Protective Effects of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* Extract on Liver Injury in Rats

Hsin-Fang Chang,[*] Yun-Ho Lin,[†] Chia-Chou Chu,[‡] Shu-Ju Wu,[‡,§]
Ya-Hui Tsai[*,‡] and Jane C.-J. Chao[*]
[*]*School of Nutrition and Health Sciences*, [†]*Department of Pathology*, *School of Medicine*
[‡]*School of Pharmacy*, *Taipei Medical University*, *Taipei 110*, *Taiwan*
[§]*Department of Nursing*, *Chang Gung Institute of Technology*, *Taoyuan 333*, *Taiwan*

Abstract: This study investigated the effects of the combined extracts of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* at different doses on hepatic antioxidant status and fibrosis in rats with carbon tetrachloride (CCl_4)-induced liver injury. Male Sprague-Dawley rats (n = 8–12 per group) were divided into the control, CCl_4, CCl_4 + silymarin (0.35%), CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1; LE), and CCl_4 + high-dose herbal extract (1.20% of the same herbal extract; HE) groups. Silymarin or herbal extract was orally given to rats a week before chronic intraperitoneal injection with CCl_4 for 6 weeks. The pathological results showed that herbal extract suppressed hepatic bile duct proliferation, and low-dose herbal extract inhibited liver fibrosis. Hepatic superoxide dismutase (SOD) activity was lower in the CCl_4 group, but there was no difference in the silymarin or herbal extract treated groups compared to the control group. Hepatic catalase activity and the ratio of reduced to oxidized glutathione were significantly higher ($p < 0.05$) in the HE group than those in the CCl_4 group. Silymarin and herbal extract reversed the impaired hepatic total antioxidant status ($p < 0.05$). Herbal extract partially reduced the elevated hepatic lipid peroxides. Hepatic transforming growth factor-β1 (TGF-β1) level decreased significantly ($p < 0.05$) in the LE group. Therefore, high-dose herbal extract improved hepatic antioxidant capacity through enhancing catalase activity and glutathione redox status, whereas low-dose herbal extract inhibited liver fibrosis through decreasing hepatic TGF-β1 level in rats with CCl_4-induced liver injury.

Keywords: *Ginkgo biloba*; *Panax ginseng*; *Schizandra chinensis*; Liver Injury; Rat.

Correspondence to: Dr. Jane C.-J. Chao, School of Nutrition and Health Sciences, Taipei Medical University, 250 Wu Hsing Street, Taipei 110, Taiwan. Tel: (+886) 2-2736-1661 (ext. 6548), Fax: (+886) 2-2737-3112, E-mail: chenjui@tmu.edu.tw

Introduction

The Department of Health, Taiwan reported that chronic liver diseases including cirrhosis have become the 7th leading cause of death in Taiwan, and over 5,600 patients died from these diseases during 2005. The infection rate of hepatitis B, the most prevalent type among virus-induced hepatitis, was 15% to 20% for persons aged over 30 years in Taiwan, with at least three million persons carrying hepatitis B surface antigen (Sung *et al.*, 1984). Hepatitis B infection has been reported to be associated with the development of cirrhosis and hepatocellular carcinoma (HCC) (Chen, 1993; Tuei *et al.*, 1994; Yu and Chen, 1994). Therefore, hepatoprotection is a major approach to prevent the development of HCC.

When liver injury occurs, quiescent stellate cells transform into myofibroblasts (Kawada, 2004). Subsequently, cell proliferation is induced in response to certain growth factors such as platelet-derived growth factor-BB, insulin-like growth factor-1, connective tissue growth factor, and fibroblast growth factor-2 (Kawada, 2004; Paradis *et al.*, 2002; Wells *et al.*, 2004). Meanwhile, increased extracellular matrix production and mononuclear cell migration are accompanied by the secretion of transforming growth factor-β (TGF-β) (Kawada, 2004; Wells *et al.*, 2004) and monocyte chemotactic protein-1 (Sprenger *et al.*, 1999; Kawada, 2004), which contributes to the pathogenesis of liver fibrosis.

Many herbs have been reported to show significant hepatoprotective activities. *Ginkgo biloba* and Han-Dan-Gan-Le, a *Ginkgo biloba*-containing herb preparation, have been demonstrated to be effective in protecting against liver fibrosis in humans (Li *et al.*, 1995) and rats (Li *et al.*, 1998, 2003), respectively. *Panax ginseng* extract or its active ingredients protected against chemical-induced hepatotoxicity in animals (Matsuda *et al.*, 1991; Jeong *et al.*, 1997; Lin *et al.*, 2003). Additionally, previous studies (Mizoguchi *et al.*, 1991; Chiu *et al.*, 2002) showed a potent antihepatotoxic activity of the lignan-enriched extract of schizandra fruit. However, it is not known whether the combination extracts of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* has a potent hepatoprotective activity compared to silymarin which is derived from milk thistle and has been traditionally used for the treatment of liver diseases. This study further investigated the protective effects of the combined herbal extracts of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* with different doses on liver injury induced by CCl_4 in rats.

Materials and Methods

Animals and Treatments

Male Sprague-Dawley rats (300–350 g) were purchased from the National Laboratory Animal Center (Taipei, Taiwan). The rats were housed individually on a 12-hour light/dark cycle at 22 ± 2°C. After one-week adaptation period, the rats were randomly divided into 5 groups (n = 8–12 per group): control, CCl_4, CCl_4 + silymarin (0.35% silymarin; SM), CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1; LE), and CCl_4 + high-dose herbal extract (1.20% of the same herbal extract; HE) groups. Silymarin was commonly used as a positive control in the model

of liver injury (Favari and Perez-Alvarez, 1997). The combined herbal extract contains standardized *Ginkgo biloba* extract (25.5% ginkgo flavone glycosides and 7.0% terpene lactones; EGb761, Chemlife Enterprise, Taipei, Taiwan), *Panax ginseng* extract (80% ginsenosides with ginsenosides-Rh1, Rh2, Rg1, Rg2, Rg3, Rd, Rc, and Rb1, Chemlife Enterprise), and *Schizandra chinensis* extract (2% schisantherins with schisantherins A and B, Chemlife Enterprise) in an equal ratio. The rats were orally given silymarin or herbal extracts mixed in the powdered feed (Laboratory Rodent Diet 5001™, PMI Nutrition International Inc., Brentwood, MO, USA) a week before CCl_4 injection at week 1 for 6 weeks. Carbon tetrachloride (20% in olive oil) was administered intraperitoneally at a single dose of 1.0 ml/kg body weight once a week from week 2 to week 5. The rats receiving the vehicle (olive oil) in a similar dose (1.0 ml/kg body weight) served as a negative control. All animal protocols were approved and conducted under the guidelines of the Institutional Animal Care and Use Committee at Taipei Medical University.

Histopathological Examination

After 6 weeks, the rats were sacrificed under ether anesthesia. Excised liver (1 cm × 1 cm) was fixed in 10% paraformaldehyde, embedded in paraffin wax, and stained with haematoxylin and eosin (H & E) or Masson's trichrome. Coded specimens were scored under a light microscope by a pathologist in a blinded fashion.

Plasma Alanine Aminotransferase (*ALT*) *and Aspartate Aminotransferase* (*AST*) *Activities*

Blood, after rats were fasted overnight, was collected into heparin-containing tubes from the tail vein at week 0, 1, 2, 4, and 6. Blood samples were centrifuged at 1400 g at 4°C for 15 min. Plasma ALT and AST activities were determined spectrophotometrically at 570 nm (RM163-K, Iatron Laboratories, Inc., Tokyo, Japan).

Plasma and Hepatic Lipid Concentrations

Plasma and hepatic cholesterol and triglyceride concentrations were determined spectrophotometrically using cholesterol esterase/peroxidase (CHOD-PAP) and lipase glycerol kinase (GPO-PAP) enzymatic methods, respectively (CH201 for cholesterol, Randox TR213 for triglycerides, Randox Laboratories Ltd., Antrim, UK). The liver was homogenized in chloroform/methanol (2:1) solution, and extracted by chloroform/methanol/water (3:48:47). The absorbance was measured at 500 nm.

Hepatic Antioxidant Enzyme Activities and Antioxidant Status

The liver was homogenized in the buffer containing 0.25 M sucrose, 10 mM Tris-HCl, and 1 mM EDTA (pH 7.4). Hepatic catalase activity was measured spectrophotometrically at

240 nm (Lück, 1983). One unit of catalase activity was defined as the amount of enzyme that decomposes 1 μmol H_2O_2 in one minute at pH 7.4. Hepatic superoxide dismutase (SOD) activity was determined colorimetrically at 525 nm (Nebot *et al.*, 1993). One unit of SOD activity was defined as the activity that doubles the auto-oxidation background in the absence of SOD. Protein content in the liver homogenate was quantitated by the modified method of Lowry *et al.* (1951). Glutathione level was measured spectrophotometrically at 412 nm (Calbiochem 371757, EMD Biosciences, Inc., San Diego, CA, USA) (Anderson, 1989). The liver was homogenized in 5% metaphosphoric acid with or without the reactant (1-methyl-2-vinyl-pyidium trifluoromethane sulfonate in HCl) at 4°C. After centrifugation, the supernatant was mixed with the chromogenic reagent (5,5′-dithio-bis-2-nitrobenzoic acid), glutathione reductase, and NADPH. The oxidized (GSSG) and total glutathione concentrations were determined at 412 nm. The reduced glutathione (GSH) concentration is calculated by subtraction of 2 GSSG from total glutathione.

Hepatic total antioxidant status was determined spectrophotometrically by the ability of the antioxidants to inhibit the oxidation of ABTS® (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate]) to $ABTS^{®•+}$ by metmyoglobin (Randox NX 2332, Randox Laboratories Ltd.) (Miller *et al.*, 1993). The liver homogenate (20 μl) was mixed with 1 ml chromogen (5 μM metmyoglobin and 500 μM ABTS® in 66 mM phosphate buffer saline, pH 7.4), and total antioxidant status was measured at 600 nm at 37°C.

Hepatic lipid peroxides were assessed colorimetrically (Calbiochem 437634, EMD Biosciences, Inc.) (Esterbauer and Cheeseman, 1990). The liver homogenate (in 50 mM Tris-HCl, pH 7.4, 200 μl) was mixed with 650 μl of reagent 1 (7.7 mM N-methyl-2-phenylindole in 75% acetonitrile and 25% methanol) and 150 μl of reagent 2 (15.4 M methanesulfonic acid) at 45°C for 60 min. Malondialdehyde (MDA) level was measured at 586 nm.

Hepatic TGF-β1 Level

Hepatic TGF-β1 level was determined by an enzyme-linked immunosorbent assay (ELISA) (Quantikine MB100, R & D Systems, Inc., Minneapolis, MN, USA). The liver was homogenized in the buffer containing 20 mM Tris-HCl (pH 7.5), 2 M NaCl, 1 mM EDTA, 1 mM PMSF, and 0.1% Tween-80. Latent TGF-β1 in the liver homogenate (200 μl) was activated by 40 μl of 1 M HCl, and neutralized by 26 μl of 1.2 M NaOH and 0.5 M HEPES. The liver homogenate (50 μl) was subsequently incubated with monoclonal anti-TGF-β1 antibody, polyclonal antibody against TGF-β1 conjugated to horseradish peroxidase, and the substrates (H_2O_2 and tetramethylbenzidine). The level of TGF-β1 was measured at 450 nm and corrected at 540 nm using an ELISA reader (Multiskan RC, Thermo Labsystems, Helsinki, Finland).

Statistical Analysis

All data are expressed as mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA) and Fisher's least significant difference test using SAS software

(version 8.2, SAS Institute Inc., Cary, NC, USA). Differences were considered significant at $p < 0.05$.

Results

Food Intake, Body Weight, and Liver Weight

Food intake (22.2 ± 2.4–22.7 ± 2.3 g/day) did not differ among the 5 groups (data not shown). The SM group had the daily intake of 71.2 ± 13.5 mg silymarin. The LE and HE groups had the daily intake of 53.4 ± 3.1 mg (140.0 ± 2.1 mg/kg body weight) and 255.6 ± 4.5 mg (720.9 ± 9.4 mg/kg body weight) herbal extract, respectively. Initial body weight did not differ among the 5 groups (Table 1). However, weight gain in the CCl_4 group decreased significantly by 23% ($p < 0.05$) compared to the control group. Similar weight gain was observed in the control and other CCl_4-treated groups. All CCl_4-treated groups decreased absolute liver weight by 5%–14% ($p < 0.05$) compared to the control group. Relative liver weight reduced significantly by 7%–10% ($p < 0.05$) in all CCl_4-treated groups except for the HE group. Absolute and relative liver weight did not differ among the CCl_4, SM, and LE groups.

Histopathological Evaluation

Rat liver sections were stained with H & E (Fig. 1) and Masson's trichrome (Fig. 2) for the evaluation of general morphology and liver fibrosis, respectively. No fat vacuoles were found in the liver biopsy of the control group (Table 2). However, fat vacuoles occurred in all CCl_4-treated groups, and increased significantly ($p < 0.05$) only in the HE group compared to the control group. The bile duct proliferation was increased in CCl_4 group ($p < 0.05$) compared to the control group. Herbal extract improved bile acid proliferation ($p < 0.05$) stimulated by CCl_4, whereas silymarin had no reversible effect on CCl_4-induced bile duct proliferation. Both histopathological stains showed that all CCl_4-treated groups

Table 1. Body Weight and Liver Weight in Rats

	Control (n = 8)	CCl_4 (n = 9)	SM (n = 12)	LE (n = 12)	HE (n = 12)
Initial weight (g)	347.2 ± 16.1^{a}	355.8 ± 14.7^{a}	350.6 ± 13.5^{a}	350.0 ± 17.0^{a}	343.9 ± 11.8^{a}
Final weight (g)	434.6 ± 22.3^{a}	418.0 ± 18.0^{ab}	427.3 ± 25.6^{a}	419.0 ± 33.3^{ab}	398.2 ± 26.7^{b}
Weight gain (g)	83.0 ± 17.0^{a}	63.7 ± 3.0^{b}	71.5 ± 19.7^{ab}	74.7 ± 1.9^{ab}	65.7 ± 23.2^{ab}
Liver weight (g)	11.2 ± 0.8^{a}	9.6 ± 0.6^{c}	10.1 ± 1.0^{bc}	10.1 ± 1.0^{bc}	10.6 ± 1.4^{ab}
Relative liver weight (g/kg)	25.4 ± 4.0^{a}	22.8 ± 1.8^{b}	22.9 ± 6.9^{b}	23.5 ± 6.9^{b}	26.4 ± 4.2^{a}

Values are mean ± SD. Values not sharing a common superscript letter within rows differ significantly ($p < 0.05$). SM: CCl_4 + silymarin (0.35% silymarin), LE: CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1), HE: CCl_4 + high-dose herbal extract (1.20% of the same herbal extract).

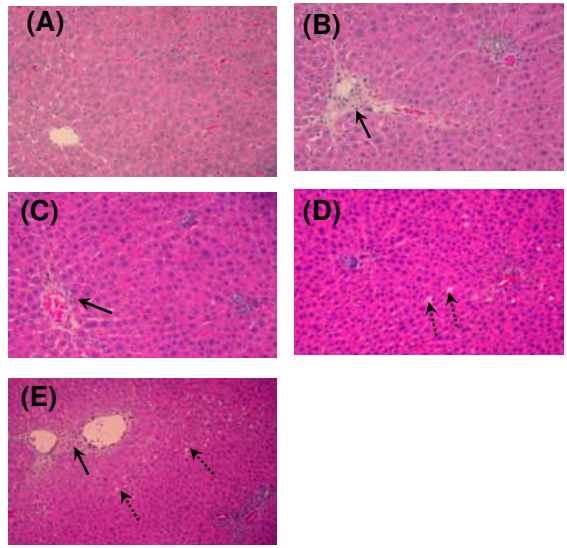

Figure 1. Rat liver sections stained with haematoxylin and eosin (H & E) for the evaluation of general morphology. (A) Control (×200), (B) CCl_4 (×200), (C) CCl_4 + silymarin (0.35% silymarin) (×200), (D) CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1) (×150), and (E) CCl_4 + high-dose herbal extract (1.20% of the same herbal extract) (×150) groups. The solid arrow represents central vein fibrosis, and the dashed arrow represents fat vacuoles.

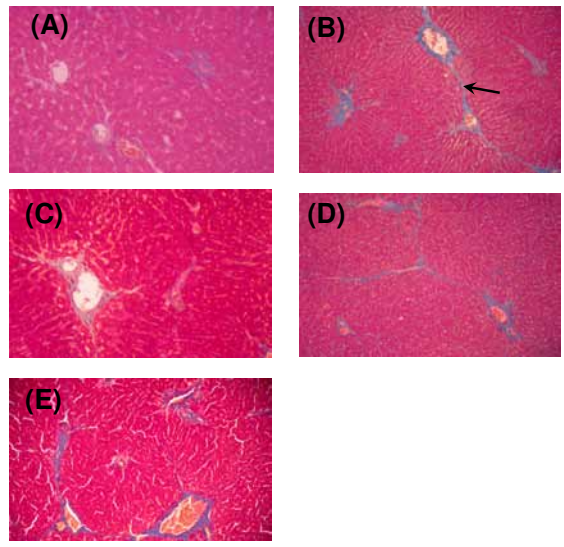

Figure 2. Rat liver sections stained with Masson's trichrome for fibrosis assessment. (A) Control (×200), (B) CCl_4 (×150), (C) CCl_4 + silymarin (0.35% silymarin) (×150), (D) CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1) (×100), and (E) CCl_4 + high-dose herbal extract (1.20% of the same herbal extract) (×150) groups. Collagen fibers appear blue, muscle, cytoplasm, and keratin are red, and nuclei are shown in a dark brown color. The solid arrow represents fibrous bridge.

Table 2. Scores for Rat Liver Biopsy Changes Stained with Haematoxylin and Eosin or Masson's Trichrome

	Control (n = 8)	CCl_4 (n = 9)	SM (n = 12)	LE (n = 12)	HE (n = 12)
Haematoxylin and eosin stain					
Fat vacuoles	0.00 ± 0.00^{a}	0.14 ± 0.07^{ab}	0.29 ± 0.07^{ab}	0.15 ± 0.05^{ab}	0.34 ± 0.13^{b}
Bile duct proliferation	0.19 ± 0.08^{ab}	0.43 ± 0.09^{c}	0.35 ± 0.07^{bc}	0.15 ± 0.06^{a}	0.21 ± 0.07^{ab}
Central vein fibrosis	0.06 ± 0.06^{a}	1.22 ± 0.15^{c}	0.92 ± 0.10^{c}	0.50 ± 0.09^{b}	0.90 ± 0.14^{c}
Portal tract fibrosis	0.11 ± 0.08^{a}	0.30 ± 0.10^{a}	0.17 ± 0.07^{a}	0.13 ± 0.07^{a}	0.17 ± 0.08^{a}
Masson's trichrome stain					
Central vein fibrosis	0.11 ± 0.04^{a}	1.22 ± 0.10^{c}	0.95 ± 0.09^{bc}	0.81 ± 0.10^{b}	1.16 ± 0.12^{c}
Portal tract fibrosis	0.06 ± 0.04^{a}	1.50 ± 0.12^{b}	0.06 ± 0.03^{a}	0.06 ± 0.03^{a}	0.25 ± 0.09^{a}

Values are mean ± SD. Fat vacuoles and bile duct proliferation were graded by 0 (none), 1 (formation in central veins), 2 (formation in central veins and middle zone, but not reaching portal tract), or 3 (formation from central veins to portal tract). Fibrosis was graded by 0 (none), 1 (fibrosis in central veins), 2 (fibrous septa formation between central veins or between central veins and portal tract), or 3 (cirrhosis). Values not sharing a common superscript letter within rows differ significantly ($p < 0.05$). SM: CCl_4 + silymarin (0.35% silymarin), LE: CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1), HE: CCl_4 + high-dose herbal extract (1.20% of the same herbal extract).

developed more severe liver fibrosis in the central veins compared to the control group. The formation of collagen fibers in the central veins was significantly eliminated ($p < 0.05$) in the LE group compared to the other CCl_4-treated groups. Liver fibrosis did not differ in the portal tract among the 5 groups by H & E stain. However, Masson's trichrome stain showed that the formation of collagen fibers in the portal tract was stimulated ($p < 0.05$) in the CCl_4 group.

Liver Function Index

With the injection of CCl_4 at week 2, both plasma ALT and AST activities increased rapidly at least by 20- and 10-fold ($p < 0.05$), respectively, and sustained until week 4 in the CCl_4 and HE groups (Table 3). The SM and LE groups tended to increase plasma ALT and AST activities. Plasma ALT and AST activities increased significantly ($p < 0.05$) in the SM and LE groups compared to those in the control group at week 4. After a week cessation of CCl_4 injection (week 6), plasma AST activity returned to the normal level in all CCl_4-treated groups, and plasma ALT activity was partially near the normal level.

Plasma and Hepatic Lipids

At week 2, CCl_4 treatment increased plasma cholesterol level by 20%–29% ($p < 0.05$) (Table 4). Plasma triglycerides elevated significantly ($p < 0.05$) in the SM group compared to the control group. Both plasma cholesterol and triglyceride levels did not differ among the five groups at week 4. At week 6, plasma cholesterol level decreased significantly

Table 3. Plasma Alanine Aminotransferase and Aspartate Aminotransferase Activities in Rats

	Control (n = 8)	CCl_4 (n = 9)	SM (n = 12)	LE (n = 12)	HE (n = 12)
Plasma alanine aminotransferase activity (U/l)					
Week 0	25.3 ± 1.7[a]	25.2 ± 0.9[a]	23.9 ± 3.1[a]	24.3 ± 1.7[a]	23.7 ± 1.0[a]
Week 1	29.0 ± 2.0[a]	29.1 ± 2.4[a]	28.9 ± 2.1[a]	29.1 ± 2.1[a]	27.1 ± 2.8[a]
Week 2	35.6 ± 7.1[a]	876 ± 582[bc]	525 ± 474[ab]	375 ± 246[a]	1186 ± 325[c]
Week 4	24.7 ± 7.9[a]	1449 ± 836[c]	1395 ± 333[bc]	792 ± 484[b]	1305 ± 713[bc]
Week 6	23.8 ± 3.7[a]	31.2 ± 3.9[b]	28.6 ± 8.3[b]	29.5 ± 4.5[b]	31.9 ± 3.8[b]
Plasma aspartate aminotransferase activity (U/l)					
Week 0	82.3 ± 9.9[a]	79.8 ± 12.9[a]	79.3 ± 8.7[a]	77.6 ± 8.7[a]	79.2 ± 7.6[a]
Week 1	70.0 ± 7.6[a]	71.7 ± 9.6[a]	72.1 ± 10.0[a]	70.0 ± 15.9[a]	69.8 ± 8.0[a]
Week 2	86.0 ± 11.3[a]	1440 ± 850[b]	564 ± 373[a]	339 ± 175[a]	1525 ± 481[b]
Week 4	70.0 ± 5.9[a]	1340 ± 641[c]	835 ± 692[b]	645 ± 270[b]	965 ± 391[bc]
Week 6	61.0 ± 5.1[a]	64.4 ± 4.2[a]	60.3 ± 2.8[a]	62.2 ± 4.8[a]	61.8 ± 7.6[a]

Values are mean ± SD. Values not sharing a common superscript letter within rows differ significantly ($p < 0.05$). SM: CCl_4 + silymarin (0.35% silymarin), LE: CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1), HE: CCl_4 + high-dose herbal extract (1.20% of the same herbal extract).

Table 4. Plasma and Hepatic Cholesterol and Triglyceride Levels in Rats

	Control (n = 8)	CCl_4 (n = 9)	SM (n = 12)	LE (n = 12)	HE (n = 12)
Plasma cholesterol (mmol/l)					
Week 0	0.93 ± 0.11[a]	0.91 ± 0.09[a]	0.90 ± 0.13[a]	1.03 ± 0.22[a]	1.01 ± 0.22[a]
Week 1	0.47 ± 0.16[a]	0.55 ± 0.18[a]	0.50 ± 0.09[a]	0.43 ± 0.12[a]	0.44 ± 0.15[a]
Week 2	1.00 ± 0.13[a]	1.26 ± 0.23[b]	1.26 ± 0.22[b]	1.20 ± 0.17[b]	1.29 ± 0.24[b]
Week 4	0.46 ± 0.13[a]	0.66 ± 0.29[a]	0.61 ± 0.42[a]	0.60 ± 0.28[a]	0.49 ± 0.18[a]
Week 6	0.96 ± 0.27[ab]	1.14 ± 0.19[a]	0.96 ± 0.33[ab]	0.89 ± 0.24[b]	0.89 ± 0.26[b]
Hepatic cholesterol (mg/g liver)	7.4 ± 2.0[a]	7.5 ± 0.6[a]	8.2 ± 1.4[a]	8.4 ± 1.4[a]	14.5 ± 2.4[b]
Plasma triglycerides (mmol/l)					
Week 0	0.50 ± 0.25[a]	0.49 ± 0.11[a]	0.50 ± 0.15[a]	0.45 ± 0.09[a]	0.40 ± 0.04[a]
Week 1	0.42 ± 0.05[a]	0.40 ± 0.06[a]	0.41 ± 0.06[a]	0.42 ± 0.09[a]	0.39 ± 0.06[a]
Week 2	0.19 ± 0.05[a]	0.24 ± 0.13[ab]	0.30 ± 0.09[b]	0.26 ± 0.11[ab]	0.27 ± 0.11[ab]
Week 4	0.45 ± 0.07[a]	0.50 ± 0.12[a]	0.46 ± 0.14[a]	0.47 ± 0.11[a]	0.48 ± 0.11[a]
Week 6	0.45 ± 0.08[a]	0.56 ± 0.08[b]	0.50 ± 0.06[ab]	0.53 ± 0.08[b]	0.55 ± 0.10[b]
Hepatic triglycerides (mg/g liver)	3.0 ± 1.1[a]	7.8 ± 4.5[b]	5.3 ± 1.7[ab]	12.1 ± 3.5[c]	12.0 ± 7.3[c]

Values are mean ± SD. Values not sharing a common superscript letter within rows differ significantly ($p < 0.05$). SM: CCl_4 + silymarin (0.35% silymarin), LE: CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1), HE: CCl_4 + high-dose herbal extract (1.20% of the same herbal extract).

($p < 0.05$) in the herbal extract treated groups, whereas hepatic cholesterol level increased significantly ($p < 0.05$) in the HE group. In all CCl_4-treated groups, except the SM group, plasma and hepatic triglycerides were inceased ($p < 0.05$) compared to the control group. Hepatic triglycerides were significantly higher ($p < 0.05$) in the herbal extract treated groups than those in the CCl_4 group.

Hepatic Antioxidant Enzyme Activities and Antioxidant Status

Hepatic catalase activity increased significantly by 2.4- and 4.6-fold ($p < 0.05$) in the SM and HE groups, respectively, compared to in the control group (Table 5). The injection of CCl_4 impaired hepatic SOD activity by 36% ($p < 0.05$). Both silymarin and herbal extract restored the hepatic SOD activity to the similar level as in the control group. The ratio of GSH to GSSG elevated significantly by 49% ($p < 0.05$) in the HE group compared to the CCl_4 group, although total glutathione, GSH, and GSSG concentrations did not differ among the five groups. The CCl_4 group impaired hepatic total antioxidant status by 15% ($p < 0.05$), but total antioxidant status in other CCl_4 treated groups did not differ from that in the control group. Hepatic MDA concentration increased significantly by 40% and 29% ($p < 0.05$) in the CCl_4 and SM groups, respectively. Hepatic MDA level in the herbal extract treated groups did not differ from that in the control group or other CCl_4-treated groups.

Table 5. Hepatic Antioxidant Enzyme Activities, Antioxidant Status, and Transforming Growth Factor-β1 (TGF-β1) Level in Rats

	Control (n = 8)	CCl_4 (n = 9)	SM (n = 12)	LE (n = 12)	HE (n = 12)
Catalase (U/mg protein)	0.79 ± 0.31^{a}	0.75 ± 0.27^{a}	1.86 ± 1.39^{b}	1.63 ± 0.76^{ab}	3.62 ± 2.22^{c}
Superoxide dismutase (mU/mg protein)	62.8 ± 28.0^{a}	40.5 ± 19.2^{b}	48.2 ± 31.5^{ab}	43.6 ± 18.4^{ab}	41.0 ± 21.5^{ab}
Total glutathione (mmol/l)	4.76 ± 1.24^{a}	4.67 ± 0.68^{a}	4.18 ± 1.10^{a}	4.92 ± 0.68^{a}	4.26 ± 0.93^{a}
Reduced glutathione, GSH (mmol/l)	4.28 ± 1.40^{a}	3.59 ± 0.66^{a}	4.38 ± 1.35^{a}	4.00 ± 0.73^{a}	3.89 ± 0.85^{a}
Oxidized glutathione, GSSG (mmol/l)	0.36 ± 0.16^{a}	0.39 ± 0.10^{a}	0.35 ± 0.11^{a}	0.36 ± 0.12^{a}	0.29 ± 0.01^{a}
GSH/GSSG	10.3 ± 3.7^{ab}	9.1 ± 2.1^{a}	13.2 ± 5.5^{ab}	12.0 ± 6.6^{ab}	13.6 ± 5.9^{b}
Total antioxidant status (mmol/l)	269.0 ± 43.0^{a}	228.6 ± 33.0^{b}	278.0 ± 39.8^{a}	281.3 ± 35.7^{a}	265.2 ± 27.4^{a}
Malondialdehyde (μmol/l)	109.3 ± 24.3^{a}	153.0 ± 46.8^{b}	140.8 ± 33.3^{b}	137.1 ± 40.5^{ab}	138.9 ± 35.7^{ab}
TGF-β1 (ng/g liver)	112.2 ± 14.7^{a}	136.3 ± 29.4^{b}	121.5 ± 8.3^{ab}	113.2 ± 8.7^{a}	128.1 ± 26.7^{ab}

Values are mean ± SD. Values not sharing a common superscript letter within rows differ significantly ($p < 0.05$). SM: CCl_4 + silymarin (0.35% silymarin), LE: CCl_4 + low-dose herbal extract (0.24% of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* extract at 1:1:1), HE: CCl_4 + high-dose herbal extract (1.20% of the same herbal extract).

Hepatic TGF-β1 Level

Hepatic TGF-β1 level was elevated significantly by 21% ($p < 0.05$) in the CCl_4 group compared to that in the control group (Table 5). Hepatic TGF-β1 level in the silymarin and herbal extract treated groups did not differ from that in the control group. The LE group reduced hepatic TGF-β1 level by 17% ($p < 0.05$) compared to the CCl_4 group.

Discussion

Exposure to CCl_4 led to a significant decrease in weight gain, although food intake was not significantly affected. The rats treated with CCl_4 alone exhibited decreased physical activity, and certain rats had a bleeding nose, which resulted in decreased weight gain. However, the rats in the CCl_4 group had constantly increased body weight, indicating CCl_4 did not affect the growth. Absolute and relative liver weight decreased significantly in the CCl_4 group probably due to an increased tendency of fat vacuole accumulation in the liver. Reduced absolute and relative liver weight was significantly reversed only in the HE group.

Following injection with CCl_4, plasma ALT and AST activities were increased dramatically, indicating acute liver damage was induced by CCl_4. At the end of the 6th week, plasma ALT and AST activities in the CCl_4 group almost or already returned to the normal levels, respectively, due to liver regeneration after the cessation of CCl_4 injection. A previous study (Clawson, 1989) showed that cell necrosis of the hepatocytes was observed within 6–8 hours after CCl_4 administration, and CCl_4-induced hepatotoxicity was sustained within 6–72 hours thereafter. Accordingly, plasma ALT and AST activities reached the maximum within 6–48 hours after intraperitoneal injection of CCl_4 (Taniguchi *et al.*, 2004). Additionally, the gene expression of hepatocyte growth factor and proliferating cell nuclear antigen was induced at 6 h and 36 h, respectively, suggesting that hepatotoxicity caused by CCl_4 concomitantly induced both the processes of acute injury and liver regeneration. Elevated plasma ALT or AST activity was partially reversed by silymarin or low-dose herbal extract, indicating that liver necrosis could be partially recovered by silymarin and low-dose herbal extract. Similarly, oral pretreatment with 200 mg/kg *Ginkgo biloba* extract for 10 days limited CCl_4-induced liver necrosis and atrophy in rats (Ozenirler *et al.*, 1997). The dammarane-type triterpene saponins with an ocotillol-type side chain in Vietnamese ginseng, such as Rh1 and Rg1, showed the protective activity against D-galactosamine/tumor necrosis factor (TNF)-α-induced cell death in primary cultured mouse hepatocytes (Tran *et al.*, 2001). Gomisin A (0.06%) extracted from *Schizandra chinensis* remarkably improved rat liver damage induced by intravenous administration of heat-killed *Propionibacterium acnes* followed by Gram-negative lipopolysaccharide (Mizoguchi *et al.*, 1991).

The administration of CCl_4 induced hepatic fatty degeneration including increases in fat vacuoles and hepatic triglycerides. The free radicals derived from the metabolites of CCl_4 by cytochrome P-450 interrupted the transport of hepatic triglycerides by very low density lipoproteins (Recknagel, 1983). An *in vitro* study (Boll *et al.*, 2001) demonstrated

that CCl_4 increased the synthesis of lipids and the rate of lipid esterification, but reduced lipolysis and fatty acid β-oxidation in primary rat hepatocytes, which caused the accumulation of triglycerides in the hepatocytes. Unlike silymarin, herbal extract increased hepatic triglycerides and maintained high plasma triglycerides, even though it resulted in lower plasma cholesterol level compared to the CCl_4 group. Fat vacuoles in the HE group were increased in parallel to the elevated hepatic cholesterol and triglycerides. However, intraperitoneal administration of ginsenoside Rb1 decreased hepatic cholesterol and triglycerides through regulating the production of c-AMP and the activity of microsomal cytochrome P-450 monooxygenase, but did not change serum triglycerides and low density lipoprotein (LDL) in normal rats (Park *et al.*, 2002). Oral administration of ginsenoside Rb decreased the ratios of cholesterol to high density lipoprotein-cholesterol (HDL-C) and LDL-C to HDL-C, and inhibited hepatic fat accumulation in hyperlipidemic rats (Zhang *et al.*, 2004). The effects of the extract or component of *Ginkgo biloba* or *Schizandra chinensis* on hepatic and blood lipids have not been reported. Inconsistent with the results of ginsenoside Rb, our herbal extract had an adverse effect on triglyceride metabolism probably through modulating the absorption of triglycerides in the intestine, the synthesis and hydrolysis of triglycerides in the liver, the transport of triglycerides by lipoproteins, and/or the clearance of blood triglycerides.

Hepatic SOD activity, total antioxidant status, and lipid peroxidation were impaired by CCl_4 treatment. Carbon tetrachloride is activated by cytochrome (CYP)2E1, CYP2B1 or CYP2B2, and possibly CYP3A in the liver, to produce the trichloromethyl radical ($CCl_3^{\bullet}$) which can bind to cellular macromolecules, such as lipids, proteins, and nucleic acids. Furthermore, the radicals react with oxygen to form the trichloromethylperoxy radical ($CCl_3OO^{\bullet}$) which can impair lipid metabolism and initiate lipid peroxidation (Weber *et al.*, 2003). Decreased hepatic SOD activity by CCl_4 could result from the consumption of SOD to compensate excessive peroxy radicals derived from the metabolite of CCl_4. Similar to silymarin, herbal extract improved the impaired total antioxidant status. Herbal extract partially reversed the impaired SOD activity and lipid peroxidation. High-dose herbal extract enhanced hepatic catalase activity and glutathione status. Ginkgo, ginseng, and schizandra fruit can act as antioxidants to prevent CCl_4-induced oxidative damage in the liver. *Ginkgo biloba* extract had antioxidative capacity to inhibit lipid peroxidation (Huang *et al.*, 2004; Sener *et al.*, 2005; He *et al.*, 2006) and increase SOD activity and GSH level (Chao *et al.*, 2004; Sener *et al.*, 2005; He *et al.*, 2006). Ginseng extract or ginsenosides showed the antioxidant property to increase SOD activity (Yang *et al.*, 1999; Zhang *et al.*, 2004) and suppress lipid peroxidation by its hydroxyl radical-scavenging activity (Zhang *et al.*, 1996, 2004; Park *et al.*, 2005). *Schizandra chinensis* extract protected against oxidative damage by inhibiting lipid peroxidation (Ma *et al.*, 2005; You *et al.*, 2006) and improving SOD activity (Yang *et al.*, 1999) and glutathione status (Chiu *et al.*, 2002). Based on the result of improving total antioxidant status, the combined extract of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* still preserved the antioxidant activity of the active components in the individual extract. However, different doses of herbal extract hid not have different effects on total antioxidant status. The mechanism for enhancing hepatic antioxidant status by the herbal extract could be strongly associated with increasing hepatic

catalase activity and glutathione status to scavenge reactive oxygen species generated by the metabolism of CCl_4.

Liver fibrosis and increased hepatic TGF-β1 level were observed in the CCl_4 group. A previous study (Weber *et al.*, 2003) reported that CCl_4 activated TNF-α, nitric oxide, TGF-α and TGF-β to drive hepatocytes toward self-destruction or fibrosis. Low-dose herbal extract inhibited CCl_4-induced fibrosis in the central veins and portal tract through decreasing hepatic TGF-β1 level. However, high-dose herbal extract partially decreased hepatic TGF-β1 level, and only suppressed liver fibrosis in the portal tract. *Ginkgo biloba* extract attenuated biliary obstruction-induced collagen fibers (Sener *et al.*, 2005) and CCl_4-induced TGF-β1 and type I collagen fibers in the liver (Luo *et al.*, 2004; Ding *et al.*, 2005; He *et al.*, 2006; Liu *et al.*, 2006). The antifibrogenic effect of *Ginkgo biloba* extract could be attributed to the inhibition of activated stellate cells through suppressing the expression of TGF-β1 and nuclear factor κBP65 (Liu *et al.*, 2006), and the stimulation of fibrinolysis through increasing metalloproteinase-1 and decreasing tissue inhibitor-1 of metalloproteinase levels (Luo *et al.*, 2004). Water extract of ginseng suppressed liver fibrosis through decreasing the formation of lipid peroxides (Park *et al.*, 2005). The lignan compounds isolated from schizandra fruit inhibited liver fibrosis and accelerated liver regeneration (Takeda *et al.*, 1987). The antifibrogenic effect of the combined extract of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* could result from the overall consequences of the active compounds through modulating the balance between fibrogenesis and fibrinolysis as well as stimulating liver regeneration.

Our results suggested that the combined extract of *Ginkgo biloba*, *Panax ginseng*, and *Schizandra chinensis* ameliorated CCl_4-induced liver injury through improving antioxidant status and suppressing liver fibrosis. High-dose herbal extract had no additive effect. However, more studies are further required to identify the active compounds in the combined extract and explore the possible mechanisms for the antifibrogenic effects.

Acknowledgments

This study was sponsored by the National Science Council, Taiwan (NSC93-2815-C-038-003- B).

References

Anderson, M.E. Enzymatic and chemical methods for the determination of glutathione. In: D. Dolphin, R. Poulson and O. Avramovic (eds.) *Glutathione: Chemical, Biochemical and Medical Aspects*, *Vol. A*. John Wiley and Sons, Inc., New York, 1989, pp. 339–365.

Boll, M., L.W. Weber, E. Becker and A. Stampfl. Pathogenesis of carbon tetrachloride-induced hepatocyte injury bioactivation of CCl_4 by cytochrome P450 and effects on lipid homeostasis. *Z. Naturforsch.* [*C*] 56: 111–121, 2001.

Chao, J.C.-J., H.C. Hung, S.H. Chen and C.L. Fang. Effects of *Ginkgo biloba* extract on cytoprotective factors in rats with duodenal ulcer. *World J. Gastroenterol.* 10: 560–566, 2004.

Chen, D.S. From hepatitis to hepatoma: lessons from type B viral hepatitis. *Science* 262: 369–370, 1993.

Chiu, P.Y., D.H. Mak, M.K. Poon and K.M. Ko. *In vivo* antioxidant action of a lignan-enriched extract of Schisandra fruit and an anthraquinone-containing extract of Polygonum root in comparison with schisandrin B and emodin. *Planta Med.* 68: 951–956, 2002.

Clawson, G.A. Mechanisms of carbon tetrachloride hepatotoxicity. *Pathol. Immunopathol. Res.* 8: 104–112, 1989.

Ding, J., J. Yu, C. Wang, W. Hu, D. Li, Y. Luo, H. Luo and H. Yu. *Ginkgo biloba* extract alleviates liver fibrosis induced by CCl_4 in rats. *Liver Int.* 25: 1224–1232, 2005.

Esterbauer, H. and K.H. Cheeseman. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.* 186: 407–421, 1990.

Favari, L. and V. Perez-Alvarez. Comparative effects of colchicine and silymarin on CCl_4-chronic liver damage in rats. *Arch. Med. Res.* 28: 11–17, 1997.

He, S.X., J.Y. Luo, Y.P. Wang, Y.L. Wang, H. Fu, J.L. Xu, G. Zhao and E.Q. Liu. Effects of extract from *Ginkgo biloba* on carbon tetrachloride-induced liver injury in rats. *World J. Gastroenterol.* 12: 3924–3928, 2006.

Huang, P., G. Feng, S. Zhang, H. Wang and Y. Jin. Effect of *Ginkgo biloba* L. leaves on oxidation of human low density lipoproteins *in vitro*. *Wei Sheng Yan Jiu* 33: 453–454, 520, 2004.

Jeong, T.C., H.J. Kim, J.I. Park, C.S. Ha, J.D. Park, S.I. Kim and J.K. Roh. Protective effects of red ginseng saponins against carbon tetrachloride-induced hepatotoxicity in Sprague Dawley rats. *Planta Med.* 63: 136–140, 1997.

Kawada, N. Molecular mechanism of stellate cell activation and therapeutic strategy for liver fibrosis. *Comp. Hepatol.* 3(Suppl. 1): S3, 2004.

Lück, H. Catalase. In: H. Bergmeye (ed.) *Methods of Enzymatic Analysis*. Academic Press, New York, 1983, pp. 885–888.

Li, C., J. Luo, L. Li, M. Cheng, N. Huang, J. Liu and M.P. Waalkes. The collagenolytic effects of the traditional Chinese medicine preparation, Han-Dan-Gan-Le, contribute to reversal of chemical-induced liver fibrosis in rats. *Life Sci.* 72: 1563–1571, 2003.

Li, C.X., L. Li, J. Lou, W.X. Yang, T.W. Lei, Y.H. Li, J. Liu, M.L. Cheng and L.H. Huang. The protective effects of traditional Chinese medicine prescription, han-dan-gan-le, on CCl_4-induced liver fibrosis in rats. *Am. J. Chin. Med.* 26: 325–332, 1998.

Li, W., Q.T. Dai and Z.E. Liu. Preliminary study on early fibrosis of chronic hepatitis B treated with *Ginkgo biloba Composita*. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 15: 593–595, 1995.

Lin, C.F., K.L. Wong, R.S. Wu, T.C. Huang and C.F. Liu. Protection by hot water extract of *Panax notoginseng* on chronic ethanol-induced hepatotoxicity. *Phytother. Res.* 17: 1119–1122, 2003.

Liu, S.Q., J.P. Yu, H.L. Chen, H.S. Luo, S.M. Chen and H.G. Yu. Therapeutic effects and molecular mechanisms of *Ginkgo biloba* extract on liver fibrosis in rats. *Am. J. Chin. Med.* 34: 99–114, 2006.

Lowry, O.H., N.J. Rosebrough, A. Farr and R.J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275, 1951.

Luo, Y.J., J.P. Yu, Z.H. Shi and L. Wang. *Ginkgo biloba* extract reverses CCl_4-induced liver fibrosis in rats. *World J. Gastroenterol.* 10: 1037–1042, 2004.

Ma, D., A. Shan, Z. Chen, J. Du, K. Song, J. Li and Q. Xu. Effect of *Ligustrum lucidum* and *Schisandra chinensis* on the egg production, antioxidant status and immunity of laying hens during heat stress. *Arch. Anim. Nutr.* 59: 439–447, 2005.

Matsuda, H., K. Samukawa and M. Kubo. Anti-hepatitic activity of ginsenoside Ro. *Planta Med.* 57: 523–526, 1991.

Miller, N.J., C. Rice-Evans, M.J. Davies, V. Gopinathan and A. Milner. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* 84: 407–412, 1993.

Mizoguchi, Y., N. Kawada, Y. Ichikawa and H. Tsutsui. Effect of gomisin A in the prevention of acute hepatic failure induction. *Planta Med.* 57: 320–324, 1991.

Nebot, C., M. Moutet, P. Huet, J.Z. Xu, J.C. Yadan and J. Chaudiere. Spectrophotometric assay of superoxide dismutase activity based on the activated autoxidation of a tetracyclic catechol. *Anal. Biochem.* 214: 442–451, 1993.

Ozenirler, S., S. Dincer, G. Akyol, C. Ozogul and E. Oz. The protective effect of *Ginkgo biloba* extract on CCl_4-induced hepatic damage. *Acta Physiol. Hung.* 85: 277–285, 1997–1998.

Paradis, V., D. Dargere, F. Bonvoust, M. Vidaud, P. Segarini and P. Bedossa. Effects and regulation of connective tissue growth factor on hepatic stellate cells. *Lab. Invest.* 82: 767–774, 2002.

Park, K.H., H.J. Shin, Y.B. Song, H.C. Hyun, H.J. Cho, H.S. Ham, Y.B. Yoo, Y.C. Ko, W.T. Jun and H.J. Park. Possible role of ginsenoside Rb1 on regulation of rat liver triglycerides. *Biol. Pharm. Bull.* 25: 457–460, 2002.

Park, W.H., S.K. Lee and C.H. Kim. A Korean herbal medicine, *Panax notoginseng*, prevents liver fibrosis and hepatic microvascular dysfunction in rats. *Life Sci.* 76: 1675–1690, 2005.

Recknagel, R.O. A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sci.* 33: 401–408, 1983.

Sener, G., L. Kabasakal, M. Yuksel, N. Gedik and Y. Alican. Hepatic fibrosis in biliary-obstructed rats is prevented by *Ginkgo biloba* treatment. *World J. Gastroenterol.* 11: 5444–5449, 2005.

Sprenger, H., A. Kaufmann, H. Garn, B. Lahme, D. Gemsa and A.M. Gressner. Differential expression of monocyte chemotactic protein-1 (MCP-1) in transforming rat hepatic stellate cells. *J. Hepatol.* 30: 88–94, 1999.

Sung, J.L., D.S. Chen, M.Y. Lai, J.Y. Yu, T.H. Wang, C.Y. Wang, C.Y. Lee, S.H. Chen and T.M. Ko. Epidemiological study on hepatitis B virus infection in Taiwan. *Chin. J. Gastroenterol.* 1: 1–9, 1984.

Takeda, S., Y. Kase, I. Arai, Y. Ohkura, M. Hasegawa, Y. Sekiguchi, A. Tatsugi, S. Funo, M. Aburada and E. Hosoya. Effects of TJN-101, a lignan compound isolated from Schisandra fruits, on liver fibrosis and on liver regeneration after partial hepatectomy in rats with chronic liver injury induced by CCl_4. *Nippon Yakurigaku Zasshi* 90: 51–65, 1987.

Taniguchi, M., T. Takeuchi, R. Nakatsuka, T. Watanabe and K. Sato. Molecular process in acute liver injury and regeneration induced by carbon tetrachloride. *Life Sci.* 75: 1539–1549, 2004.

Tran, Q.L., I.K. Adnyana, Y. Tezuka, T. Nagaoka, Q.K. Tran and S. Kadota. Triterpene saponins from Vietnamese ginseng (*Panax vietnamensis*) and their hepatocytoprotective activity. *J. Nat. Prod.* 64: 456–461, 2001.

Tuei, J., F. Okoth, A. Kasomo, E. Ireri, D. Kaptich and A. Watahi. Prevalence of hepatocellular carcinoma, liver cirrhosis and HBV carriers in liver disease patients referred to Clinical Research Centre, Kemri. *Afr. J. Health Sci.* 1: 126–128, 1994.

Weber, L.W., M. Boll and A. Stampfl. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.* 33: 105–136, 2003.

Wells, R.G., E. Kruglov and J.A. Dranoff. Autocrine release of TGF-β by portal fibroblasts regulates cell growth. *FEBS Lett.* 559: 107–110, 2004.

Yang, Y., T. Wu, K. He and Z.G. Fu. Effect of aerobic exercise and ginsenosides on lipid metabolism in diet-induced hyperlipidemia mice. *Zhongguo Yao Li Xue Bao* 20: 563–565, 1999.

You, J.S., T.L. Pan and Y.C. Hou. *Schisandra chinensis* protects against adriamycin-induced cardiotoxicity in rats. *Chang Gung Med. J.* 29: 63–70, 2006.

Yu, M.W. and C.J. Chen. Hepatitis B and C viruses in the development of hepatocellular carcinoma. *Crit. Rev. Oncol. Hematol.* 17: 71–91, 1994.

Zhang, D., T. Yasuda, Y. Yu, P. Zheng, T. Kawabata, Y. Ma and S. Okada. Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. *Free Radic. Biol. Med.* 20: 145–150, 1996.

Zhang, X.M., S.C. Qu, D.Y. Sui, X.F. Yu and Z.Z. Lv. Effects of ginsenoside-Rb on blood lipid metabolism and anti-oxidation in hyperlipidemia rats. *Zhongguo Zhong Yao Za Zhi* 29: 1085–1088, 2004.