Protective effects of silica hydride against carbon tetrachloride-induced hepatotoxicity in mice

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A B S T R A C T

The protective effects of MegaHydrate™ silica hydride against liver damage were evaluated by its attenuation of carbon tetrachloride (CCl4)-induced hepatotoxicity in mice. Male ICR mice were orally treated with silica hydride (104, 208 and 520 mg/kg) or silymarin (200 mg/kg) daily, with administration of CCl4 (1 mL/kg, 20% CCl4 in olive oil) twice a week for eight weeks. The results showed that oral administration of silica hydride significantly reduced the elevated serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), and cholesterol and the level of malondialdehyde (MDA) in the liver that were induced by CCl4 in mice. Moreover, the silica-hydride treatment was also found to significantly increase the activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), as well as increase the GSH content, in the liver. Liver histopathology also showed that silica hydride reduced the incidence of liver lesions induced by CCl4. The results suggest that silica hydride exhibits potent hepatoprotective effects on CCl4-induced liver damage in mice, likely due to both the increase of antioxidant-defense system activity and the inhibition of lipid peroxidation.

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1. Introduction

Liver diseases often progress from subclinical icteric hepatitis to necroinflammatory hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Vitaglione et al., 2004; Cristovao et al., 2007). Documented evidence has been reported that reactive oxygen species (ROS), including singlet oxygen, superoxide, and hydroxyl radicals, are known to play an important role in liver-disease pathology and progression (Vitaglione et al., 2004), and ROS have been proven to be associated with carbon tetrachloride (CCl4)-induced hepatotoxicity (Slater and Sawyer, 1971). Hepatotoxins, such as ethanol, acetaminophen, and CCl4, cause liver damage that is characterized by varying degrees of hepatocyte degeneration and cell death (Wu et al., 1999). CCl4 has been commonly used as a hepatotoxin in experimental hepatopathy (Hsu et al., 2008; Geetha et al., 2008) because experimentally induced cirrhotic response in animals by CCl4 has been shown to be superficially similar to human cirrhosis of the liver (Taira et al., 2004; Lee et al., 2007; Rudnicki et al., 2007). Therefore, CCl4-induced hepatic injury has been extensively used in animal models to evaluate the therapeutic potential of drugs and dietary antioxidants. The metabolites of CCl4, trichloromethyl free radicals, are capable of binding to DNA, lipids, proteins or carbohydrates and eventually lead to membrane-lipid peroxidation and finally to cell death (Recknagel et al., 1989; Weber et al., 2003; Basu, 2003). Many studies have reported that antioxidant supplements are efficacious in preventing oxidative-stress-related liver pathologies due to particular interactions and synergisms (Bhathal et al., 1983; Vitaglione et al., 2004). Additionally, one of the major defense mechanisms for the prevention and treatment of liver damage includes reducing the production of reactive
metabolites using antioxidants (Wu et al., 1999; Bansal et al., 2005). Antioxidants appear to act against disease processes by elevating the levels of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), thus decreasing lipid peroxidation (Aruoma, 1994; Wu et al., 1999; Bansal et al., 2005).

Silica hydride is a novel silica-based polymeric colloid that has interstitially enclosed hydride anions. The silica hydride synthesis process appears to cluster the organosilicate subunits into hydrogen-bonded aggregates (Stephanson and Flanagan, 2003a,b). In an aqueous environment, silica hydride is characterized by the features of stable release of the hydride ion for an extended length of time, slightly alkaline pH, and low oxidation–reduction potential. Recent publications have shown that silica hydride is nontoxic and safe as a dietary supplement (Carlise, 1982; Purdy-Lloyd et al., 2001; Stephanson and Flanagan, 2004a). Several studies have reported that silica hydride acts as an effective antioxidant that has the ability to scavenge free radicals and thus protect mammalian cells against strong oxidative stress (Stephanson et al., 2002; Stephanson and Flanagan, 2003a). Stephanson and Flanagan (2003b) have also shown that silica hydride decreased oxidative stress and effectively protected against ROS-induced pathological changes. In an in vitro study, silica hydride increased the ratio of [NADH]/[NAD+] in the mitochondria of Chinese hamster ovary cells as a function of increased cellular ATP production (Stephanson and Flanagan, 2004). In addition, a clinical study reported (Purdy-Lloyd et al., 2001) that a silica mineral supplement effectively decreased blood lactate concentrations after exercise. Altogether, these studies suggest that silica hydride is an incredibly effective radical scavenger and helps reduce oxidative stress due to its minimal size and high reduction potential.

Based on the excellent antioxidant potential of silica hydride found in vitro, it was of interest to evaluate its protective effects in vivo. However, it is not known if silica hydride can prevent or alleviate liver injury induced by CCl₄ and the mechanisms by which silica hydride may protect against CCl₄-induced hepatotoxicity are unclear. In the present study, male ICR mice were orally treated with silica hydride or silymarin (as a standard drug) daily accompanied by CCl₄ administration twice a week for 8 weeks. Hepatic GSH and MDA levels, as well as serum activities of AST, ALT, and ALP and SOD, catalase, GSH-Px, and GSH-Rd levels in liver tissues, were measured to monitor liver injury. The extent of CCl₄-induced liver injury was also analyzed through histopathological observations.

2. Materials and methods

2.1. Chemicals

Silymarin was obtained from the Sigma Chemical Co. All other chemicals and reagents used were obtained from local sources and were of analytical grade.

2.2. Material

Commercially available preparations of MegaHydrate™ silica hydride from New I Ten Rin Enterprise Co., Ltd. (Changhua City, Taiwan) were dissolved in distilled water prior to use. The quality of silica hydride was described and provided by the company. In accordance with the company-provided data, the constituents in the silica hydride powder included potassium citrate, silica, potassium carbonate, oleic acid, vitamin C, and negative hydrogen ions.

2.3. Animals

Male ICR mice (20 ± 2 g) were obtained from the Animal Department of BioASCO Taiwan Company and were quarantined and allowed to acclimate for a week prior to experimentation. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature- and humidity-controlled room. Food and water were available ad libitum. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guidelines.

2.4. Treatment

The animals were randomly divided into seven groups each consisting of ten mice. Group I served as the normal control and was given normal saline daily for a period of 8 weeks. Group II served as the vehicle control and was given olive oil daily for a period of 8 weeks. For inducing hepatotoxicity (in vivo), animals of Groups III, IV, V, VI, and VII were orally administered 1 mL/kg body weight of carbon tetrachloride (20% CCl₄ in olive oil) twice a week for a period of eight weeks. After CCl₄ intoxication, Group III served as the CCl₄ control. Group IV served as the positive control and was orally administered silymarin (200 mg/kg) daily for a period of eight weeks. Groups V, VI and VII were orally administered silica hydride dissolved in olive oil at doses of 104, 208, and 520 mg/kg, respectively, daily for a period of 8 weeks. At the end of the experiment, the animals were sacrificed by cervical dislocation. Blood was collected into heparinized tubes (50 U/mL). Liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible, and then they were immediately stored at −70 °C until analysis. An extra sample of each liver was excised and fixed in a 10% formalin solution for histopathological analysis.

2.5. Measurement of serum ALT, AST, cholesterol and triglyceride (TG) levels

Liver damage was assessed by the estimation of serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using commercially available test kits from Randox Laboratories Ltd. (UK). The results were expressed as units/liter (U/L). In addition, the serum levels of cholesterol and TG were estimated in the experimental animals using kits produced by Randox Laboratories Ltd. (UK).

2.6. Measurement of SOD, catalase, GSH-Px, GSH-Rd, and GSH in liver homogenate

Liver homogenates were prepared in cold Tris–HCl (5 mmol/L, containing 2 mmol/L EDTA, pH 7.4) using a homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10,000g for 10 min at 4 °C. The supernatant was used immediately for the assays for SOD, catalase, GSH-Px, GSH-Rd, and GSH. The activities of all of these enzymes were determined following the instructions in the Randox Laboratories kit.

2.7. Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation was done by measuring the concentration of thiobarbituric acid reactive substances (TBARS) in liver using the method of Berton et al. (1998). The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. In brief, samples were mixed with TBA reagent consisting of 0.375% TBA and 15% trichloroacetic acid in 0.25 N hydrochloric acid. The reaction mixtures were placed in a boiling-water bath for 30 min and centrifuged at 1811 g for 5 min. The supernatant was collected, and its absorbance was measured at 535 nm with an ELISA plate reader (Quant, BioTek, Vermont, USA). The results were expressed as nmole/mg protein using the molar extinction coefficient of the chromophore (1.56 × 10⁻¹⁰ M⁻¹ cm⁻¹).

2.8. Assessment of liver damage

The livers were preserved in neutral buffered formalin and were processed for paraffin embedding, following the standard microtechniques. Four- to five-micron sections of livers, stained with hematoxylin and eosin for the estimation of hepatocyte necrosis and vacuolization, as well as Masson trichrome stain and Sirius red stain for hepatocyte fibrosis, were observed under the microscope (IX71S8F-2, Olympus, Tokyo, Japan).

2.9. Statistical analysis

All values are expressed as means ± SD. Comparison between any two groups was performed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using a computer program SPSS. Statistically significant differences between groups were defined as p < 0.05.

3. Results

3.1. Effect of silica hydride on CCl₄-induced hepatotoxicity

The serum biochemical data for the evaluation of CCl₄-induced hepatotoxicity are summarized in Table 1. There was a significant elevation of serum ALT, AST, and ALP activities in the CCl₄-treated group as compared to the vehicle control (p < 0.05), indicating CCl₄-induced damage to the hepatic cells. However, treatment
with silica hydride at a dose of 520 mg/kg significantly decreased the percentages of ALT, AST, and ALP by 43%, 35%, and 14%, respectively, compared to the CCl4-treated group. Furthermore, there was a significant reduction (p < 0.05) of lipid parameters (TG and cholesterol) in serum, up to 17% in the silica hydride-treated group at a dose of 520 mg/kg, but silica-hydride treatment at a dose of 104 mg/kg did not significantly affect lipid parameters compared to the CCl4-treated group. In contrast to the CCl4-treated group, mice treated with silica hydride at doses of 104, 208, and 520 mg/kg showed significantly increased GSH levels, by 23%, 28%, and 36%, respectively; similar results were also found with the dose of 200 mg/kg of silymarin. The MDA level is widely used as a marker of free-radical mediated lipid peroxidation. The results of the MDA assays in the livers showed significantly higher values in the CCl4-treated group (10.55 ± 0.58 nmol/mg protein) than in the vehicle-control group (5.26 ± 0.83 nmol/mg protein, p < 0.05). Consistent with the liver levels of SOD, catalase and GSH-Px, administration of silica hydride significantly decreased CCl4-induced hepatic lipid peroxidation. The MDA levels in the silica hydride-treated group at doses of 208 and 520 mg/kg were significantly lower, by at least 28%, than that in the CCl4-treated control group (p < 0.05). Silymarin also inhibited the elevation of MDA levels after CCl4 administration. These findings indicated that silica hydride provides protection against CCl4-induced liver injury.

3.2. Hepatic antioxidant enzyme activities

SOD, catalase, GSH-Px and GSH-Rd were measured as an index of antioxidant status of tissues. Significantly lower activities of hepatic antioxidant enzymes were observed in the CCl4-treated group compared to the vehicle-control group. The group treated with silica hydride at a dose of 104 mg/kg showed a significantly increased (p < 0.05) SOD activity (by 21%), but the catalase and GSH-Px activities were not significantly affected compared to the CCl4-treated group. The GSH-Rd activity was not different between the CCl4-treated group and the each silica-hydride treatment groups (Table 2).

3.3. Effect of silica hydride on GSH level and lipid peroxidation

GSH acts as a nonenzymatic antioxidant in the detoxification pathway that reduces the reactive toxic metabolites of CCl4. The hepatic GSH levels in the mouse livers are shown in Table 3. Treatment with CCl4 significantly decreased the GSH levels in the liver as compared to the vehicle-control group. In contrast to the CCl4-treated group, mice treated with silica hydride at doses of 104, 208, and 520 mg/kg showed significantly increased GSH levels, by 23%, 28%, and 36%, respectively; similar results were also found with the dose of 200 mg/kg of silymarin. The MDA level is widely used as a marker of free-radical mediated lipid peroxidation. The results of the MDA assays in the livers showed significantly higher values in the CCl4-treated group (10.55 ± 0.58 nmol/mg protein) than in the vehicle-control group (5.26 ± 0.83 nmol/mg protein, p < 0.05). Consistent with the liver levels of SOD, catalase and GSH-Px, administration of silica hydride significantly decreased CCl4-induced hepatic lipid peroxidation. The MDA levels in the silica hydride-treated group at doses of 208 and 520 mg/kg were significantly lower, by at least 28%, than that in the CCl4-treated control group (p < 0.05). Silymarin also inhibited the elevation of MDA levels after CCl4 administration. These findings indicated that silica hydride provides protection against CCl4-induced liver injury.

### Table 1

<table>
<thead>
<tr>
<th>Design of Treatment</th>
<th>ALT (Units/L)</th>
<th>AST (Units/L)</th>
<th>ALP (Units/L)</th>
<th>TG (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
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<tr>
<td>Normal Control</td>
<td>63.00 ± 0.45b</td>
<td>20.15 ± 1.12b</td>
<td>42.17 ± 8.84b</td>
<td>147.77 ± 8.58b</td>
<td>274.37 ± 27.17b</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>61.05 ± 0.45a</td>
<td>15.00 ± 1.20a</td>
<td>37.00 ± 7.65a</td>
<td>142.37 ± 8.58a</td>
<td>261.37 ± 28.17a</td>
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<tr>
<td>CCl4 Control (1 mL/kg)</td>
<td>61.05 ± 1.20</td>
<td>20.15 ± 1.12</td>
<td>42.17 ± 8.84</td>
<td>147.77 ± 8.58</td>
<td>274.37 ± 27.17</td>
</tr>
<tr>
<td>Silymarin (200 mg/kg) + CCl4</td>
<td>61.05 ± 0.45b</td>
<td>20.15 ± 1.12b</td>
<td>42.17 ± 8.84b</td>
<td>147.77 ± 8.58b</td>
<td>274.37 ± 27.17b</td>
</tr>
<tr>
<td>Silica hydride (104 mg/kg) + CCl4</td>
<td>61.05 ± 1.20</td>
<td>20.15 ± 1.12</td>
<td>42.17 ± 8.84</td>
<td>147.77 ± 8.58</td>
<td>274.37 ± 27.17</td>
</tr>
<tr>
<td>Silica hydride (208 mg/kg) + CCl4</td>
<td>61.05 ± 1.20</td>
<td>20.15 ± 1.12</td>
<td>42.17 ± 8.84</td>
<td>147.77 ± 8.58</td>
<td>274.37 ± 27.17</td>
</tr>
<tr>
<td>Silica hydride (520 mg/kg) + CCl4</td>
<td>61.05 ± 1.20</td>
<td>20.15 ± 1.12</td>
<td>42.17 ± 8.84</td>
<td>147.77 ± 8.58</td>
<td>274.37 ± 27.17</td>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Design of Treatment</th>
<th>SOD (Units/mg protein)</th>
<th>Catalase (Units/mg protein)</th>
<th>GSH-Px (nmole/10 min/mg protein)</th>
<th>GSH-Rd (nmole/10 min/mg protein)</th>
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<tr>
<td>Normal Control</td>
<td>9.45 ± 0.59b</td>
<td>20.15 ± 1.12b</td>
<td>286.11 ± 22.89b</td>
<td>4.97 ± 0.48b</td>
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<tr>
<td>Vehicle Control</td>
<td>8.93 ± 0.67b</td>
<td>19.93 ± 2.08b</td>
<td>289.09 ± 35.06b</td>
<td>4.99 ± 0.73b</td>
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<tr>
<td>CCl4 Control (1 mL/kg)</td>
<td>6.42 ± 0.62c</td>
<td>12.24 ± 1.43c</td>
<td>185.13 ± 22.60c</td>
<td>4.31 ± 0.66c</td>
</tr>
<tr>
<td>Silymarin (200 mg/kg) + CCl4</td>
<td>8.56 ± 0.71b</td>
<td>16.40 ± 1.52b</td>
<td>240.40 ± 26.88b</td>
<td>4.41 ± 0.32b</td>
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<tr>
<td>Silica hydride (104 mg/kg) + CCl4</td>
<td>8.20 ± 0.37b</td>
<td>13.85 ± 1.50b</td>
<td>209.32 ± 22.58b</td>
<td>4.36 ± 0.65b</td>
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<tr>
<td>Silica hydride (208 mg/kg) + CCl4</td>
<td>8.74 ± 0.29b</td>
<td>17.15 ± 1.75b</td>
<td>268.15 ± 37.15b</td>
<td>4.79 ± 0.70b</td>
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<tr>
<td>Silica hydride (520 mg/kg) + CCl4</td>
<td>9.27 ± 0.77b</td>
<td>17.31 ± 1.35b</td>
<td>256.57 ± 29.28b</td>
<td>4.45 ± 0.81b</td>
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### Table 3

<table>
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<th>Design of Treatment</th>
<th>GSH (µmol/g wet weight)</th>
<th>MDA-TBA (µmol/mg protein)</th>
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<tbody>
<tr>
<td>Normal Control</td>
<td>12.40 ± 0.91c</td>
<td>6.47 ± 0.87c</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>13.03 ± 0.64c</td>
<td>5.26 ± 0.83c</td>
</tr>
<tr>
<td>CCl4 Control (1 mL/kg)</td>
<td>8.89 ± 0.66b</td>
<td>10.55 ± 0.58bc</td>
</tr>
<tr>
<td>Silymarin (200 mg/kg) + CCl4</td>
<td>15.58 ± 0.99b</td>
<td>5.11 ± 0.54b</td>
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<tr>
<td>Silica hydride (104 mg/kg) + CCl4</td>
<td>11.65 ± 1.18b</td>
<td>9.59 ± 1.07bc</td>
</tr>
<tr>
<td>Silica hydride (208 mg/kg) + CCl4</td>
<td>12.46 ± 1.28bc</td>
<td>7.32 ± 0.82b</td>
</tr>
<tr>
<td>Silica hydride (520 mg/kg) + CCl4</td>
<td>13.99 ± 0.83bc</td>
<td>7.59 ± 1.06b</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 10.

a p < 0.05 compare with vehicle control.

b p < 0.05 compare with CCl4.

c p < 0.05 compare with silymarin.
the free radicals being released in the liver were effectively scavenged by silica hydride.

3.4. Histopathological examination

Histopathological studies also provided important evidence supporting the biochemical analysis and liver antioxidant status. In the normal control and vehicle-control animals, liver sections showed normal hepatic cells, i.e., with a well-preserved cytoplasm and a prominent nucleus, nucleolus and central vein (Fig. 1A and B). The livers of CCl₄-intoxicated mice revealed moderate to severe hepatocellular vacuolization, hepatic necrosis and swelling, bile-duct hyperplasia, and increasing cellular mitosis (Fig. 1C). Compared with the lesions observed in the CCl₄ control group,

Fig. 1. Effect of the silica hydride on hepatic morphological analysis in CCl₄-intoxicated mice. Livers were sectioned and stained with hematoxylineosin by standard techniques (200×). (A) Normal control, (B) vehicle control, (C) CCl₄ control, (D) silymarin (200 mg/kg) + CCl₄, (E) silica hydride (104 mg/kg) + CCl₄, (F) silica hydride (208 mg/kg) + CCl₄, (G) silica hydride (520 mg/kg) + CCl₄.
the lesions of the silymarin-treated mice were of a much milder degree (Fig. 1D). These animals showed slight to moderate diffuse necrosis of hepatocytes and slight to mild hepatocellular vacuolization, whereas a slight to mild degree of hepatocellular necrosis and slight to moderate degree of hepatocellular vacuolization were observed in the livers of silica hydride-treated mice at 104, 208, and 520 mg/kg (Fig. 1E–G).

Furthermore, histopathological changes (fibrosis) occurred in CCl₄-intoxicated mouse livers, and their prevention by treatment with silica hydride was observed, as shown in Fig. 2 (Masson Trichrome stain) and Fig. 3 (Sirius red stain). In the normal control and vehicle-control groups, liver sections showed normal hepatic cells without fibrosis (Figs. 2A and B and 3A and B). The livers of mice treated with CCl₄ showed numerous hepatic lobules

**Fig. 2.** Histopathological changes of fibrosis occurred in CCl₄-intoxication and prevention by the treatment with silica hydride (Masson Trichrome stain, 200X). (A) Normal control, (B) vehicle control, (C) CCl₄ control, (D) silymarin (200 mg/kg) + CCl₄, (E) silica hydride (104 mg/kg) + CCl₄, (F) silica hydride (208 mg/kg) + CCl₄, (G) silica hydride (520 mg/kg) + CCl₄.
surrounded by thick fibrotic tissue, resulting in the formation of continuous fibrotic septa. The collagen of these fibrotic tissues showed a blue color when stained by Masson’s Trichrome (Fig. 2C) and a red color when stained by Sirius red (Fig. 3C). The lesions of silymarin-treated mice were observed to a much milder degree (Figs. 2D and 3D) than in the CCl$_4$ control group. The groups intoxicated with CCl$_4$ and treated with 104, 208, and 520 mg/kg of silica hydride showed mild to moderate degrees of fibrosis and the formation of incomplete septa from portal tract to central vein (Figs. 2E–G and 3E–G).

Histopathological examinations such as hepatocyte necrosis, vacuolization, and hepatocyte fibrosis are recorded and scored in Table 4. In this semi-quantitative assessment, all scores of histopathological examinations in the CCl$_4$ control group were

![Fig. 3. Histopathological changes of fibrosis occurred in CCl$_4$-intoxication and prevention by the treatment with silica hydride (Sirius red stain, 200×). (A) Normal control, (B) vehicle control, (C) CCl$_4$ control, (D) silymarin (200 mg/kg) + CCl$_4$, (E) silica hydride (104 mg/kg) + CCl$_4$, (F) silica hydride (208 mg/kg) + CCl$_4$, (G) silica hydride (520 mg/kg) + CCl$_4$.](image-url)
significantly higher than that of the normal control (p < 0.05), indicating that CCl4-induced severe damage to the hepatic cells. All of the tested doses of silica hydride significantly decreased (p < 0.05) the scores of hepatocyte necrosis as compared to the CCl4 control group; in contrast, mice treated with silica hydride at doses of 104, 208, and 520 mg/kg showed reduced scores for vacuolization and hepatocyte fibrosis, although these did not amount to statistically significant differences, except for those treated with 208 mg/kg silica hydride with respect to vacuolization. The positive control drug, silymarin, significantly reduced the scores for hepatocyte necrosis, vacuolization, and hepatocyte fibrosis, although these did not amount to statistically significant differences, except for those treated with 208 mg/kg silica hydride with respect to vacuolization. The positive control drug, silymarin, significantly reduced the scores for hepatocyte necrosis, vacuolization, and hepatocyte fibrosis compared to the CCl4 control group. When silica hydride-treated groups were compared to silymarin-treated groups, the overwhelming majority of scores were not significantly different (p > 0.05) between each histopathological examination.

According to the microscopic examinations, severe liver damage induced by CCl4 was remarkably reduced by the administration of silica hydride, which was in good correlation with the results of the liver-functional parameters of the serum and hepatic antioxidant enzyme activities and hepatic lipid peroxidation.

### 4. Discussion

Several studies have reported that silica hydride possesses free-radical scavenging activities for species including singlet oxygen, hydroxyl radical, and superoxide radical and protects cells against strong oxidative stress (Stephanson et al., 2002; Stephanson and Flanagan, 2003a). Stephanson et al. (2003) have also shown that silica hydride decreases oxidative stress and effectively protects against ROS-induced pathological changes. In addition, a clinical study reported (Purdy-Lloyd et al., 2001) that a silica mineral supplement was effective in decreasing the postexercise blood lactate concentrations in bicycle-training subjects. Therefore, we considered that silica hydride may be useful in the prevention of various hepatic damages induced by oxidative stress. In the present study, the capability of silica hydride to protect against CCl4-induced hepatotoxicity and oxidative stress were investigated.

Bioretransformed metabolites of CCl4 formed by cytochrome P-450 2E1, including trichloromethyl radical (CCl3) and trichloromethyl peroxyl radical (CCl3O2), have been demonstrated to initiate peroxidation (Recknagel et al., 1989) and affect liver pathogenesis (Recknagel, 1967). Both radicals have been shown to cause numerous cellular anomalies, such as DNA alteration, protein damage, lipid peroxidation, cell necrosis, and liver fibrosis (Brattin et al., 1985; Recknagel et al., 1989; Basu, 2003). Several reports have indicated that an important mechanism in hepatoprotective effects may be related to the capacities of antioxidants to scaveng reactive oxygen species (Naik and Panda, 2007; Tsai et al., 2009) and/or alleviate CCl4-induced toxic effects by the prevention of lipid peroxidation (Hsu et al., 2009). In the present study, we found that treatment with silica hydride significantly prevented CCl4-induced liver damage as evidenced by decreased serum activities of AST, ALT, and ALP and reduced serum concentrations of TG and cholesterol.

The balance of intracellular ROS depends on both their production within cells during normal aerobic metabolism and their removal by the antioxidant-defense system that includes nonenzymatic antioxidants (e.g., GSH, bilirubin, and vitamins E and C) and enzymatic antioxidants such as SOD, catalase, GSH-Px, and GSH-Rd in mammalian cells (Halliwell and Gutteridge, 1990; Sreelatha et al., 2009). Therefore, the enzymatic antioxidant activities and/or the inhibition of free-radical generation are important in terms of protecting the liver from CCl4-induced damage (Campos et al., 2001). A decrease in antioxidant enzyme activity is related to an increase in free radical production in CCl4 toxicity. SOD converts the dismutation of superoxide anions into hydrogen peroxide (H2O2) (Reiter et al., 2000) and catalase decomposes H2O2 to oxygen and water. GSH-Px metabolizes H2O2 and hydroperoxides to nontoxic products and terminates the chain reaction of lipid peroxidation by removing lipid hydroperoxides from the cell membrane (Jung and Henke, 1996). GSH-Rd is involved in the detoxification of a range of xenobiotic compounds by their conjugation with GSH (Baudrimont et al., 1997; Naik and Panda, 2007). These antioxidant enzymes are easily deprived of their activity by lipid peroxides or

### Table 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grades</th>
<th>Design of treatment</th>
<th>Parameter</th>
<th>Grades</th>
<th>Design of treatment</th>
</tr>
</thead>
<tbody>
<tr>
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A Grades are as follows: −, absent; +, trace (1–25%); ++, weak (26–50%); ++++, moderate (50–75%); ++++, severe (75–100%).

B The numerical score of histopathology were the result of adding the number per grade of affected mice and dividing by the total number of examined mice.

C For estimation of fibrosis, livers were sectioned and stained with Masson Trichrome by standard techniques.
free radicals, resulting in their decreased activities in CCl4 toxicity (Szymonik-Lesiuk et al., 2003). The results of the present study indicate that SOD, catalase, GSH-Px, and GSH-Rd activities were significantly decreased in the liver in response to CCl4 treatment alone compared with normal control mice, suggesting increased oxidative damage to the liver. In contrast, SOD, catalase, and GSH-Px levels were significantly elevated by administration of silica hydride to CCl4-intoxicated mice, suggesting that it has the ability to restore and/or maintain the activities of hepatic enzymes in CCl4-damaged liver. However, administration of silica hydride to CCl4-intoxicated mice could not significantly increase the activity of GSH-Rd. GSH-Rd and NADPH can rapidly reduce GSSG to form GSH, which is prone to oxidation to glutathione disulfide (GSSG) by xenobiotics. Mendieta-Wejebe et al., 2008 indicated that treatment with silica hydride elevated the amount of NADPH in rabbit hepatic cells. Therefore, the mechanism by silica hydride elevated the GSH content may be mainly due to the increased amount NADPH instead of an increase in the activity of GSH-Rd.

Previous studies on the mechanism of CCl4-induced hepatotoxicity showed that GSH acts as a nonenzymatic antioxidant that reduces H2O2, hydroperoxides (ROOH), and xenobiotic toxicity (Kadiska et al., 2000). In particular, the amount of GSH depletion is substantially correlated with the degree of liver necrosis (Dambach et al., 2006). Therefore, it appears that GSH conjugation is critically required for attenuating CCl4-induced liver injury. GSH is easily oxidized to GSSG by xenobiotic compounds, and there may additionally be reaction with any of the selenium-containing GSH-Px isozymes that may subsequently result in the reduction of GSH levels. GSSG is either rapidly reduced by GSH-Rd and NADPH or utilized in the protein-folding process in the endoplasmic reticulum. Because of these recycling mechanisms, GSH is an extremely efficient intracellular antioxidant for oxidative stress (Cantin et al., 2007). A study reported that silica hydride has the capability to regenerate some amount of NADPH from the reduction of NADP+ in rabbit hepatic cells (Mendieta-Wejebe et al., 2008), suggesting that GSH content in the liver may be elevated by the administration of silica hydride. In the present study, the hepatic content of GSH was significantly decreased in CCl4-intoxicated mice compared with control mice. Conversely administration of silica hydride to CCl4-intoxicated mice significantly elevated GSH content in the liver compared to the untreated group, indicating that silica hydride can protect against the CCl4-induced depletion of hepatic GSH.

CCl4 biotransformed metabolites have been demonstrated to cause lipid peroxidation (Recknagel et al., 1989), which is one of the principal mechanisms of CCl4-induced liver injury (Castor et al., 1974). Moreover, the initiation of oxidative stress related to various tissue injuries, cell death, and the progression of many acute and chronic diseases is generally believed to be induced by increased lipid peroxidation (Halliwell, 1997). Lipid peroxidation generates a variety of more-or-less stable toxic products and many of these are aldehydes (Esterbauer, 1982; Brattn et al., 1985). MDA is the major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acids (Vaca et al., 1988). An increase in MDA levels in the liver suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant-defense mechanisms to prevent the formation of excessive free radicals (Naik, 2003). In the present study, CCl4-induced toxicity caused an increase in liver MDA levels compared to the normal control group. Treatment with silica hydride significantly reversed these changes. The administration of silica hydride caused a significant decrease in MDA levels compared to the CCl4-induced toxicity group.

Silymarin, an antioxidant flavonoid complex isolated from the seed of milk thistle (Silybum marianum, Compositae), has been used to treat hepatotoxicity diseases in clinical practice for at least two decades. Silymarin has powerful free-radical scavenging properties and regulates intracellular GSH levels (Kren and Walterova, 2005; Shaker et al., 2010). The mechanism by which silymarin prevents against CCl4-induced lipid peroxidation and hepatotoxicity is either by decreasing the metabolic activation of CCl4 or by acting as a chain-breaking antioxidant for scavenging free radicals, or by a combination of these effects (Lettéron et al., 1990). In fact, a considerable body of experimental work in animal models has reported that silymarin, as a positive control, reduced CCl4-induced hepatotoxic effects by the prevention of lipid peroxidation (Wang et al., 2004; Naik and Panda, 2007; Shyu et al., 2008; Srleelatha et al., 2009). In the present study, silymarin acted as an effective positive control as evidenced by decreasing the ALT, AST, and ALP serum levels and increasing the SOD and catalase, GSH-Px activities and GSH content in liver while decreasing the hepatic MDA content when compared with the CCl4 control group.

The intracellular antioxidant status is always maintained at an equilibrium in mammalian cells. Dietary supplementation with extra natural antioxidants mainly assists the intracellular antioxidant-defense system in protecting cells and organs against ROS-induced oxidative damage. Indeed, allochthonous antioxidants usually have no effect on normal intracellular antioxidant status, as evidenced by Mansour (2000) and Lee et al. (2004), who reported that the hepatic antioxidant status was not affected by treatment with samples alone. Undoubtedly, silica hydride is an effective antioxidant for protecting mammalian cells against strong oxidative stress (Stephanson et al., 2002; Stephanson and Flanagan, 2003a). In the present study, we provide evidence that silica hydride has the ability to restore and/or maintain the levels of antioxidant status in CCl4-damaged liver. In addition, our previous study evaluating the safety of silica hydride showed that administration of silica hydride alone via gavage to ICR mice at dosage levels of 500, 1250, 2500, and 5000 mg/kg body weight did not cause treatment-related adverse effects on liver weight, serum liver-function tests (AST, ALT, and ALP) or histopathological changes of the liver. The results of the safety evaluation provide evidence that silica hydride is nontoxic (Tsai et al., submitted for publication). Therefore, silica hydride alone has no effect on liver structure, function, and/or antioxidant status.

In the histological examinations in this study, liver damage including hepatocyte necrosis and vacuolization was evaluated by hematoxylin and eosin staining as well as Masson trichrome and Sirius red staining for hepatocyte fibrosis. In clinical diagnosis and experimental examination, liver fibrosis has often depended upon microscopic detection of the collagen fibers, and Masson trichrome and Sirius red stains are generally the routine staining techniques for detecting collagen fibers (Marceau et al., 1999; Yang et al., 2008; Fang and Lin, 2008). Using routine staining techniques such as Sirius red and/or Masson’s trichrome has its advantages in that experienced pathologists have reliably and consistently identified liver damage in formalin-fixed, paraffin-embedded tissue sections. Collagen fibers stained with Masson’s trichrome show a conspicuous blue color, and when stained with Sirius red the fibers show an obvious red color. In our histological examination for hepatocyte fibrosis, we not only observed collagen by Masson trichrome staining but also confirmed it by Sirius red staining.

Histological examination of CCl4-treated mouse liver showed significant hepatotoxicity characteristics, such as necrosis in hepatic lobules, vacuolization, Kupffer cells around the central vein, and hepatocyte fibrosis. However, treatment with silica hydride significantly decreased these hepatotoxicity characteristics in mouse liver, suggesting that silica hydride provided protection against CCl4-induced liver injury. The histological examinations of the hepatocyte fibrosis assessment also found that both staining methods showed identical results in all liver sections, indicating that there was no difference between Masson trichrome stain and Sirius red stain with respect to histopathology. Through a semi-quantita-
tive assessment (see Table 4), the results were in agreement with the histological observations that administration of silica hydride significantly reduced hepatotoxicity in CCl₄-induced mice.

Stephanson and Flanagan (2004a) reported that silica hydride in an aqueous environment has several unique characteristics, such as the ability to stably release the hydride ion over an extended length of time, a slightly alkaline pH, and a low oxidation-reduction potential, but this study used silica hydride dissolved in olive oil. In fact, when silica hydride powder is dissolved in water there is a small amount of carbon dioxide released to the aqueous environment. It is well known that stomach acid in mice causes release of CO₂ from some otherwise nontoxic products and that the pressure can kill mice because mice and rats cannot burp, belch or release gas from their stomachs. Therefore, it was routine in the laboratory to add silica hydride to food-grade oil before testing. The silica hydride was absorbed without evolving CO₂ gas. However, the small amount of CO₂ released from a silica hydride solution is not a problem in humans. In the present study, dissolving silica hydride in olive oil not only retained the unique features of silica hydride in an aqueous environment but also solved the problem of CO₂ release. In addition, dissolving silica hydride in olive oil allowed the use of the same vehicle as for CCl₄ and silymarin.

Silica hydride has been verified as an effective antioxidant that plays an important role in protecting cells and organisms against the harmful effects of free radicals. The primary mechanism of action of this phenomenon appears to be the ability of silica hydride to quench superoxide reactive species, hydroxyl radical species and singlet oxygen (Stephanson et al., 2002; Stephanson and Flanagan, 2003a). In addition, silica hydride regenerated the NADPH produced by the reduction of NADP⁺ and decreased the catalytic activity of cytochrome P-450 in rabbit hepatic cells (Mendieta-Wejebe et al., 2008), suggesting that silica hydride protected hepatic cells against oxidative stress mediated by CCl₄-biotransformation metabolites. Therefore, silica hydride was expected to protect against CCl₄-induced liver damage.

In conclusion, the results of this study demonstrate that silica hydride was effective for the prevention of CCl₄-induced hepatic damage in ICR mice. Our results show that the hepatoprotective effects of silica hydride may be due to both an increase in the activity of the antioxidant-defense system and an inhibition of lipid peroxidation. This is the first report of the hepatoprotective effects of silica hydride in vivo. According to the results of the present study, silica hydride possesses a potent antioxidant activity. Oxidation is known to be involved in the pathogenesis of many diseases in which treatment with silica hydride is claimed to be effective. The inhibitory effects of dietary silica hydride may be useful as a hepatoprotective agent against chemical-induced hepatotoxicity in vivo.

Conflict of interest

The authors declare that there are no conflicts of interest.

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