JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 197–204

Received June 24, 2000
Accepted October 4, 2000
ISSN 0022-3573

Anti-fibrotic effects of a hot-water extract from *Salvia miltiorrhiza* roots on liver fibrosis induced by biliary obstruction in rats

Ji-Xing Nan, Eun-Jeon Park, Hee-Chul Kang, Pil-Hoon Park, Ji-Young Kim and Dong Hwan Sohn

Abstract

The anti-fibrotic effects of a hot-water extract form the traditional Chinese medicinal herb *Salvia miltiorrhiza* (Labiatae) on liver fibrosis induced by biliary obstruction was studied in rats. Liver fibrosis was induced in male Sprague-Dawley rats by bile duct ligation and scission (BDL). After surgery, the hot-water extract of *S. miltiorrhiza* roots (100 mg kg $^{-1}$, p.o.) was administered daily for 28 days. The concentrations of aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin and total cholesterol in serum and hydroxyproline and malondialdehyde contents in liver were significantly increased in BDL rats. Treatment with the extract of *S. miltiorrhiza* significantly reduced ($P < 0.01$) the serum aspartate transaminase, alanine transaminase, alkaline phosphatase, and total cholesterol concentrations in BDL rats. The liver hydroxyproline content in BDL rats treated with extract was also reduced to 68% of that in BDL control rats ($P < 0.01$). The liver malondialdehyde content in BDL rats treated with the extract was also reduced to 47% of that in BDL control rats ($P < 0.01$). The morphological characteristics of fibrotic livers were improved in BDL rats treated with extract. Immunohistochemical examination of fibrotic liver showed that the extract of *S. miltiorrhiza* markedly reduced protein expression of α-smooth muscle cell-like actin, which indicates that hepatic stellate cell activation was inhibited during liver fibrosis development. The results indicate that the hot-water extract of *S. miltiorrhiza* roots inhibits fibrosis and lipid peroxidation in rats with liver fibrosis induced by biliary obstruction.

College of Pharmacy, Medicinal Resources Research Center, Wonkwang University, Iksan, Cheonbuk 570–749, South Korea

Ji-Xing Nan, Eun-Jeon Park, Hee-Chul Kang, Pil-Hoon Park, Ji-Young Kim, Dong Hwan Sohn
Correspondence: D. H. Sohn, College of Pharmacy, Wonkwang University, Iksan, Cheonbuk 570–749, South Korea. E-mail: dhsohn@wonkwang.ac.kr
Funding: This work was supported by the Medicinal Resources Research Center (98-16-02-01-A-3) sponsored by the Korea Science and Engineering, Chollabuk-Do Provincial Government and Wonkwang University.

Introduction

Chronic liver injury leads to excessive deposition of collagen in liver resulting in fibrosis/cirrhosis (Chojkier & Brenner 1988; Park et al 1997). Prevention or suppression of fibrotic changes in liver, or protection from and treatment of liver fibrosis/cirrhosis are therefore important (Chojkier & Brenner 1988; Friedman 1993). Therapeutic attempts with anti-fibrotic drugs are still at an experimental stage (Park et al 1997; Nan et al 2000a). The problems associated with anti-fibrotics are toxicity due to chronic administration and the reduced therapeutic effects when tested in clinical studies. Developing anti-fibrotics from natural products used in traditional medicine may reduce the risk of toxicity and maintain the therapeutic effectiveness when the drug is used clinically (Wasser et al 1998; Li & Friedman 1999; Shimizu et al 1999).

Table 1 Changes in bodyweight and liver weight in fibrotic rats induced by bile duct ligation and scission (BDL), treated with the hot-water extract of *Salvia miltiorrhiza* roots (100 mg kg $^{-1}$, p.o.) daily for 28 days.

Group	n	Bodyweight (g)		Liver weight (g)	Liver weight/100 g bodyweight
		Day 0	Day 28		
Control sham-operated	4	245 ± 10	327 ± 19	11.7 ± 1.2	3.6 ± 0.3
Sham-operated + extract	4	247 ± 12	329 ± 27	11.8 ± 1.3	3.6 ± 0.2
Control BDL	8	240 ± 20	312 ± 24	23.6 ± 3.3*	7.6 ± 0.8*
BDL + extract	8	243 ± 15	309 ± 28	17.8 ± 2.8*,†	5.8 ± 0.7*,††

Data are mean ± s.d. *$P < 0.001$, significantly different compared with control sham-operated group. †$P < 0.05$, significantly different compared with control BDL group. ††$P < 0.01$, significantly different compared with control BDL group.

Salvia miltiorrhiza (Labiatae; Danshen in Chinese) is an important ingredient of certain recipes used in traditional oriental medicine for the treatment of cardiovascular disorders and liver diseases. It has been reported that the hot-water extract of the root of *S. miltiorrhiza* has pharmacological actions, such as anti-platelet aggregation and thrombus formation, improving cardio–cerebral circulation, and antioxidant activity against peroxidative damage to liver microsomes, hepatocytes and erythrocytes of rats (Huang & Zhang 1992; Wu et al 1998). It has also been shown to inhibit acute liver injury induced by D-galactosamine (Qi 1991) and to reduce carbon tetrachloride (CCl_4)-induced liver fibrosis in rats (Wasser et al 1998).

We investigated the anti-fibrotic effect of *S. miltiorrhiza* on liver fibrosis induced by bile duct ligation and scission (BDL) in rats. Although *S. miltiorrhiza* has been previously reported to reduce fibrosis, the study involved liver fibrosis induced by chemical toxicants (e.g. carbon tetrachloride) which is aetiologically and pathogenically different from liver fibrosis in man. In this study, we used a liver fibrosis model induced by biliary obstruction, which resembles the biliary fibrosis in man. We also determined the effects of *S. miltiorrhiza* on hepatic stellate cells that are responsible for collagen synthesis in liver (Maher & McGuire 1990).

Materials and Methods

Animals

Male Sprague-Dawley rats, 200–220 g, were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea). They were fed a normal standard chow diet (Jae Il Chow, Korea) and had free access to tap water. Throughout the experiment, the rats were housed four or five per cage in laminar flow cages maintained at 22 ± 2°C and 50–60 % relative humidity, with a 12-h light–dark cycle. The rats were maintained in these facilities for at least 1 week before the experiment. The experiment was carried out in accordance with the Guiding Principles in the Use of Animals in Toxicology adopted by the Society of Toxicology.

Preparation of hot-water extract from *S. miltiorrhiza*

S. miltiorrhiza roots were purchased from Sungboe Farm, Co. (Ahnsung, Kyunggi-do, Korea) and authenticated by Professor Y. C. Kim, College of Pharmacy, Wonkwang University. The hot-water extract was prepared by boiling the dried roots with distilled water for 5 h. The extract was filtered, freeze-dried, and kept at 4°C. The yield of extraction was approximately 8.1 % (w/w). The dried extract was dissolved in distilled water before use.

Induction of liver fibrosis

Rats were anaesthetized with ketamine/rompun and the common bile duct was exposed and ligated by double ligatures with suture silk. The first ligature was made below the junction of the hepatic ducts and the second ligature was made above the entrance of the pancreatic ducts. Finally, the common bile duct was resected between the double ligatures. In sham-operated rats, an incision was made in the abdomen, which was then closed without any treatment. The number of the rats used in each group is shown in Table 1.

Table 2 Serum biochemical values in fibrotic rats induced by bile duct ligation and scission (BDL), treated with the hot-water extract of *Salvia miltiorrhiza* roots (100 mg kg^{-1}, p.o.) daily for 28 days.

Group	n	Aspartate transaminase (IU L^{-1})	Alanine transaminase (IU L^{-1})	Alkaline phosphatase (IU L^{-1})	Total cholesterol (mg L^{-1})	Total bilirubin (mg dL^{-1})
Control sham-operated	4	86 ± 18	49 ± 6	603 ± 104	84 ± 23	0.3 ± 0.2
Sham-operated + extract	4	87 ± 10	31 ± 15	716 ± 106	80 ± 11	0.3 ± 0.2
Control BDL	8	670 ± 95*	153 ± 11*	1326 ± 172*	143 ± 11*	8.6 ± 0.7*
BDL + extract	8	454 ± 96*,†	98 ± 15*,†	846 ± 110*,†	115 ± 10*,†	8.1 ± 0.5*

Data are mean ± s.d. *$P < 0.001$, significantly different compared with control sham-operated group. †$P < 0.01$, significantly different compared with control BDL group.

Animal treatment

The hot-water extract was diluted with distilled water and given orally, using an intubation needle, to rats for 28 days, at a daily dose of 100 mg kg^{-1} (corresponding to crude drug, 1.2 g/100 mg). The control groups received equal amounts of distilled water given orally, using an intubation needle, for 28 days.

Determination of serum biochemical parameters

After 28 days of treatment, rats were anaesthetized with ether and blood was obtained by cardiac puncture for serum biochemical testing. Blood samples were kept at room temperature for 1 h and centrifuged at 3000 rev min^{-1} for 30 min to obtain sera. Sera were kept at −20°C until further assay. The activity of serum aspartate transaminase, alanine transaminase, alkaline phosphatase, levels of total cholesterol and total bilirubin were measured by the Autodry chemistry analyser (Spotchem SP4410, Arkray, Japan).

Determination of hydroxyproline content in liver

The hydroxyproline content in liver was determined by the method of Jamall et al (1981). Briefly, specimens of the liver were weighed and completely hydrolysed in 6 M HCl. A fraction of the samples was derivatized using chloramine T solution and Erhlich reagent and measured at 558 nm. The standard calibration curve was prepared using trans-4-hydroxy-L-proline (Sigma Chem. Co.).

Determination of lipid peroxidation in liver

Lipid peroxidation was quantified by the thiobarbituric acid (TBA) method (Plaa & Charbonneau 1994) using the S9 fraction of liver homogenates as enzyme source. All manipulations were rapidly made on ice to avoid peroxidation. The S9 fraction of homogenate (approx. 5 mg protein) was reacted with TBA solution in a boiling water bath for 10 min and then extracted with *n*-butanol for 10 s. The organic phase containing coloured complex was collected for fluorescence measurement. Excitation and emission wavelengths were 532 and 553 nm, respectively. The calibration curve was prepared using 1,1,3,3-tetraethoxypropane (Sigma Chemical Co.), a chemical that releases malondialdehyde in acidic conditions.

Histological and immunohistochemical examination of liver

The portion of removed liver was rapidly fixed with 10% neutralized formalin (pH 7.4), stained with haematoxylin and eosin to determine histological changes in liver fibrosis induced by BDL. α-Smooth muscle cell actin for detection of activated hepatic stellate cells was immunohistologically assessed by the strepavidin–biotin–peroxidase complex method using Lsab 2 Kit (Dako Co., USA) and anti-α-smooth muscle cell actin monoclonal antibody (Boehringer Mannheim, Germany).

Statistical analysis

The results were evaluated by one-way analysis of variance and Tukey's multiple comparison tests. Values of $P < 0.05$ were considered to indicate a significant difference.

Results

BDL rats showed the symptoms of jaundice within five days and developed fibrosis/cirrhosis 28 days after the

Table 3 Hydroxyproline and thiobarbituric reactive substance values in rat liver induced by bile duct ligation and scission (BDL), treated with the hot-water extract of *Salvia miltiorrhiza* roots (100 mg kg $^{-1}$, p.o.) daily for 28 days.

Group	n	Hydroxyproline (μg (g liver)$^{-1}$)	Thiobarbituric reactive substance (pmol (mg protein)$^{-1}$)
Control sham-operated	4	341 ± 23	149 ± 8
Sham-operated + extract	4	321 ± 29	118 ± 10
Control BDL	8	1077 ± 195*	396 ± 36*
BDL + extract	8	727 ± 121*,†	188 ± 42*,††

Data are mean ± s.d. *$P < 0.001$, significantly different compared with control sham-operated group. †$P < 0.05$, significantly different compared with control BDL group. ††$P < 0.01$, significantly different compared with control BDL group.

operation. All rats showed a slight decrease in bodyweight during the first week due to the operation, but then returned to normal weight. There was no significant difference in bodyweight between control BDL rats and BDL rats treated with extract (Table 1). Liver weights were significantly increased in BDL rats compared with sham-operated rats ($P < 0.001$). The liver-to-body weight ratio of BDL rats treated with extract was significantly lower than that of the control BDL rats ($P < 0.01$) (Table 1).

Serum biochemical parameters are shown in Table 2. The activity of serum aspartate transaminase, alanine transaminase, alkaline phosphatase, total cholesterol and levels of total bilirubin were significantly elevated in control BDL rats ($P < 0.01$). In BDL rats treated with extract, serum aspartate transaminase, alanine transaminase, alkaline phosphatase and total cholesterol levels were reduced to 68, 64, 64 and 80% that of control BDL rats, respectively ($P < 0.01$). In sham-operated rats treated with extract, there were no significant changes in serum parameters compared with control sham-operated rats (Table 2).

As shown in Table 3, the liver content of hydroxyproline increased about 3.5-fold 28 days after BDL operation ($P < 0.01$). Compared with the control BDL rats, treatment with the extract reduced the hydroxyproline content to 68% that of control BDL rats ($P < 0.01$). In sham-operated rats treated with extract, there were no significant changes in hydroxyproline content compared with control sham-operated rats. MDA levels increased 2.5-fold in fibrotic rat liver induced by BDL, indicating that 28 days of bile duct obstruction significantly increases lipid peroxidation in liver ($P < 0.001$) (Table 3). In BDL rats treated with extract, the MDA level was reduced to 47% that of control BDL rats ($P < 0.01$). In sham-operated rats treated with extract, there were no significant changes in MDA content compared with control sham-operated rats (Table 3).

Histological analysis of liver sections showed that 28 days of biliary obstruction is accompanied by an increase in collagen deposition around the portal triad, excessive bile duct proliferation and inflammatory cell deposition resulting in destruction of the lobular architecture (Figure 1B). In BDL rats treated with extract (Figure 1C), there was a tendency towards less pronounced destruction of the liver architecture, bile duct proliferation and fibrosis compared with control BDL rat liver. Liver from sham-operated rats treated with extract showed no histological alterations compared with sham-operated rats (Figure 1A and D).

Using an antibody against α-smooth muscle cell actin, a marker of stellate cell activation, we assayed expression of this protein in liver immunohistochemically. In sham-operated livers, vascular smooth muscle cells around the vessel were positive for α-smooth muscle cell actin, whereas stellate cells positive for α-smooth muscle cell actin were rarely observed (Figure 2A). Activated hepatic stellate cells, which express α-smooth muscle cell like actin, showed marked proliferation in the livers of control BDL rats (Figure 2B). In contrast, treatment with the extract markedly reduced the numbers of α-smooth muscle cell actin positive stellate cells (Figure 2C) in fibrotic liver induced by BDL.

Discussion

We have screened a number of liver protective and anti-fibrotic agents from natural products used in traditional

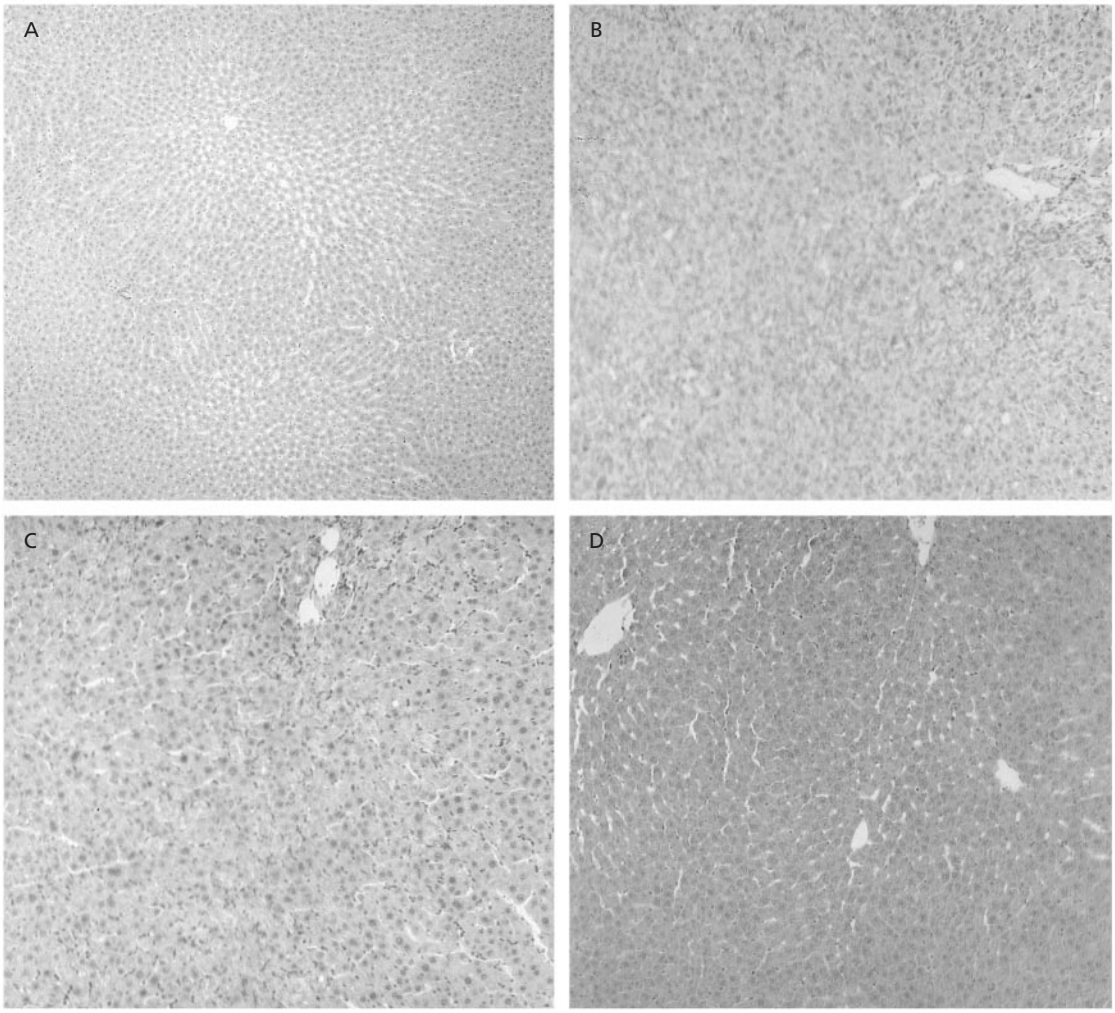

Figure 1 Light microscopic appearance of fibrotic rat liver induced by bile duct ligation and scission (BDL) and treated with the hot-water extract of *Salvia miltiorrhiza* roots for 28 days (H&E, magnification × 100). A, Control sham-operated group; B, control BDL group; C, BDL group treated with extract; D, sham-operated group treated with extract.

oriental folk medicine for the treatment of liver diseases (Park et al 1997, 2000a, b, c; Song et al 1998; Nan et al 2000a, b). *S. miltiorrhiza* roots are inexpensive and widely available, and have traditionally been used for the treatment of cardiovascular and liver diseases in China, Japan and Korea. The hot-water extract of *S. miltiorrhiza* roots contains a mixture of natural phenolic compounds, which have antioxidant effects in-vitro and in-vivo (Liu et al 1992, 1999; Wu et al 1998). These compounds are reported to be salvianolic acid A, salvianolic acid B, protocathechualdehyde, protocathechuic acid, caffeic acid, D(+)β 3,4-dihydroxyphenol lactic acid, danshensu and rosmarinic acid. *S. miltiorrhiza* has been reported to improve experimental liver damage induced in rats by D-galactosamine (Qi 1991) and CCl_4 (Hu et al 1997; Wasser et al 1998). Moreover, a recent report indicated that *S. miltiorrhiza* prevented liver fibrosis formation and even reversed the cirrhotic change induced by carbon tetrachloride in rats (Wang & Wang 1995). It is also reported to exhibit anti-tumour properties, inducing apoptosis in HepG2, HL60, A549 and K562 cells (Sung et al 1999; Yoon et al 1999; Liu et al 2000). Magnesium lithospermate in an aqueous extract of *S. miltiorrhiza* was also reported to reduce newly synthesized skin collagen when administered orally (Shigematsu et al 1994).

In this study, liver fibrosis was induced by bile duct ligation and scission in rats. To evaluate the anti-fibrotic

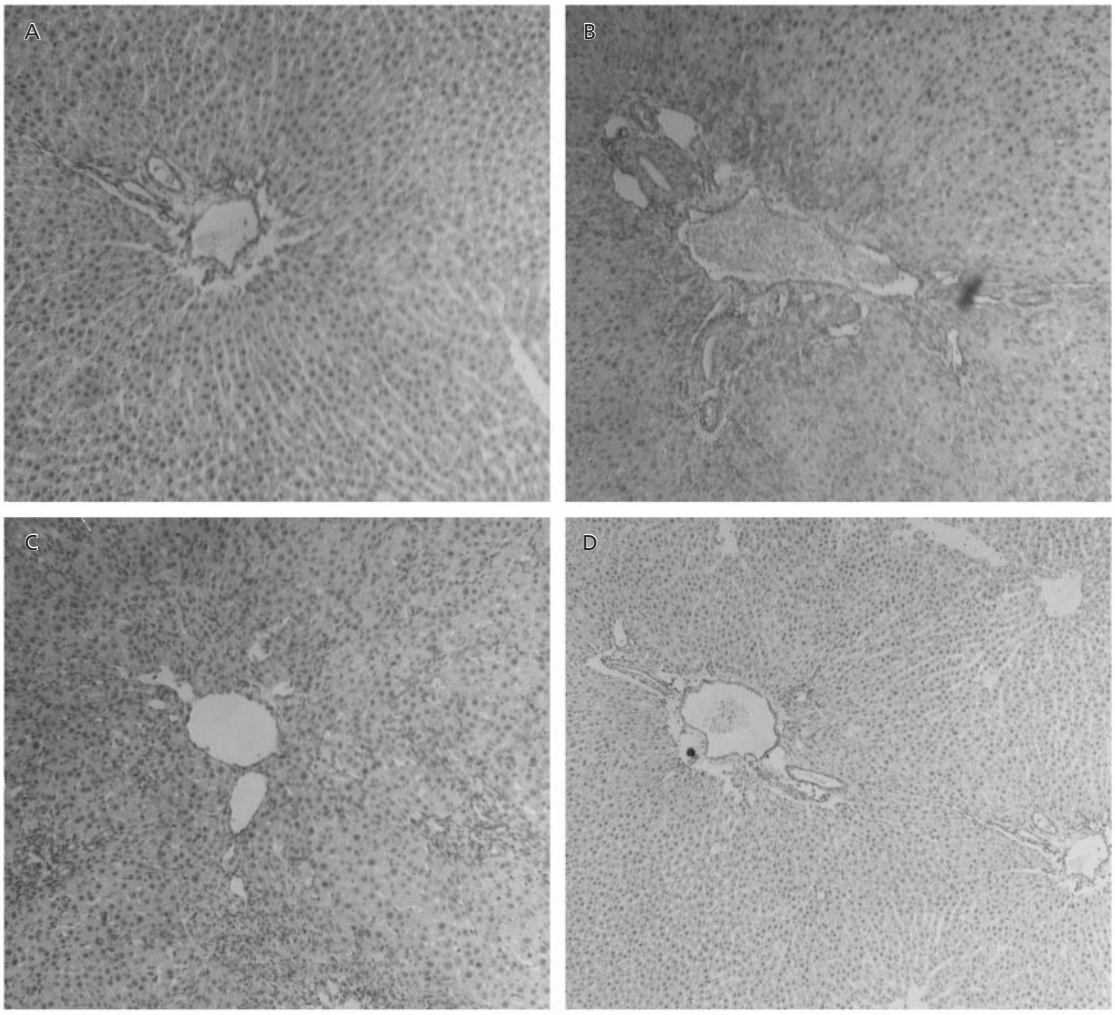

Figure 2 Immunohistochemical appearance of fibrotic rat liver stained against α-smooth muscle cell-like actin (magnification $\times$ 100). A, Control sham-operated group; B, control bile duct ligation and scission (BDL) group; C, BDL group treated with hot-water extract of *Salvia miltiorrhiza* root for 28 days; D, sham-operated group treated with extract for 28 days.

potential of a given agent, the selection of appropriate animal models that most closely represent human chronic liver diseases is important. Most studies have used models of injury induced by free radicals, severe necrosis or inflammation such as CCl_4- or dimethylnitrosamine-induced liver fibrosis (Perez 1984). These models do not represent the common human chronic liver diseases, which are often characterized by moderate or even absent inflammation and necrosis in spite of progression to fibrosis/cirrhosis.

Bile duct ligation and scission induces a type of liver fibrosis aetiologically and pathogenically different from the experimental liver fibrosis induced by toxicants such as CCl_4 (Kountouras et al 1984). Extra-hepatic obstruction of the bile duct leads to dilation of bile canaliculi, reduction of canalicular microvilli, widening of the pericanalicular space and increased lysosomal activity. Biliary obstruction for 28 days or more leads to fibrosis and resembles the biliary fibrosis/cirrhosis in man (Milani et al 1990).

In this study, there were marked increases in liver weight, serum bilirubin, and the activity of serum aspartate transaminase, alanine transaminase, alkaline phosphatase, as well as in hydroxyproline and malondialdehyde content in liver after 28 days of bile duct ligation and scission. The elevation of these serum biochemical parameters and the distortion of normal lobular architecture in liver showed the major charac-

teristics of biliary liver fibrosis (Kountouras et al 1984). Treatment with the hot-water extract of *S. miltiorrhiza* roots for 28 days significantly reduced deposition of hydroxyproline and malondialdehyde in fibrotic liver ($P < 0.01$). These results indicate that the extract significantly inhibited collagen accumulation and lipid peroxidation in liver fibrosis induced by biliary obstruction. Treatment with hot-water extract of *S. miltiorrhiza* roots reduced the activity of serum aspartate transaminase, alanine transaminase, alkaline phosphatase and level of total cholesterol in rats with liver fibrosis ($P < 0.01$). From the immunohistological appearances, hot-water extract of *S. miltiorrhiza* roots reduced the α-smooth muscle cell actin-positive area in liver, showing inhibition of hepatic stellate cell activation during the fibrogenic process.

Although the mechanism of liver fibrosis is not fully understood, activated hepatic stellate cells play an important role in connective tissue synthesis and deposition during fibrogenesis. The pharmacological inhibition of new connective tissue formation and deposition appears to be a promising therapeutic approach. Thus, the extract of *S. miltiorrhiza* roots, which suppressed hepatic stellate cell activation and reduced collagen in biliary liver fibrosis, could be a promising anti-fibrotic agent in biliary liver fibrosis. Furthermore, it has been reported that *S. miltiorrhiza* roots strongly inhibited the proliferation and induced apoptosis in human hepatoma cell line, HepG2 (Liu et al 2000). Thus, the hot-water extract of *S. miltiorrhiza* roots may have a cancer-preventive action, as liver fibrosis/cirrhosis can develop into cancer.

In conclusion, this study demonstrates that the hot-water extract of *S. miltiorrhiza* roots can effectively improve the liver fibrosis induced by bile duct ligation and scission as monitored by reduced levels of connective tissue, lipid peroxidation and hepatic stellate cell activation in liver. Further systematic study is needed to reveal the anti-fibrotic mechanism and to identify the active compounds in the hot-water extract of *S. miltiorrhiza* roots that inhibit liver fibrosis induced by biliary obstruction in rats.

References

Chojkier, M., Brenner, D. A. (1988) Therapeutic strategies for hepatic fibrosis. *Hepatology* **8**: 176–182

Friedman, S. L. (1993) The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. *N. Engl. J. Med.* **328**: 1828–1835

Hu, Y. Y., Liu, P., Xu, L. M., Liu, C. H., Zhu, D. Y., Huang, M. F. (1997) Actions of salvianolic acid A on CCl_4-poisoned liver injury and fibrosis in rats. *Acta. Pharma. Sinica.* **18**: 478–480

Huang, Y. S., Zhang, J. T. (1992) Antioxidative effect of three water-soluble components isolated from *Salvia miltiorrhiza* in vitro. *Acta. Pharma. Sinica.* **27**: 96–100

Kountouras, J., Billing B. H., Scheuer, P. J. (1984) Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. *Br. J. Exp.* **65**: 305–311

Jamall, I. S., Finelli, V. N., Que Hee, S. S. (1981) A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. *Anal. Biochem.* **112**: 70–75

Li, D., Friedman, S. (1999) Liver fibrosis and the role of hepatic stellate cells: new insights and prospects for therapy. *J. Gastroenterol. Hepatol.* **14**: 618–633

Liu, G. T., Zhang, T. M., Wang, B. E., Wang, Y. W. (1992) Protective action of seven natural phenolic compounds against peroxidative damage to biomembranes. *Biochem. Pharmacol.* **43**: 147–152

Liu, J., Yang, C. F., Lee, B. L., Shen, H. M., Ang, S. G., Ong, C. N. (1999) Effect of *Salvia miltiorrhiza* on aflatoxin B1-induced oxidative stress in cultured rat hepatocytes. *Free Radic. Res.* **31**: 559–568

Liu, J., Shen, H., Ong, C. (2000) *Salvia miltiorrhiza* inhibits cell growth and induces apoptosis in human hepatoma HepG(2) cells. *Cancer Lett.* **153**: 85–93

Maher, J. J., McGuire, R. F. (1990) Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. *J. Clin. Invest.* **98**: 175–184

Milani, S., Herbst, H., Schuppan, D., Kim, K. Y., Riecken, E. O., Stein, H. (1990) Procollagen expression by non-parenchymal rat liver cells in experimental biliary fibrosis. *Gastroenterology* **98**: 175–184

Nan, J.-X., Park, E.-J., Kim, H. J., Ko, G., Sohn, D. H. (2000a) Antifibrotic effects of the methanol extract of *Polygonum aviculare* in fibrotic rats induced by bile duct ligation and scission. *Biol. Pharm. Bull.* **23**: 240–243

Nan, J.-X., Park, E.-J., Yang, B.-K., Song, C.-H., Ko, G., Sohn, D. H. (2000b) Antifibrotic effect of *Stephania tetrandra* on experimental liver fibrosis induced by bile duct ligation and scission in rats. *Arch. Pharm. Res.* **23**: 501–506

Park, E. J., Ko, G., Kim, J., Sohn, D. H. (1997) Antifibrotic effects of a polysaccharide extracted from *Ganoderma lucidum*, glycyrrhizin, and pentoxifylline in rats with cirrhosis induced by biliary obstruction. *Biol. Pharm. Bull.* **20**: 417–420

Park, E. J., Jeon, C. H., Ko, G., Kim, J., Sohn, D. H. (2000a) Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J. Pharm. Pharmacol.* **52**: 437–440

Park, E. J., Nan, J.-X., Kim, J. Y., Kang, H. C., Choi, J. H., Lee, S. J., Lee, B. H., Kim, S. J., Lee, J. H., Kim, Y. C., Sohn, D. H. (2000b) Ethanol soluble part of hot-water extract from *Artemisia iwayomogi* inhibits liver fibrosis induced by carbon tetrachloride in rats. *J. Pharm. Pharmacol.* **52**: 875–881

Park, P.-H., Nan, J.-X., Park, E.-J., Kang, H. C., Kim, J. Y., Ko, G., Sohn, D. H. (2000c) Effect of tetrandrine on experimental hepatic fibrosis induced by bile duct ligation and scission in rats. *Pharmacol. Toxicol.* **87**: 261–268

Perez, T. R. (1984) Is cirrhosis of the liver experimentally produced by CCl_4 an adequate model of human cirrhosis? *Hepatology* **3**: 112–120

Plaa, G. L., Charbonneau, M. (1994) Detection and evaluation of chemically induced liver injury. In: Hayes, A. W. (ed.) *Principles and Methods of Toxicology*. 3rd edn. Raven Press Ltd, New York, pp 839–870

Qi, X. G. (1991) Protective mechanism of *Salvia miltiorrhiza* and *Paeonia lactiflora* for experimental liver damage. *Chin. J. Integr. Trad. West. Med.* **11**: 102–104

Shigematsu, T., Tajima, S., Nishikawa, T., Murad, S., Pinnell, S. R., Nishioka, I. (1994) Inhibition of collagen hydroxylation by lithospermic acid magnesium salt, a novel compound isolated from *Salviae miltiorrhizae* Radix. *Biochim. Biophys. Acta* **1200**: 79–83

Shimizu, I., Ma, Y. R., Mizobuchi, Y., Liu, F., Miura, T., Nakai, Y., Yasuda, M., Shiba, M., Horie, T., Amagaya, S., Kawada, N., Hori, H., Ito, S. (1999) Effects of sho-saiko-to, a Japanese herbal medicine, on hepatic fibrosis in rats. *Hepatology* **29**: 149–160

Song, C. H.,Yang, B. K., Ra, K. S., Sohn, D. H., Park, E. J., Ko, G., Kim, Y. H. (1998) Hepatoprotective effect of extracellular polymer produced by submerged culture of *Ganoderma lucidum* WK003. *J. Microbiol. Biotechnol.* **8**: 277–279

Sung, H. J., Choi, S. M., Yoon, Y., An, K. S. (1999) Tanshinone IIA, an ingredient of *Salvia miltiorrhiza* Bunge, induces apoptosis in human leukemia cell lines through the activation of caspase-3. *Exp. Mol. Med.* **31**: 174–178

Wang, H. J., Wang, B. E. (1995) Long-term follow-up result of compound Dan Shen granule (861 Chong Fu Ji) in treating hepatofibrosis. *Chin. J. Integr. Trad. West. Med.* **5**: 4–5

Wasser, S., Ho, J. M., Ang, H. K., Tan, C. E. (1998) *Salvia miltiorrhiza* reduces experimentally-induced hepatic fibrosis in rats. *J. Hepatol.* **29**: 760–771

Wu, Y. J., Hong, C. Y., Lin, S. J., Wu, P., Shiao, M. S. (1998) Increase of vitamin E content in LDL and reduction of atherosclerosis in cholesterol-fed rabbits by a water-soluble antioxidant-rich fraction of *Salvia miltiorrhiza*. *Arterioscler. Thromb. Vasc. Biol.* **18**: 481–486

Yoon, Y., Kim, Y. O., Jeon, W. K., Park, H. J., Sung, H. J. (1999) Tanshinone IIA isolated from *Salvia miltiorrhiza* Bunge induced apoptosis in HL60 human premyelocytic leukemia cell line. *J. Ethnopharmacol.* **68**: 121–127