression was measured by RT-PCR. Actin mRNA level was used as an internal control. **(B, C)** Total RNA was isolated, and αSMA **(B)** and COL1A1 **(C)** mRNA expression was measured by Q-PCR. Relative fold changes were normalized to those of GAPDH

mRNA. Data are presented as the mean  $\pm$  SD ( $n = 3$ ). \*\*  $P = 0.009$ ; \*\*\*  $P < 0.001$  by ANOVA followed by Dunnett's multiple comparisons test. **(D)** HSCs were treated with 10 ng/mL TGFβ for different time periods. Whole cell lysates were prepared, and immunoblot analysis was performed with antibody against COL1A1. GAPDH level was measured as an internal control

### Quantitative real time-PCR (Q-PCR)

The relative expression levels of αSMA and GAPDH mRNAs were measured with a TaqMan-iQ<sup>TM</sup> supermix kit (Bio-Rad) using the Bio-Rad iCycler iQ<sup>TM</sup> according to the manufacturer's instructions. TaqMan<sup>TM</sup> fluorogenic probes and PCR probes were synthesized by Metabion International (Martinsried, Germany) as follows: COL1A1 TaqMan probe, 5'-FAM-CATCGTGGCTTCTCTGGTCTCC-BHQ-1-3'; αSMA TaqMan probe, 5'-FAM-TAGAACACGGCATCATCACTCACTG-BHQ-1-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Taqman probe, 5'-YY-CGTCGCCAGCCGAGCCACATCGC-BHQ-1-3' (FAM, 6-carboxyfluorescein; BHQ, *tert*-butylhydroquinone; YY, Yakima Yellow). Expression values were normalized to that of GAPDH mRNA in the same samples using the software program provided by the manufacturer.

### Statistical analysis

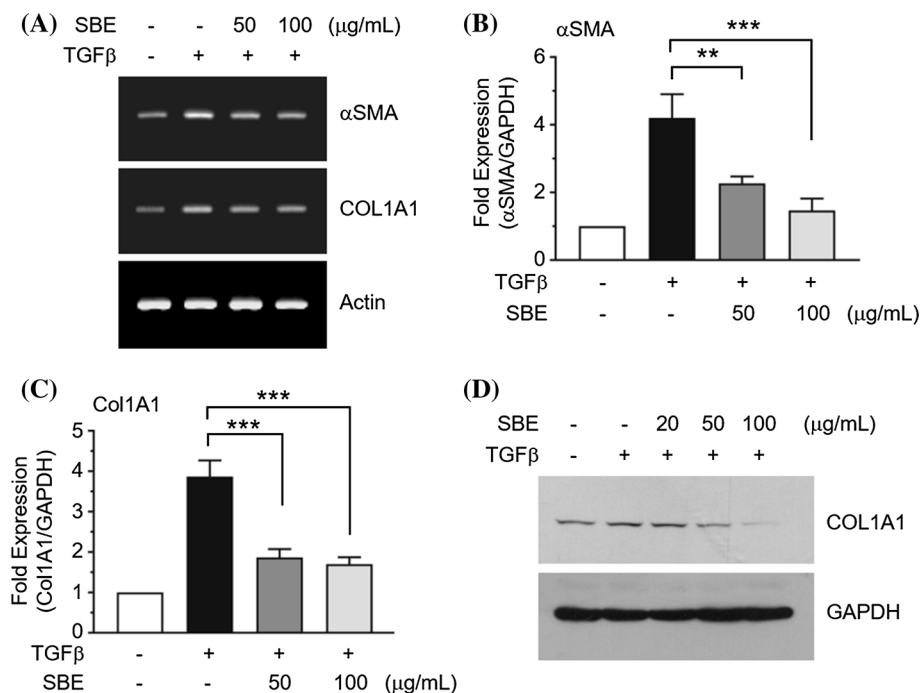
Statistical significance was determined by one-way analysis of variance followed by Sidak's multiple comparisons

test using GraphPad Prism version 7.02 (GraphPad Software, Inc., La Jolla, CA, USA).

## Results and discussion

### Effect of *Schisandra* berry ethanolic extract (SBE) on TAA-induced liver injury

When liver cells are damaged, liver ALT and AST enzymes are released into the blood. Therefore, increases in plasma ALT and AST levels are commonly used as a key biochemical marker for hepatotoxicity. Thioacetamide (TAA) is a hepatotoxin that causes acute hepatic failure in exposed animals [13]. To test the potential of SBE in protecting against liver injury, mice were divided into three groups and treated with vehicle, TAA, or TAA in combination with SBE for 8 weeks: G1, vehicle only; G2, TAA alone; and G3, SBE for 10 days, followed by SBE plus TAA (Fig. 1A). Treatment with TAA alone (3 times/week for 8 weeks; G2 group) significantly elevated the serum levels of ALT (Fig. 1B) and AST (Fig. 1C) compared to those in the control G1 group, indicating that TAA causes



**Fig. 4** Inhibitory effect of *Schisandra* berry ethanolic extract (SBE) on TGFβ-induced expression of αSMA and COL1A1 mRNAs. **(A)** HSCs were either untreated or treated with 50 or 100 μg/mL SBE for 30 min, followed by stimulation with 10 ng/mL TGFβ. After 12 h, total RNA was isolated and αSMA and COL1A1 mRNA expression was measured by RT-PCR. Actin mRNA level was used as an internal control. **(B, C)** HSCs were treated as in **(A)**, and total RNA was isolated. αSMA **(B)** and COL1A1 **(C)** mRNA expression was

measured by Q-PCR. The relative fold changes were normalized to GAPDH mRNA. Data are presented as the mean ± SD (n = 3). \*\*  $P = 0.002$ ; \*\*\*  $P < 0.001$  by ANOVA followed by Dunnett's multiple comparisons test. **(D)** HSCs were either untreated or treated with 20, 50 or 100 μg/mL SBE for 30 min, followed by stimulation with 10 ng/mL TGFβ. After 12 h, whole cell lysates were prepared and immunoblot analysis was performed with antibody against COL1A1. GAPDH level was measured as an internal control

hepatotoxicity under our experimental conditions. However, in the presence of SBE, TAA-induced ALT and AST activities were significantly (all  $P < 0.05$  by Sidak's multiple comparison test) reduced compared to those in the TAA-treated G2 group. These data suggest that SBE may have a beneficial effect against acute TAA-induced hepatotoxicity.

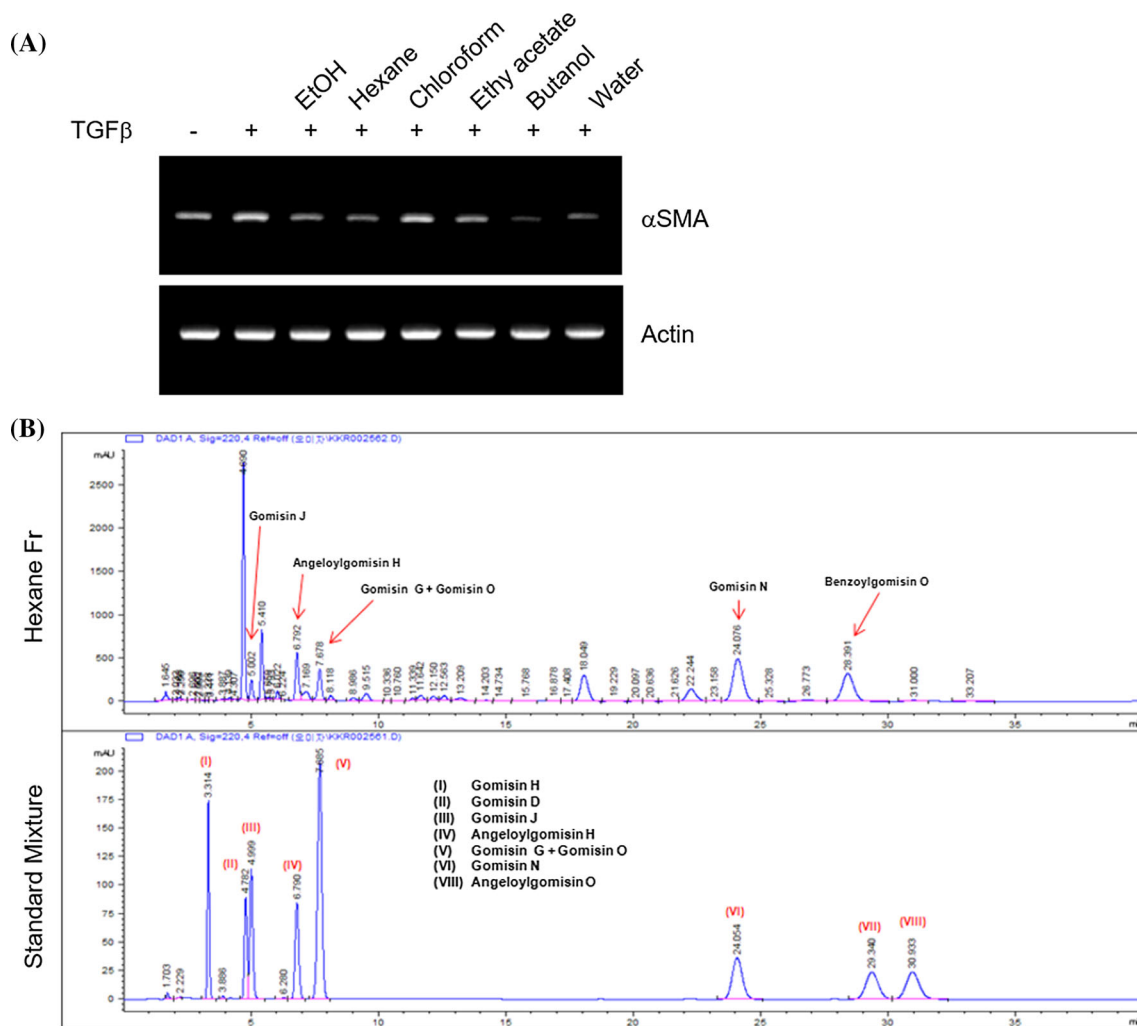
### SBE ameliorates TAA-induced hepatic fibrosis

In TAA-treated livers, morphological features of parenchymal cells and lobular architectures were severely altered compared to those of the vehicle-treated control, whereas in the SBE-administered group, liver damage by TAA remarkably improved (Fig. 2A). It has been demonstrated that TAA-induced liver injury causes hepatic fibrosis [14]. The most characteristic feature of hepatic fibrosis is excessive deposition of extracellular matrix proteins, particularly collagen fibers. To determine the effect of SBE on the protection of hepatic fibrosis, collagen accumulation was visualized by modified Masson's trichrome staining (blue staining; Fig. 2B) and Picro Sirius Red staining (red staining; Fig. 2C). In TAA-treated hepatic tissue, severe deposition of collagen was observed.

However, TAA-induced collagen fibers were substantially decreased in SBE-treated liver tissue. These data suggest that SBE inhibits the deposition of collagen fibers induced by TAA in the mouse liver.

### TGFβ increases αSMA and COL1A1 expression in HSCs

HSCs play a crucial role in the production of collagens in response to liver injury [3]. HSCs are in the perisinusoidal space between sinusoid endothelial cells and hepatocytes. TGFβ, which is secreted from injured hepatocytes and Kupffer cells, triggers activation of quiescent HSCs into myofibroblast-like cells [15]. Activated HSCs expressing αSMA enhance the synthesis of matrix proteins, including COL1A1, and promote hepatic fibrosis [16]. To assess the effect of TGFβ on HSC activation, we examined the expression of αSMA and COL1A1 mRNAs by reverse transcription-polymerase chain reaction (RT-PCR). Following TGFβ stimulation, the αSMA mRNA level increased within 6 h and was sustained for at least 12 h and COL1A1 expression gradually increased for up to 24 h after treatment (Fig. 3A). Q-PCR showed that αSMA mRNA levels increased by  $4.6 \pm 0.56$ -,  $5.3 \pm 0.32$ -, and



**Fig. 5** Effect of sequential fractions of *Schisandra* berry on TGFβ-induced expression of αSMA mRNA. **(A)** HSCs were either untreated or treated with 20 μg/mL fractions for 30 min, followed by stimulation with 10 ng/mL TGFβ. After 12 h, total RNA was isolated and

αSMA mRNA expression was measured by RT-PCR. Actin mRNA level was used as an internal control. **(B)** HPLC chromatogram of the *n*-hexane fraction (top) and chromatogram of the authentic lignans (bottom)

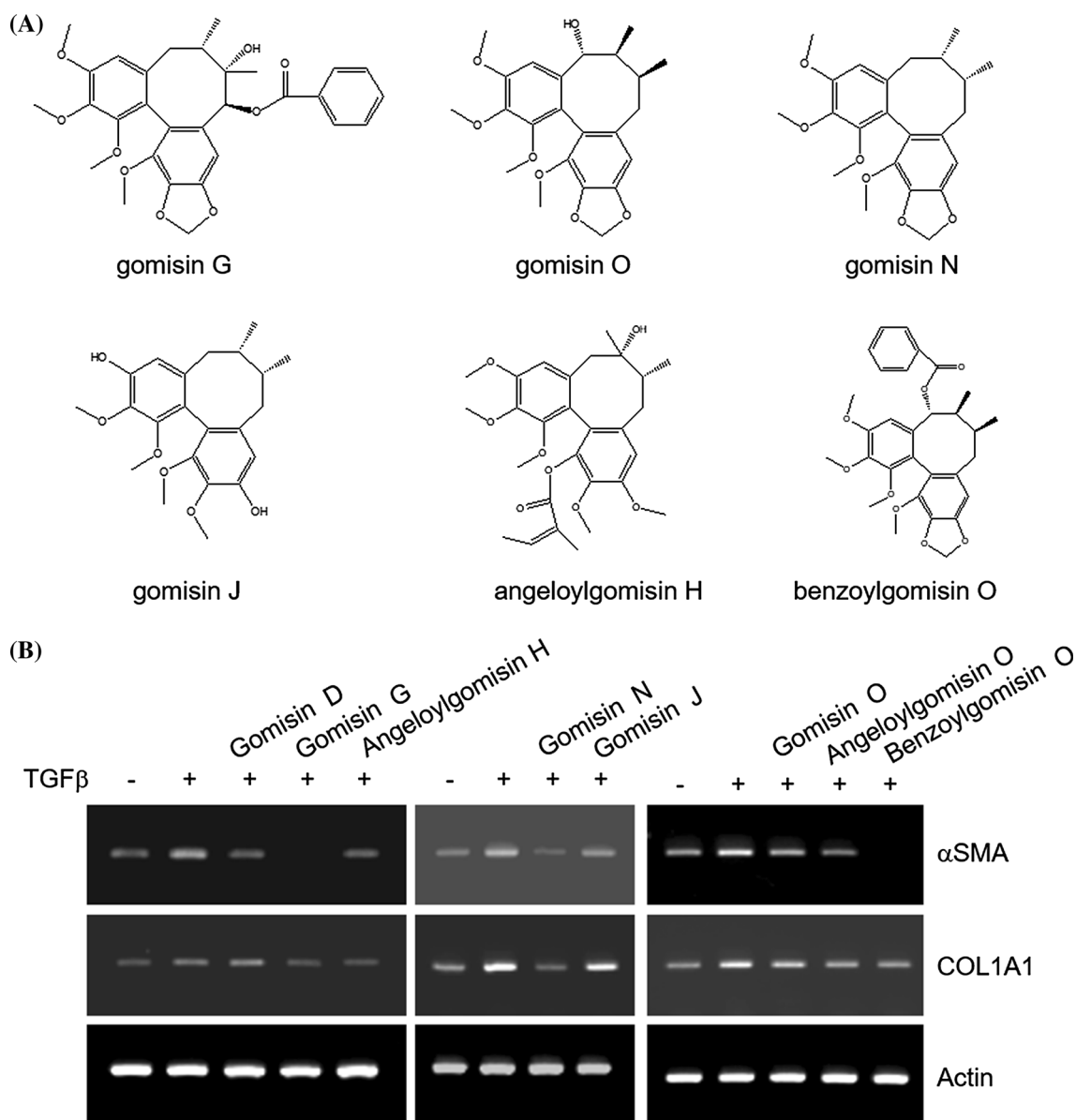
$2.3 \pm 0.38$ -fold (all  $P < 0.01$ ) (Fig. 3B), whereas COL1A1 mRNA levels were increased by  $2.7 \pm 0.20$ -,  $3.67 \pm 0.21$ -, and  $4.2 \pm 0.30$ -fold (all  $P < 0.001$ ) (Fig. 3C), respectively, after 6, 12, and 24 h of TGFβ stimulation. TGFβ-induced elevation of COL1A1 protein levels was confirmed by immunoblotting (Fig. 3D). These data demonstrate that TGFβ stimulates HSCs in a time-dependent manner under our experimental conditions.

### SBE inhibits TGFβ-induced activation of hepatic stellate cells

We next investigated whether SBE can inhibit HSCs stimulated by TGFβ. HSCs were pre-treated with 50 and 100 μg/mL SBE before the addition of TGFβ. RT-PCR showed that TGFβ-induced αSMA and COL1A1 expression was considerably attenuated in the presence of SBE

(Fig. 4A). To precisely quantify the inhibitory activity of SBE on TGFβ-induced HSC activation, Q-PCR analysis was performed. Expression levels of αSMA (Fig. 4B) and COL1A1 (Fig. 4C) mRNAs were increased by  $4.2 \pm 0.70$ - and  $3.5 \pm 0.35$ -fold (all  $P < 0.001$ ), respectively, compared to those of the control group by TGFβ stimulation. SBE treatment attenuated these TGFβ inducibilities of both αSMA and COL1A1 mRNAs in a dose-dependent manner. Inhibitory effect of SBE on TGFβ-induced COL1A1 expression was confirmed by immunoblotting (Fig. 4D). These results suggest that SBE inhibits TGFβ-induced HSC activation.





**Fig. 6** Inhibitory effect of lignans contained in *Schisandra chinensis* on TGFβ-induced expression of αSMA and COL1A1 mRNAs. (A) The structures of six lignans identified in the *n*-hexane fraction. (B) HSCs were either untreated or treated with 100 μg/mL solvent

extracts for 30 min, followed by stimulation with 10 ng/mL TGFβ. After 12 h, total RNA was isolated and αSMA and COL1A1 mRNA expression was measured by RT-PCR. Actin mRNA level was used as an internal control

### Identification of functional components of SBE responsible for suppression of TGFβ-induced activation of HSCs

To identify the functional components of SBE that inhibit HSCs, SBE was further partitioned successively with *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and water. HSCs were treated with each fraction before TGFβ stimulation, and then αSMA mRNA levels were measured by RT-PCR. We found that the *n*-hexane and *n*-butanol fractions of SBE had strong inhibitory effects on TGFβ-induced αSMA expression (Fig. 5A). We chose the *n*-hexane

fraction because more components were observed in the chromatogram of this fraction compared to those in the *n*-butanol fraction. Because many lignans have been reported to be contained in *S. chinensis* as mentioned above, authentic samples of nine lignans including gomisin D, gomisin G, gomisin H, gomisin J, gomisin N, gomisin O, angeloylgomisin H, angeloylgomisin O, and benzoylgomisin O were prepared at a concentration of 1 g/L in methanol. For HPLC analysis, 700-μL samples were collected and mixed. The chromatogram obtained is shown in Fig. 5B bottom. Six lignans including gomisin G, gomisin J, gomisin N, gomisin O, angeloylgomisin H, and



benzoylgomisin O were observed in the chromatogram of the *n*-hexane fraction (Fig. 5B, top). Although many studies have reported the hepatoprotective effects of lignan compounds isolated from *S. chinensis*, little information is available regarding the inhibitory effect on HSCs.

To determine whether lignan components exhibit inhibitory activity on TGF $\beta$ -stimulated HSCs, we used commercially available lignans (Fig. 6A). HSCs were treated with different lignans before stimulation with TGF $\beta$ , and  $\alpha$ SMA and COL1A1 mRNA levels were measured. RT-PCR revealed that six lignans (gomicin G, gomicin O, gomisin J, gomicin N, angeloylgomisin H, and benzoylgomisin O) reduced TGF $\beta$ -induced expression of both  $\alpha$ SMA and COL1A1 mRNA (Fig. 6B). These data suggest that at least six lignans in SBE have the potential to prevent TGF $\beta$ -induced activation of HSCs.

HSCs are primary cells that produce type-I collagen, a predominant extracellular matrix protein in liver fibrosis [17]. TGF $\beta$  is the most potent factor in promoting liver fibrosis by expressing extracellular matrix proteins [16, 18]. During hepatic injury, TGF $\beta$  is secreted from hepatocytes, platelets, Kupffer cells, and sinusoidal endothelial cells [19]. It activates HSCs and then stimulates Smad2 and Smad3 transcription factors that regulate transcription of various target-genes including COL1A1 [20]. Type-I collagen is composed of the  $\alpha$ 1 and  $\alpha$ 2 chains encoded by the COL1A1 and COL1A2 genes, respectively [21]. The previous study has reported that overexpression of Smad3 is sufficient to express COL1A1, suggesting that Smad transcription factors are essential in TGF $\beta$ -induced COL1A1 expression.

Previously, Park et al. [22] have demonstrated that p38 and JNK mitogen-activated protein kinase (MAPK) pathways stimulate Smad2/3 and gomicin N (also called schisandrin B) protects TGF $\beta$ -induced vascular fibrosis through inhibition of p38 and JNK MAPKs in rat aortic smooth muscle cell. Also, Chen et al. [12] have reported that gomicin N protects carbon tetrachloride (CCL<sub>4</sub>)-induced liver fibrosis by inhibition of TGF $\beta$ -mediated Smad signaling pathway. Similarly, it is possible that SBE inhibits TGF $\beta$ -induced COL1A1 expression through inhibition of Smad signaling pathway. Oxidative stress is closely associated with liver fibrosis [23]. Indeed, TGF $\beta$ -induced oxidative stress up-regulates COL1A1 gene expression via the activation of a CCAAT/enhancer binding protein- $\beta$  (C/EBP $\beta$ ) [24]. It has been reported that gomisin A and gomicin N inhibit ROS production in lipopolysaccharide-treated microglial cells [25] and doxorubicin-treated mouse heart [26]. Thus, it is also possible that lignans in SBE may abrogate TGF $\beta$ -induced COL1A1 expression through reducing of ROS accumulation in HSCs. Whether each lignan targets the same signaling pathway warrants further investigation. Future studies should explore which

signaling pathway is responsible for mediating the inhibitory effect of each lignan on COL1A1 expression in HSCs.

In summary, this study showed that SBE inhibits TGF $\beta$ -induced  $\alpha$ SMA and COL1A1 mRNA expression in HSCs. We also found that at least six lignans (gomicin G, gomicin O, gomicin N, gomisin J, angeloylgomisin H, and benzoylgomisin O) in the *n*-hexane fraction of the SBE were functionally involved in inhibiting TGF $\beta$ -induced HSC activation. Given that activation of HSCs plays a critical role in hepatic fibrogenesis in the injured liver, SBE can be utilized as a functional ingredient for preventing hepatic fibrosis.

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