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Effect of Schisandrin B and Sesamin Mixture on CCl_4-induced Hepatic Oxidative Stress in Rats

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To study the effects of schisandrin B and sesamin mixture on carbon tetrachloride (CCl_4)-induced hepatic oxidative stress in male Sprague-Dawley rats. The rats were randomly assigned to five groups: control group (olive oil injection), CCl_4 group (CCl_4 injection), silymarin group (CCl_4 injection combined with supplementation of silymarin, 7.5 mg/kg/day), low dose group (CCl_4 injection combined with supplementation of schisandrin B and sesamin mixture at a low dose, 43 mg/kg/day) and high dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixture at a high dose, 215 mg/kg/day). The hepatic superoxide dismutase and glutathione peroxidase activities of rats in the low dose and high dose groups were increased significantly compared with those in the CCl_4 group. The hepatic reduced glutathione concentration in the silymarin, low dose and high dose groups were increased significantly (48%, 45% and 53%, respectively) when compared with those of the CCl_4 group. In addition, the concentration of glutathione in the erythrocytes of the low dose group was significantly higher than the CCl_4 group by 25%. These results suggest that the schisandrin B–sesamin mixture exerted a hepatoprotective effect by improving the antioxidative capacity in rats under CCl_4-induced hepatic oxidative stress. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: schisandrin B; sesamin; hepatoprotective; carbon tetrachloride; oxidative stress.

INTRODUCTION

According to the statistics tabulated by the Department of Health, Taiwan in 2005, chronic liver disease and cirrhosis together are the 7th leading cause of death in Taiwan. Unfortunately, there are certain limitations in the treatment of hepatitis. Recently, many reports indicated that phytochemicals in herbs, such as the lignans of schizandra and sesame may help to prevent liver diseases (Ohtaki *et al.*, 1996; Kang *et al.*, 1999). Schizandra (*Schisandra chinesnesis*) is a commonly used herb in traditional Chinese medicine. The extract of schizandra is traditionally used to treat various diseases including asthmatic cough, excessive sweating, insomnia and amnesia (Ko *et al.*, 1995). Previous study further demonstrated that the liver protective effect of schizandra against CCl_4-induced liver injury is attributed to its lignans such as schisandrin, deoxyschisandrin, schisantherin and schisandrol (Zhu *et al.*, 2000). Besides, schisandrin B, the most abundant active dibenzocyclooctadiene derivative isolated from the fruit of schizandra, can increase the glutathione antioxidant status in mice challenged with CCl_4 (Chiu *et al.*, 2002).

Sesamin is one of the lignans found in minute quantity in sesame seeds. It has been reported to exert several pharmacological effects such as antioxidation, regulation of lipid metabolism, antihypertension and mitigation of liver injury induced by CCl_4 and ethanol (Hirose *et al.*, 1991; Matsumura *et al.*, 1998; Akimoto *et al.*, 1993). Sesamin, at a level of 100 mg/kg body weight, may prevent liver lipid accumulation and normalize plasma ALT and AST activities in mice challenged with CCl_4 (Zhu *et al.*, 1999).

According to Chinese medicine, the intake of a combination of Chinese medicine is better than a single herb treatment for physiological disorders. Therefore, the present study investigated the hepatoprotective activity of a combination of sesamin and schisandrin B against chronic CCl_4-induced liver injury in rats. In addition, silymarin derived from milk thistle has been used widely for the treatment of liver disease because of its antioxidative capacity (Flora *et al.*, 1998). Therefore, silymarin was used as a positive control in this study.

MATERIALS AND METHODS

Animals and diets. Fifty male Sprague Dawley (SD) rats, weighing 120–150 g, were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The rats were housed in a temperature and humidity regulated environment (22 ± 2 °C, 65% ± 5% RH) with a 12 h dark/light cycle. The animals were allowed free access to standard powdered diet (Rodent Diet 5001, PMI Nutrition International,

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Richmond, IN, USA) and water. The standard powdered diet contains 28.5% of energy as protein, 13.5% as fat and 58.0% as carbohydrate, and provides the basal requirement of vitamins and mineral for rodents. The animals were acclimatized for 1 week prior to experiment. Then, the rats were randomly assigned to five groups: the control group (olive oil injection), CCl_4 group (CCl_4 injection), silymarin group (CCl_4 injection combined with supplementation of silymarin, 7.5 mg/kg/day), low dose group (CCl_4 injection combined with supplementation of schisandrin B and sesamin mixture at a low dose, 43 mg/kg/day) and high dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixture at a high dose, 215 mg/kg/day). The rats were fed powdered diet admixed with silymarin and schisandrin B–sesamin combination for 1 week prior to CCl_4 injection and throughout the experiment. Rats in the CCl_4, silymarin, low dose and high dose groups were subcutaneously injected with 0.75 mL/kg of 40% CCl_4 dissolved in olive oil once a week, while rats in the control group were injected with olive oil only. During the 8-week experimental period, the amounts of silymarin and schisandrin B-sesamin mixture were administered according to the weekly body weight.

The schisandrin B and sesamin mixture were provided by Cerebos Taiwan Ltd. This mixture contains 0.358 mg sesamin, 0.012 mg schisandrin B, 8 mg protein, 85 mg carbohydrate, 4 mg lipid and vitamin E (0.58 IU/100 mg).

Preparation of tissues. At the end of the experiment, a heparinized blood sample was drawn from the abdominal aorta of the ether-anesthetized rats following 12 h fasting. Plasma samples were obtained by centrifuging heparinized blood tubes. The liver was immediately perfused with ice-cold normal saline, then carefully removed, rinsed in ice-cold normal saline, blotted dry and weighed. One piece of liver tissue ($1 \times 1 \times 1$ cm^3) was cut from the largest right lobe and fixed in 40 g/L formaldehyde solution for histological preparation.

Measurements of plasma AST and ALT activities. Plasma AST and ALT activities were measured using a spectrophotometric method with a latrozyme TA-LQ kit (Iatron Laboratories Inc., Tokyo, Japan).

Measurements of antioxidant enzymes, reduced glutathione and malondialdehyde in blood and liver. The glutathione peroxidase (GPX) activities of erythrocyte hemolysates and liver samples were determined with a commercial kit (Calbiochem 354104; Calbiochem-Novabiochem, La Jolla, CA, USA). A 70 μL of diluted sample was added to 350 μL of 0.2 mM NADPH, 350 μL of buffer (50 mM Tris-HCl, 5 mM EDTA pH 7.6) and 350 μL of 0.22 mM tert-butyl hydroperoxide. The GPX activity was measured at 37 °C on a Hitachi U-2000 spectrophotometer at 340 nm for 3 min. The activity was expressed as U/mg protein in erythrocytes and liver samples.

The glutathione reductase (GRD) activities of erythrocyte hemolysates and liver tissue samples were measured with a commercial kit (Calbiochem 359962; Calbiochem-Novabiochem, La Jolla, CA, USA). Two hundred μL of diluted sample was added to 400 μL of 2.4 mmol/L GSSG buffer (dissolved in 125 mmol/L potassium phosphate buffer, pH 7.5, 2.5 mmol/L EDTA). Four hundred μL of 0.55 mmol/L NADPH (dissolved in deionized water) was added to the mixture and the GRD activity was measured at 340 nm for 5 min on a Hitachi U-2000 spectrophotometer. The activity was expressed as mU/mg protein in erythrocytes and liver samples.

Superoxide dismutase (SOD) activities of erythrocyte hemolysates and liver tissue samples were measured with a commercial kit (SD 125, Randox Laboratories, Antrim, UK). Fifty μL of diluted sample was added to 1.7 mL of mixed substrate (50 μmol/L xanthine and 25 μmol/L 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride, INT). Then 250 μL of xanthine oxidase was added to the mixture and the SOD activity was measured at 37 °C on a Hitachi U-2000 spectrophotometer at 505 nm for 3 min. The activity was expressed as U/mg protein in erythrocytes and liver samples.

Catalase (CAT) activities of erythrocyte hemolysates and liver samples were determined at 25 °C with a Hitachi U-2000 spectrophotometer UV-VIS spectrophotometer by the method previously described by Beers and Sizer (1952). The diluted sample was added to 59 mmol/L hydrogen peroxide (dissolved in 50 mmol/L potassium phosphate buffer, pH 7.0) and CAT activity was measured at 240 nm for 3 min. One unit of CAT activity was defined as the amount of H_2O_2 degraded (mmol/min/mg protein). The activity was expressed in U/mg protein in erythrocytes and liver samples.

The concentrations of glutathione (GSH) in erythrocyte hemolysates and liver tissue samples were spectrophotometrically assayed at 400 nm using a commercial kit (Calbiochem 354102; Calbiochem-Novabiochem, La Jolla, CA, USA). The concentration was expressed in μmol/mg protein in erythrocytes and in μmol/mg protein in tissue samples.

The malondialdehyde (MDA) concentrations of plasma and liver samples were assessed colorimetrically at 586 nm using a commercial kit (Calbiochem 437634; Calbiochem-Novabiochem, La Jolla, CA, USA). The concentration was expressed as μmol/L in plasma and as μmol/mg protein in liver samples.

Histological examinations. Paraffin sections of liver tissue were subjected to three kinds of histopathological stain; hematoxylin-eosin (H&E) stain to evaluate chronic liver damage including gross hepatocyte necrosis, fatty change and fibrosis; Masson stain for collagenous fibers; reticulum silver stain for reticular fibers. A semiquantitative histological evaluation was carried out by a pathologist blinded to the treatment groups in order to assess the degree of tissue inflammation, fatty change, necrosis of hepatocytes and bile duct hyperplasia. The grading ranged from 0 to 4 where 0 is absent, 1, trace, 2, mild, 3, moderate, 4, severe. The scale for semiquantitation of hepatic tissue fibrosis was as follows: 0 means no collagen; 1 means the existence of collagen but no septal; 2, the existence of collagen and septum, but no connective tissue; 3, the existence of collagen with a few thin connective tissue septa; 4, the existence of collagen with thick connective tissue septal.

Values were expressed as mean ± SD. To evaluate differences between the groups studied, one-way analysis of variance (ANOVA) with Fisher's *post hoc* test was used. The SAS software (Ver. 8.2, SAS Institute Inc., Cary, NC, USA) was used to analyse all data. Differences were considered statistically significant when $p < 0.05$.

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Table 1. Initial body weight, final body weight, initial food intake, final food intake and liver weight of rats in each group

Group	Control	CCl_4	Silymarin	Low dose	High dose
Initial body weight (g)	172 ± 24	178 ± 9	178 ± 8	178 ± 8	178 ± 8
Final body weight (g)	460 ± 29[b]	414 ± 21[a]	427 ± 27[a]	421 ± 22[a]	409 ± 21[a]
Initial food intake (g)	24.5 ± 0.1	24.4 ± 0.4	24.3 ± 0.7	24.4 ± 0.4	24.4 ± 1.5
Final food intake (g)	23.5 ± 0.5	23.1 ± 0.5	23.3 ± 0.5	23.0 ± 0.4	23.5 ± 0.7
Hepatosomatic index (%)	3.2 ± 0.5[a]	4.3 ± 0.4[b]	3.7 ± 0.7[a]	3.7 ± 0.6[a]	3.7 ± 0.5[a]

Data are presented as mean ± SD (n = 10).
Rats were assigned to the control group, CCl_4 group (CCl_4 injection), silymarin group (CCl_4 injection combined with the supplementation of silymarin, 7.5 mg/kg/day), low dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixtures at a relatively low dose, 43 mg/kg/day) and high dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixtures at a relatively high dose, 215 mg/kg/day).
Values with different superscripts indicate significantly different at $p < 0.05$. Hepatosomatic index = (Liver weight/body weight) × 100.

RESULTS

Body weight, food intake and liver weight

There was no significant difference in the initial body weight of the rats in all the groups. At the end of the study, the final body weight of rats in the CCl_4 group was decreased significantly compared with rats in the control group. However, there was no significant difference in the final body weight between CCl_4, silymarin, low dose and high dose groups (Table 1). The initial and final food intake in each group was also not significantly different. The liver weight of CCl_4 group was increased significantly when compared with the control group. Rats in the silymarin, low dose and high dose groups showed a significantly lower liver weight than rats in the CCl_4 group (Table 1).

The plasma AST and ALT activities

The plasma AST and ALT activities of the CCl_4 group were significantly higher than those of the control group. However, rats in the silymarin, low dose and high dose groups showed significantly lower plasma AST and ALT activities than those in the CCl_4 group ($p < 0.05$) (Table 2).

Antioxidant capacity

The GRD, GPX and CAT activities in the erythrocytes showed no differences in each group (data not shown). The rats in the CCl_4 group showed a significant reduction only in the hepatic GPX activity when compared with the control group. On the other hand, hepatic SOD and GPX activities of the low dose and high dose groups were significantly increased compared with those in the CCl_4 group. The hepatic SOD and GPX activities of the silymarin group showed no significant difference compared with the CCl_4 group. No significant differences in hepatic GRD activity were observed amongst the groups. The hepatic CAT activity in the silymarin and high dose groups showed no significant difference compared with the CCl_4 group. However, hepatic CAT activity in the low dose group was significantly decreased compared with the CCl_4 group (Table 3).

It was also found that the hepatic GSH content was elevated following administration of both low and high doses of schisandrin B and sesamin mixture (Table 4). In comparison with the CCl_4 group, the erythrocyte

Table 2. Effects of schisandrin B and sesamin mixtures on the plasma AST and ALT activities

Group	Control	CCl_4	Silymarin	Low dose	High dose
AST (Karmen unit/L)	58.8 ± 5.2[a]	280.7 ± 116.9[b]	72.1 ± 22.0[a]	75.6 ± 34.5[a]	89.1 ± 20.4[a]
ALT (Karmen unit/L)	39.3 ± 6.5[a]	300.1 ± 75.4[b]	73.9 ± 38.4[a]	82.3 ± 55.7[a]	77.0 ± 46.6[a]

Data are mean ± SD (n = 10).
Details are the same as described in Table 1.

Table 3. Effects of schisandrin B and sesamin mixtures on the hepatic SOD, GRD, GPX and CAT activities

Group	Control	CCl_4	Silymarin	Low dose	High dose
SOD (U/mg protein)	78.4 ± 13.4[abc]	66.5 ± 11.7[a]	73.8 ± 12.3[ab]	80.1 ± 9.3[bc]	86.5 ± 20.3[c]
GRD (mU/mg protein)	1472 ± 651[a]	2077 ± 591[ab]	2106 ± 1139[ab]	2066 ± 911[ab]	2461 ± 520[b]
GPX (mU/mg protein)	2663 ± 1026[cd]	1453 ± 297[a]	1991 ± 565[ab]	2267 ± 585[bc]	3282 ± 887[d]
CAT (U/mg protein)	202 ± 35[ab]	235 ± 63[b]	191 ± 75[ab]	162 ± 34[a]	193 ± 34[ab]

Data are mean ± SD (n = 10).
Details are the same as described in Table 1.

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Table 4. Effects of schisandrin B and sesamin mixtures on the GSH and MDA concentrations

Group[2]	Control	CCl_4	Silymarin	Low dose	High dose
GSH (μmol/L)					
Erythrocyte	75 ± 30[a]	85 ± 22[ab]	109 ± 39[bc]	113 ± 20[c]	83 ± 24[a]
Hepatic	263 ± 271[ab]	219 ± 75[a]	419 ± 146[c]	399 ± 164[bc]	465 ± 108[c]
MDA (μmol/L)					
Plasma	6.6 ± 1.4[a]	9.2 ± 2.6[b]	6.5 ± 1.7[a]	7.7 ± 2.3[ab]	7.3 ± 2.0[a]
Hepatic	8.4 ± 1.6[a]	11.2 ± 2.4[b]	10.5 ± 3.0[b]	10.7 ± 1.7[b]	10.4 ± 2.3[b]

Data are mean ± SD (n = 10).
Details are the same as described in Table 1.

GSH content was increased significantly in the low dose group but not in the high dose group. The MDA levels of plasma and liver were significantly higher in the CCl_4 group than those in the control group. When compared with the CCl_4 group, the plasma MDA concentration in the silymarin and high dose groups were significantly decreased by 42% and 25%, respectively. The plasma MDA concentration in the low dose group was similar to that of the CCl_4 group. Furthermore, there were no statistical differences in the hepatic MDA levels amongst the four groups with CCl_4 injection.

Histopathology examination

The elevation of plasma ALT and AST levels was correspondingly reflected in the histopathological findings. As shown in Figs 1 and 2, the gross histology showed that chronic CCl_4 intoxication damaged the hepatic architecture characterized by vacuolar and fatty degeneration, necrosis and fibrosis. Treatment with the low dose schizandrin B–sesamin mixture and silymarin moderately reduced the vacuolar degeneration and fibrosis while the highest dose group showed the least vacuolar degeneration and fibrosis. The semi-quantitative histological assessment showed an attenuation in bile duct proliferation and inflammation in rats in the low and high dose groups but not the silymarin group. However, the scores of fatty change, necrosis and fibrosis were similar among the four CCl_4 treated groups. The bile duct proliferation scores of the CCl_4 group (0.4 ± 0.6) were significantly increased when compared with the control group (0.1 ± 0.2). The bile duct proliferation scores of the low (0.1 ± 0.2) and high dose groups (0.0 ± 0.0) were significantly lower than that of the CCl_4 group (0.4 ± 0.6). However, the bile duct proliferation score showed no difference between the CCl_4 group and silymarin group (0.4 ± 0.6 versus 0.2 ± 0.3). The inflammation score of the CCl_4 group was significantly increased compared with the control group (0.7 ± 0.5 versus 0.3 ± 0.3). The inflammation scores of the low and high dose groups (both groups: 0.4 ± 0.4) were significantly lower than that of the CCl_4 group (0.7 ± 0.5) while no difference was observed between the CCl_4 group and silymarin group (0.6 ± 0.5).

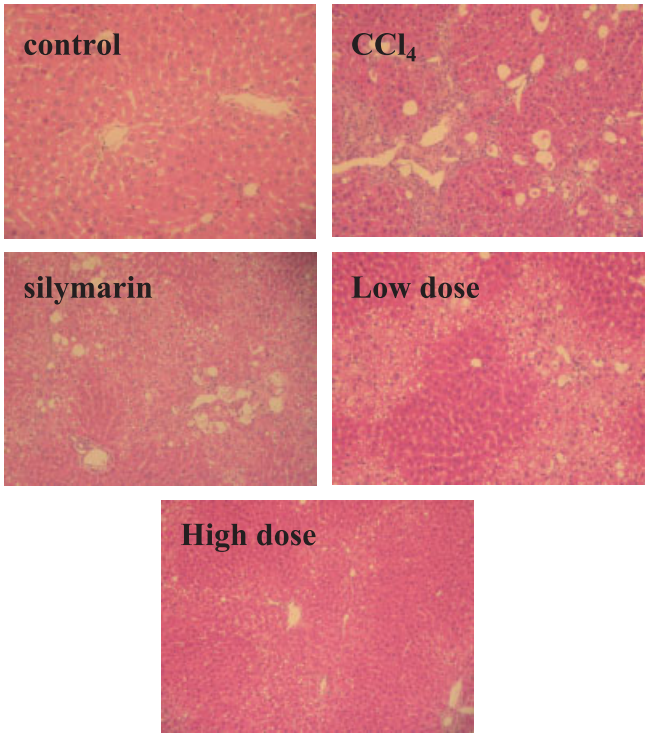

Figure 1. Representative photomicrographs of livers (× 100) from group A to group E with H & E stain. Rats were assigned to the control group (olive oil injection), CCl_4 group (CCl_4 injection), silymarin group (CCl_4 injection combined with the supplementation of silymarin, 7.5 mg/kg/day), low dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixtures at a low dose, 43 mg/kg/day) and high dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixtures at a high dose, 215 mg/kg/day). The control group showed no histopathological change. Vacuolar degeneration and fatty change was found in the rat receiving CCl_4. Silymarin and schisandrin B–sesamin mixtures suppressed the CCl_4-induced hepatocyte damage.

DISCUSSION

This study demonstrates the hepatoprotective action of the schisandrin B–sesamin mixture against chronic CCl_4-induced liver injury as evidenced by reduced plasma AST and ALT levels and histopathology findings. Carbon tetrachloride is a widely used chemical to induce liver injury in experimental studies, and its toxicity has been studied extensively. Numerous pathophysiological changes observed in CCl_4-intoxicated rats can be ascribed to damage caused by trichloromethyl free radical (CCl_3^-) generated by cytochrome P450 2E1 (CYP2E1) in liver (Zhu *et al.*, 1999). CCl_3^- initiates lipid peroxidation of the membrane of the endoplasmic reticulum and causes a chain reaction. In addition, hepatic necrosis induced by CCl_4 is usually associated with elevated plasma AST and ALT activities (Pilichos *et al.*, 2004; He *et al.*, 2004),

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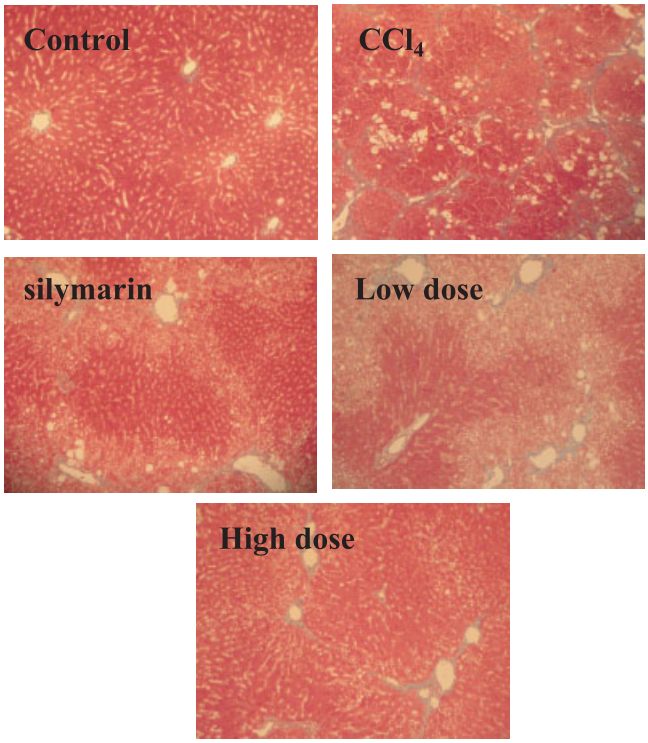

Figure 2. Representative photomicrographs of livers (× 100) from group A to group E with Masson stain. Rats were assigned to the control group, CCl_4 group (CCl_4 injection), silymarin group (CCl_4 injection combined with the supplementation of silymarin, 7.5 mg/kg/day), low dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixtures at a low dose, 43 mg/kg/day) and high dose group (CCl_4 injection combined with the supplementation of schisandrin B and sesamin mixtures at a high dose, 215 mg/kg/day). The control group showed no histopathological change. The rat receiving CCl_4 showed obvious fibrosis while rats receiving silymarin and schisandrin B–sesamin mixture showed no fibrosis.

due to leakage of cytosolic proteins and enzymes into the blood circulation when the hepatocyte membrane is compromised.

Rats fed with low and high doses of the schisandrin B and sesamin mixture showed significantly lower plasma AST and ALT activities compared with those administered with CCl_4 only (Table 2). These results are in agreement with Akimoto and co-workers (1993) who showed that sesamin, at 100 mg/kg body weight, could prevent lipid accumulation in hepatocytes of mice and attenuated plasma AST and ALT activities elevated by CCl_4.

CCl_4 generates free radicals which upset the redox balance maintained by the hepatic antioxidant enzyme system. CCl_4 has been reported to cause a significant reduction in hepatic GPX activities and GSH content in mice and rats (Manno *et al.*, 1985; Abraham *et al.*, 1999; Szymonik-Lesiuk *et al.*, 2003; Guven *et al.*, 2003). In our study, CCl_4 was shown to interfere with the hepatic antioxidant enzyme system (Table 3) although not affecting erythrocyte antioxidant enzyme activities. A comparison between the control group and the CCl_4-treated group showed a significant reduction in hepatic GPX activity. CCl_4-treatment also caused reductions in SOD, GRD, CAT enzyme activities and GSH content although not statistically significant (Tables 3 and 4).

The liver oxidative stress caused by CCl_4 intoxication leads to the mass production of free radicals, including superoxides (O_2^-) and hydrogen peroxides (H_2O_2) (Szymonik-Lesiuk *et al.*, 2003). Both GPX and CAT can react with H_2O_2. GPX is thought to be more active in the removal of H_2O_2, because of its dual location (mitochondria and cytosol) (Diplock, 1991). Therefore, this theory suggests that GPX should be more sensitive and more easily altered than CAT in a situation of CCl_4 intoxication. In addition, free radicals generated from CCl_4 also inhibit specific enzyme synthesis and activity, such as GPX (Szymonik-Lesiuk *et al.*, 2003). This explains the depressed GPX activity in rats exposed to chronic CCl_4 administration.

In this study, hepatic SOD and GPX activities of rats fed low and high doses of the schisandrin B–sesamin mixture were significantly higher than those in the CCl_4 group. The hepatic GSH content was also elevated by the schisandrin B and sesamin mixture. This finding was consistent with the previous study which reported that the transport of GSH from the cytosol to the mitochondrion was enhanced by schisandrin B (Chiu *et al.*, 2003). However, silymarin, the known hepatoprotective agent (Hall *et al.*, 1994), only increased the hepatic GSH content but not SOD and GPX activities. This indicates that the schisandrin B–sesamin mixture may counter the toxicity of CCl_4 via the removal of free radicals by an enhanced glutathione level, and SOD and GPX activities.

Studies have demonstrated that acute or chronic CCl_4 administration to experimental animals increased the formation of lipid peroxidation products, such as malondialdehyde (MDA) (Abraham *et al.*, 1999; Szymonik-Lesiuk *et al.*, 2003; Guven *et al.*, 2003). In our study, rats fed with high dose of schisandrin B–sesamin mixture showed a lower plasma MDA concentration than rats receiving CCl_4 only (Table 4). In a previous study, sesamin showed antioxidant capacity by scavenging the free radicals generated from oxidized LDL *in vitro* (Kang *et al.*, 2000). Besides, a lignan extract of *Schisandra chinensis* can inhibit superoxide formation during *in vitro* oxidation of xanthine (Lu and Liu, 1992). It is possible that the schisandrin B–sesamin mixture inhibited MDA production by interfering with the chain reaction of lipid peroxidation.

The reduction in vacuolar degeneration and fibrosis (Figs 1 and 2) observed in the histology indicates that the schisandrin B–sesamin mixture could reduce the degree of damage to the hepatic architecture. The semiquantitative histopathological examination also showed lower bile duct hyperplasia and liver tissue inflammation in rats receiving the schisandrin B–sesamin mixture. An unexpected finding is that rats fed with silymarin were not protected against bile duct proliferation and inflammation. This suggests that the schisandrin B–sesamin mixture could be just as effective as, if not stronger than, silymarin in protecting against the hepatotoxicity caused by CCl_4. Previous studies have reported that silymarin contains silibinin, silidianin and silicristin and can block the free radical chain reaction under CCl_4 challenge (Letteron *et al.*, 1990). On the other hand, the schisandrin B–sesamin mixture contains both schisandrin B and sesamin. Schisandrin B protects against carbon tetrachloride toxicity by enhancing the mitochondrial glutathione redox status in mouse liver (Ip *et al.*, 1996). Sesamin protects low-density lipoprotein against oxidative

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damage *in vitro* (Kang *et al.*, 2000). In this study, the schisandrin B–sesamin mixture groups showed higher GPX and SOD activities than those of silymarin group. In addition, the schisandrin B–sesamin mixture, but not silymarin, protected against bile duct proliferation and inflammation of CCl_4 toxicity. This finding suggests that the combination effect of schisandrin B and sesamin may be more effective in hepatoprotection because of its antioxidant capacity.

In conclusion, the schisandrin B and sesamin mixture has hepatoprotective action by enhancing the hepatic antioxidative capacity as evidenced by elevated hepatic SOD, GPX activities and GSH content.

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