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Hepatoprotection of tea seed oil (*Camellia oleifera* Abel.) against CCl_4-induced oxidative damage in rats

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Abstract

The oil of tea seed (*Camellia oleifera* Abel.) is used extensively in China for cooking. This study was designed to evaluate the effects of tea seed oil on CCl_4-induced acute hepatotoxicity in rats. Male SD rats (200 ± 10 g) were pre-treated with tea seed oil (50, 100, and 150 g/kg diet) for six weeks before treatment with a single dose of CCl_4 (50% CCl_4, 2 mL/kg of bw, intraperitoneally), the rats were sacrificed 24 h later, and blood samples were collected for assaying serum biochemical parameters. The livers were excised for evaluating peroxidation products and antioxidant substances, as well as the activities of antioxidant enzymes. Pathological histology was also performed. The results showed that a tea seed oil diet significantly ($p < 0.05$) lowered the serum levels of hepatic enzyme markers (alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase), inhibited fatty degeneration, reduced the content of the peroxidation product malondialdehyde, and elevated the content of GSH. Pre-treatment of animals with tea seed oil (150 g/kg diet) could increase the activities of glutathione peroxidase, glutathione reductase and glutathione *S* transferase in liver when compared with CCl_4-treated group ($p < 0.05$). Therefore, the results of this study show that a tea seed oil diet can be proposed to protect the liver against CCl_4-induced oxidative damage in rats, and the hepatoprotective effect might be correlated with its antioxidant and free radical scavenger effects.

Keywords: Antioxidant; Tea seed oil; CCl_4; Hepatic damage; Hepatoprotection

1. Introduction

The liver is the largest organ in the vertebrate body, and is the major site of xenobiotic metabolism. Liver injury can be caused by toxic chemicals, drugs, and virus infiltration from ingestion or infection. Carbon tetrachloride (CCl_4) has been widely used in animal models to investigate chemical toxin-induced liver damage. The most remarkable pathological characteristics of CCl_4-induced hepatotoxicity are fatty liver, cirrhosis and necrosis, which have been thought to result from the formation of reactive intermediates such as trichloromethyl free radicals ($CCl_3^{\cdot}$) metabolized by the mixed function cytochrome p450 in the endoplasmic reticulum (Recknagel et al., 1989). Reactive oxygen species (ROS) can bind to macromolecules, such as proteins, lipids, and DNA, resulting in physiologic dysfunction. According to the free radical theory, blocking or retarding the chain reaction of oxidation is one of the practicable strategies to preventing oxidative stress-induced hepatotoxicity. Therefore, intake of oxygen radical scavengers (antioxidants and phytochemicals) from vegetables and fruits may be a good defense mechanism for hepatoprotection. Furthermore, improvement of phase II detoxifying and antioxidant enzymes and elevation of the antioxidant substance content is one of the mechanisms to ameliorate the antioxidant status.

Tea seed oil is commonly used as a cooking oil in China, and used as an adjuvant in medicine. Chen et al. (1998) stated that the active compound, saponin, which was identified from tea seed oil, could lower the content of cholesterols, triglycerides and low density-lipoproteins in

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the blood of rats. The major fatty acids of tea seed oil are oleic and linolic acid (Shyu et al., 1990). Tea seed oil is rich in oleic acid similar to olive oil, which is the principal source of fat in Mediterranean diets which have shown functional effects against several degenerative pathologies, including cardiovascular diseases and cancer (Yu et al., 1999). Recently, we have isolated and identified two active compounds in tea seed oil: sesamin and a novel compound B (2,5-bis-benzo[1,3]dioxol-5-yl-tetrahydro-furo [3,4-*d*] [1,3] dioxine), both of which show potent antioxidant effects *in vitro* (Lee and Yen, 2006). The methanol extracts of tea seed oil (METSO) could reduce the formation of intercellular ROS, inhibit LDL oxidation, and protect lymphocytes against H_2O_2-induced genetic injury. Silymarin has been used for over 20 years in clinical practice for the treatment of toxic liver diseases (Messner and Brissot, 1990). Silymarin extract from the seeds of the plant Silybum marianum, also called ''milk thistle''. It has been described to be an antioxidant and exhibits anticarcinogenic, anti-inflammatory, hepatoprotection and growth-modulatory effects (Flora et al., 1998; Skottova et al., 2003). In this study, silymarin was used as a positive control to against the CCl_4-induced acute hepatic damage in rats. However, the physiological function of METSO on oxidative stress-mediated hepatotoxicity has not been investigated yet. In the present study, the model of CCl_4-induced oxidative damage in rat liver was established to investigate the potential effects of tea seed oil on hepatoprotection. The effect of tea seed oil on the expression of phase II detoxifying and antioxidant enzymes was also examined.

2. Materials and methods

2.1. Materials and chemicals

The sample of tea seed (*Camellia oleifera* Able.) was supplied from the Hsin-I country farmer's association (Nantou, Taiwan). β-Nicotinamide adenine dinucleotide phosphate (β-NADPH), butylated hydroxytoluene (BHT), sodium acetate–ammonium acetate, glutathione (GSH), glutathione reductase (GRd), sodium chloride, 1,1,3,3-tetramethoxypropane (TMP), thiobarbituric acid (TBA), 2,4-dinitrofluorobenzene (DNFB), sodium bicarbonate, and kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solvents were purchased from E. Merck Co. Ltd. (Darmstadt, Germany). Tris and protein assay kits were purchased from Bio-Rad Laboratory Ltd. (Watford, Herts, UK).

2.2. Preparation of tea seed oil

The tea seeds were sealed in a plastic bag, and stored at −20 °C until used. Tea seeds were roasted at 120 °C for 20 min and then pressed, deposited and filtered to obtain the tea seed oil (Lee and Yen, 2006).

2.3. Animal treatment

Male Sprague–Dawley rats (200 ± 10 g) were used for the experiments. The rats were randomly divided into six groups (six to eight rats/group) and provided with food and water ad libitum. To study the protective effect against CCl_4-induced acute hepatic damage, high dose (150 g tea seed oil/kg diet), medium dose (100 g tea seed oil/kg diet and 50 g soybean oil/kg diet) or low dose (50 g tea seed oil/kg diet and 100 g soybean oil diet) tea seed oil was given daily for six consecutive weeks. The control group was given a basal diet (150 g soybean oil/kg diet). Table 1 shows the composition of test diets administered. The diets differed only in the type of fat used; however the fat content remained constant (15%, w/w). In this study, we used the soybean oil for a positive energy balance. Silymarin (positive control) were given a basal diet and an additional 4 g/kg. On the last day of treatment, 50% CCl_4 (2 mL/kg of bw) was administrated intraperitoneally (Geier et al., 2002). The rats were sacrificed 24 h post-injection. Blood samples were immediately collected in tubes, kept at room temperature for 1 h, and centrifuged at 1000*g* for 10 min to obtain serum. Serum was stored at −20 °C until it was used in biochemical assays. The livers were excised and assayed for malondialdehyde (MDA) formation and pathological histology, according to the procedures described below. This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Chung Hsing University, Taichung, Taiwan.

Table 1
Composition of the test diets (g/kg)

Composition	Basal diet	Silymarin	Tea seed oil (g/kg diet)		
			50	100	150
Casein	197	197	197	197	197
D,L-Methionine	3	3	3	3	3
Tea seed oil	–	–	50	100	150
Soybean oil	150	150	100	50	–
Silymarin	–	4	–	–	–
Corn starch	572	572	572	572	572
Cellulose	30	30	30	30	30
AIN-76 Vitamin mix[a]	10	10	10	10	10
AIN-76 Mineral	35	35	35	35	35
Choline	3	3	3	3	3

[a] The composition of AIN-76 Vitamin mixture and AIN-76 Mineral mixture is as described in AIN (AIN, 1977).

2.4. Serum biochemical assays

Blood were placed at room temperature for 1 h, and then centrifuged at 1000*g* for 10 min to obtain serum. Serum biochemical parameters (GOT, GPT, LDH and ALP) were assayed by the method of Reitman and Frankel (1957) using commercially available kits, product of Randox Laboratories Co. (Antrum, UK).

2.5. Histopathologic studies

Liver and kidney tissues were fixed in 10% buffered formaldehyde and processed for histological examination by conventional methods and stained with hematoxylin and eosin (H&E). The liver pathology was scored as described by French et al. (2000) as follows: 0 = no visible cell damage; 1 = focal hepatocyte damage on <25% of tissue; 2 = focal hepatocyte damage on <25–50% of the tissue; 3 = extensive, but focal, hepatocyte lesion; 4 = global hepatocyte necrosis. The morphology of any lesions observed was classified and registered (Gray, 1964). The histopathological examinations were blinded to the study treatments.

2.6. Measurement of lipid peroxidation products

Liver tissues were homogenized in ice-cold 20 mM Tris–HCl (pH 7.4) (1:10, w/v). The homogenate was centrifuged at 2500*g* for 30 min at 4 °C. Aliquots of the homogenate were collected and stored at −80 °C for the following experiments. Determination of MDA by thiobarbituric acid (TBA) was used as an index of the extent of lipid peroxidation according to the methods of Buege and Aust (1978). The supernatant of liver tissue homogenate (1 ml) was mixed with 1 ml of 7.5% (w/v) cold trichloroacetic

acid (TCA) to precipitate proteins and then centrifuged at 1500 rpm. The supernatant was reacted with 1 ml of 0.8% (w/v) TBA in a boiling water bath for 45 min. After cooling, the lipid peroxidation product (MDA) was assayed according to an improved thiobarbituric acid reactive substances (TBARS) fluorometric method after excitation at 555 nm and emission at 515 nm using 1,1,3,3-tetraethoxypropane (TEP) as the standard. The protein concentration was determined using a standard commercial kit (Bio-Rad Laboratories, Hercules, CA, USA). The results were expressed as MDA formation per milligram of protein.

2.7. Assay of glutathione (GSH)

Liver tissues were homogenized in ice-cold potassium phosphate buffer (pH = 7.4). The homogenate was centrifuged at 2500*g* for 30 min at 4 °C. Aliquots of the homogenate were collected and stored at −80 °C for the following experiments. The content of reduced GSH was determined by modifying the method of Van Dam et al. (1999). Liver homogenate and a 5% TCA mixture was pre-incubated for 5 min at 4 °C, and then centrifuged at 8000*g* for 10 min at 4 °C. Aliquots of the homogenate were collected to which 5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) was immediately added and incubated for 5 min at 4 °C. The absorbance was measured at 412 nm, and the concentration of GSH was calculated using the absorbance of 1 M of product with $E_{412} = 13\,600\ M^{-1}\ cm^{-1}$.

2.8. Assay for antioxidant enzymes

The glutathione peroxidase (GPx) activity was determined spectrophotometrically, according to the method of Mohandas et al. (1984). The following solutions were pipetted into a cuvette: 0.1 mL of homogenate and 0.8 mL of 100 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 unit/mL GSH reductase, and 1 mM GSH. The mixture was pre-incubated for 5 min at room temperature. Thereafter, the overall reaction was initiated by adding 0.1 mL of 2.5 mM H_2O_2. Enzyme activity was calculated by the change of the absorbance at 340 nm for 5 min. GPx activity could be expressed as nanomoles of NADPH per minute per milligram of protein.

The glutathione reductase (GRd) assay was performed through monitoring the consumption of NADPH for reducing glutathione disulfide (GSSG) (Bellomo et al., 1987). The following solutions were pipetted into a 1 cm spectrophotometric cuvette: 0.1 mL of homogenate and 0.9 mL of 0.10 M phosphate buffer, pH 7.0, containing 1 mM $MgCl_2$–$6H_2O$, 50 mM GSSG, and 0.1 mM NADPH. This mixture was pre-incubated for 5 min at room temperature. GRd activity was calculated by the change of the absorbance at 340 nm for 5 min; GRd activity was expressed as nanomoles of NADPH per minute per milligram of protein.

The catalase (CAT) activity was determined following the method of Cohen et al. (1970). A mixture of 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2 and cell lysate in a final volume of 3 ml was incubated at room temperature for 2 min. The change in absorbance at 240 nm in 2 min was calculated. The catalase activity was expressed as mmole/min/mg protein.

2.9. Statistical analysis

All data are expressed as means ± SD. ANOVA was used to evaluate the difference between multiple groups. If significance was observed between the groups, Dunnett's test was used to compare the means of two specific groups. $p < 0.05$ was considered to be significant.

3. Results

3.1. Effect of tea seed oil on the tissue weight of rats

Table 2 shows the liver and kidney weights of rats in each group. Compared with controls, there was no significant difference of the kidney weight between CCl_4-treated rats and the control group. It was observed that CCl_4-treated rats showed a significant increase ($p < 0.05$) in the relative liver weight when compared with the control group. However, feeding of high dose of tea seed oil (150 g/kg of diet) for six weeks could significantly decrease ($p < 0.05$) the relative liver weight when compared with CCl_4 treated group.

Table 2
Body weight and relative organ weights of acute CCl_4-treated rats with or without tea seed oil

Groups[a]	Body weight (g)	Relative organ weight (g/100 g of bw)	
		Liver	Kidney
Control	357 ± 36	2.87 ± 0.23	0.73 ± 0.15
CCl_4 (50% CCl_4/olive oil)	377 ± 31	4.05 ± 0.30[b]	0.64 ± 0.05
Silymarin			
4 g/kg diet + CCl_4	351 ± 43	4.27 ± 0.63	0.73 ± 0.13
Tea seed oil			
50 g/kg diet + CCl_4	385 ± 16	4.11 ± 0.34	0.62 ± 0.06
100 g/kg diet + CCl_4	388 ± 28	3.73 ± 0.25	0.69 ± 0.08
150 g/kg diet + CCl_4	375 ± 33	3.24 ± 0.42[c]	0.68 ± 0.09

[a] Values are mean ± SD of 6–8 rats.
[b] Significantly different from the control group.
[c] Significantly different from the group treated with CCl_4 only, $p < 0.05$.

3.2. Pathological histology of the liver

Fig. 1 shows that CCl_4-induced liver injury caused the concave liver surface and lymphocytic infiltration in the central vein. The hepatic cells were found to be fatty degeneration, necrosis, cytoplasmic vacuolization and mitosis in CCl_4-treated group. Table 3 summarizes the data relating to liver damage induced by CCl_4 in pathological histology. The level of fatty degeneration, necrosis and vacuole formation were obvious after acute CCl_4 treatment. Pre-administration of high and medium doses of tea seed oil and silymarin could reduce the injury score of fatty degeneration and necrosis. The presence of mitotic figures in hepatocytes was assessed as an index of liver proliferative capacity in response to toxin-induced injury (Sigala et al., 2006). It showed slight variations in vacuole formation and mitosis score of liver. Histological examination showed a preventive effect of tea seed oil on CCl_4-induced hepatoxicity.

3.3. Effect of tea seed oil on CCl_4-induced hepatic damage

Following this, we analyzed whether the supplementation of tea seed oil could protect rats against CCl_4-induced acute liver destruction. The effects of pre-treatment with tea seed oil on the CCl_4-induced elevation of serum AST, ALT, and the other biomarkers are shown in Table 4. Pre-treatment of animals with tea seed oil could significantly ($p < 0.05$) reduce serum AST, ALT and LDH when compared with CCl_4-treated group. On the other hand,

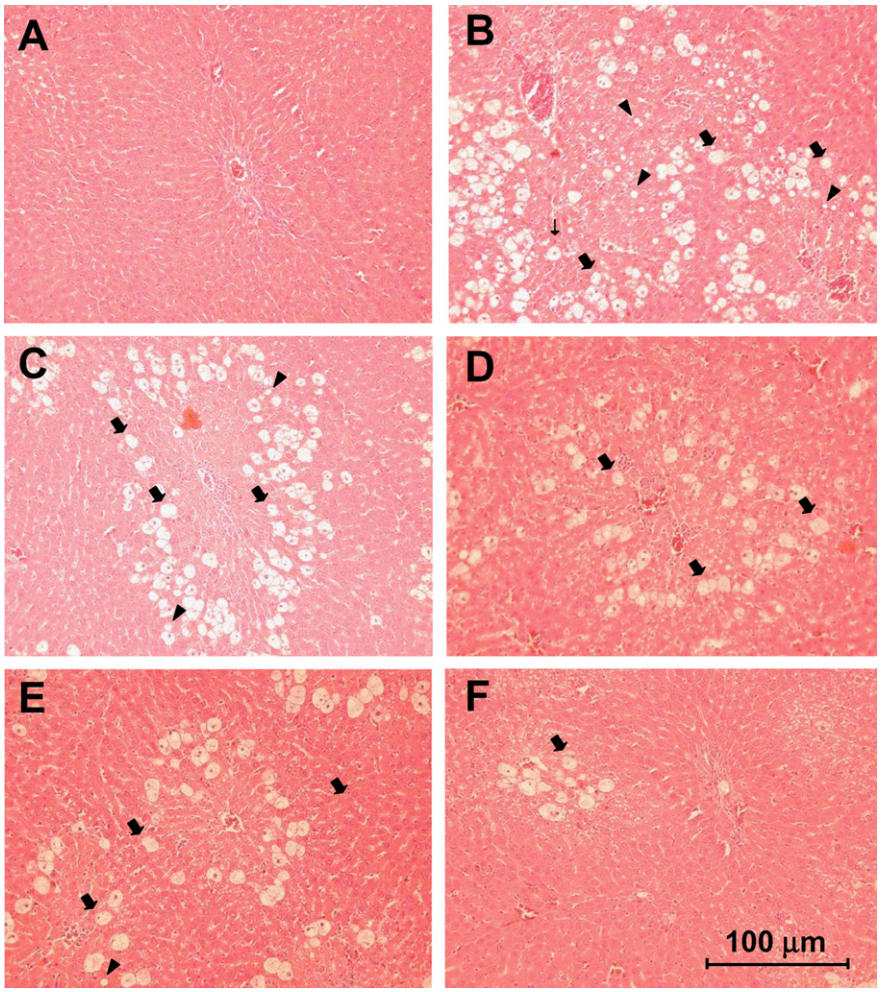

Fig. 1. Effect of tea seed oil on CCl_4-induced liver damage of Sprague–Dawley rats: (A) control group; (B) animals treated with 50% CCl_4 (2 mL/kg of bw) showed severe fatty degeneration (big arrow), cell necrosis (arrow) and vacuole formation (small arrowhead); (C) animals pre-treated with silymarin (4 g/kg diet) and then treated with 50% CCl_4 (2 mL/kg of bw); (D) animals pre-treated with tea seed oil (50 g/kg diet) and then CCl_4 (2 mL/kg of bw); (E) animals pre-treated with tea seed oil (100 g/kg diet) and then CCl_4 (2 mL/kg of bw); (F) animals pre-treated with tea seed oil (150 g/kg diet) and then CCl_4 (2 mL/kg of bw).

different diets with the same lipid contents did not affect TG (triglyceride), cholesterol, and biological parameters in the 6 week period of pre-treatment (data not shown). However, CCl_4 injection significantly increased plasma AST, ALT, and LDH activities except for ALP. This injury was significantly suppressed by the pre-treatment with tea seed oil ($p < 0.05$).

3.4. Effect of tea seed oil on hepatic phase II detoxifying and antioxidant enzymes

Table 5 shows the effects of tea seed oil on hepatic GSH-related enzymes in CCl_4-induced damage in rats. After injection of CCl_4, the activities of GPx and GRd were significantly decreased as compared with the control group. Pre-treatment of animals with different doses of tea seed oil (50–150 g/kg of diet) or silymarin (4 g/kg of feed) for 6 weeks significantly elevated the expression of GPx as compared with the group of CCl_4-treated alone ($p < 0.05$). The activity of GRd was found to be increased with pre-administration of high doses of tea seed oil and silymarin compared to the group of CCl_4-treated alone.

In contrast, neither GST nor CAT was influenced by the treatment of CCl_4, silymarin, or with tea seed oil. However, pre-treatment with high dose of tea seed oil could restore the antioxidant capacity exhausted by CCl_4.

3.5. Effect of tea seed oil on hepatic GSH content

Fig. 2 shows the effect of tea seed oil on the content of GSH in CCl_4-induced hepatotoxicity in rats. The administration of a single dose of CCl_4 (50%, i.p.) to rats resulted in a decline of total GSH content in the liver homogenate. Both pre-treatment of silymarin and tea seed oil diets significantly inhibited the depletion of GSH, compared to the group of CCl_4-treated rats alone ($p < 0.05$). Interestingly, supplementation of tea seed oil could protect GSH content depletion induced by CCl_4.

Table 3
Histological injury score of liver under different doses of tea seed oil in rats treated with CCl_4

Groups	Injury of score[a]			
	Fatty degeneration	Necrosis	Vacuole formation	Mitosis
Control	0	0	0	1
CCl_4 (50% CCl_4/ olive oil)	4	2	3	2
Silymarin				
4 g/kg diet + CCl_4	2	1	2	1
Tea seed oil				
50 g/kg diet + CCl_4	3	1	3	2
100 g/kg diet + CCl_4	2	1	3	2
150 g/kg diet + CCl_4	2	1	2	1

[a] Livers were scored for hepatic injury via light microscopy with score 0 = no visible cell damage; score 1 = focal hepatocyte damage on less than 25% of the tissue; score 2 = focal hepatocyte damage on 25–50% of the tissue; score 3 = extensive, but focal, hepatocyte lesions; score 4 = global hepatocyte necrosis.

Table 4
Effect of tea seed oil on activities of serum AST, ALT, LDH and ALP in rats treated with CCl_4

Groups	Activity (U/L)[a]			
	AST[b]	ALT	LDH	ALP
Control	121 ± 10	30 ± 3	866 ± 256	143 ± 24
CCl_4 (50% CCl_4/ olive oil)	1810 ± 486[c]	1231 ± 445[c]	3154 ± 960[c]	156 ± 49
Silymarin				
4 g/kg diet + CCl_4	1730 ± 652	893 ± 200[d]	1636 ± 231[d]	149 ± 45
Tea seed oil				
50 g/kg diet + CCl_4	1724 ± 301	663 ± 62[d]	1402 ± 534[d]	138 ± 35
100 g/kg diet + CCl_4	1265 ± 242[d]	659 ± 180[d]	1103 ± 386[d]	155 ± 30
150 g/kg diet + CCl_4	1062 ± 267[d]	795 ± 184[d]	1525 ± 328[d]	176 ± 29

[a] Values are mean ± SD of 6–8 rats.
[b] AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase.
[c] Significantly different from the control group.
[d] Significantly different from the group treated with CCl_4 only, $p < 0.05$.

Table 5
Effect of tea seed oil on antioxidant enzymes activities in the liver of rats treated with CCl_4

Groups	*n* mol/min/mg protein[a]			
	GPx[b]	GRd	GST	CAT
Control	186 ± 37	9.06 ± 1.18	243 ± 30	34.24 ± 15.04
CCl_4 (50% CCl_4/ olive oil)	129 ± 13[c]	5.06 ± 1.24[c]	224 ± 12	32.84 ± 1.74
Silymarin				
4 g/kg diet + CCl_4	170 ± 19[d]	8.38 ± 3.53[d]	252 ± 18[d]	35.16 ± 7.44
Tea seed oil				
50 g/kg diet + CCl_4	154 ± 14[d]	5.70 ± 2.05	225 ± 18	30.91 ± 2.12
100 g/kg diet + CCl_4	148 ± 31[d]	6.21 ± 2.69	227 ± 16	34.36 ± 5.37
150 g/kg diet + CCl_4	180 ± 21[d]	8.10 ± 3.13[d]	235 ± 8[d]	34.39 ± 5.10

[a] Values are means ± SD of 6–8 rats.
[b] GPx: glutathione peroxidase, GRd: glutathione reductase, GST: glutathione *S* transferase, CAT: catalase.
[c] Significantly different from the control group, $p < 0.05$.
[d] Significantly different from the group treated with CCl_4 only, $p < 0.05$.

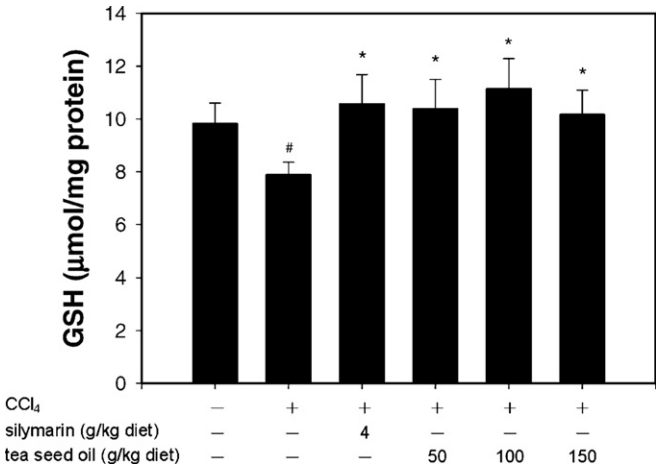

Fig. 2. Effect of tea seed oil on glutathione content in liver of rats treated with CCl_4. The data represent the mean ± SD of 6–8 rats. #Significantly different from the control group. *Significantly different from the group treated with CCl_4 alone, $p < 0.05$.

3.6. Effect of tea seed oil on CCl_4-induced oxidative damage

Fig. 3 shows the formation of TBARs in the liver of rats with different treatments. Malondialdehyde (MDA) is the product of lipid peroxidation and is a common marker of lipid peroxidation. The content of MDA was significantly increased in the liver of CCl_4-treated rats as compared with the control group. High dose tea seed oil treated animals (150 g/kg of diet) significantly suppressed the content of liver TBARs. These results suggest that oxidative stress induced by CCl_4 was blocked by the supplementation of tea seed oil.

4. Discussion

It is well known that one of the functional benefits from olive, sesame, and berry seed oils is protection against cardiovascular disease and oxidative stress. In our previous study, the data showed that tea seed oil contains sesamin and a novel compound exhibiting remarkable antioxidant capacity (Lee and Yen, 2006). However, the biological

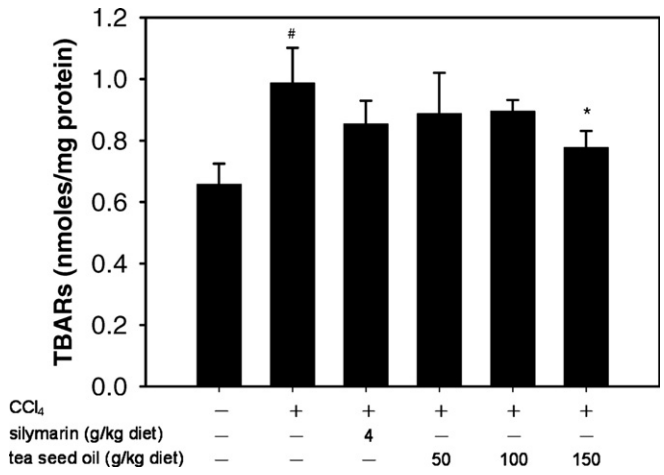

Fig. 3. Effect of tea seed oil on hepatic lipid peroxidation induced by CCl_4. The data represent the mean ± SD of 6–8 rats. #Significantly different from the control group. *Significantly different from the group treated with CCl_4 alone, $p < 0.05$.

and physiological functions were not well known. We induced hepatotoxicity in rats with CCl_4 to establish a situation in order to evaluate the hepatoprotective effect of tea seed oil against chemical toxin-mediated acute oxidative injury in the liver. In the design of this study, we focused on the intake of tea seed oil could be beneficial for acute oxidative injury in the liver. In this study, we used the soybean oil for a positive energy balance. The report indicated that the dilution solvent of CCl_4 were used soybean oil (Pereira et al., 1997). However, there is no data published for hepatic protection of soybean oil. Our study were eliminated the hepatoprotective effect of soybean oil on CCl_4-induced oxidative damage in rats.

Phase 1 reactions that often mediate the metabolic activation of carcinogens, phase II detoxifying and antioxidant enzymes provide the antioxidant capacity to catalyze conjugation through sulfation, glucuronidation, and glutathiolation to neutralize electrophilic metabolites, facilitating the expulsion of those carcinogens (Kwak et al., 2004). To investigate the correlation between oxidative stress and hepatotoxicity, the chemical toxin, CCl_4, is a well-defined and studied alkane used to elucidate the mechanisms of action of hepatotoxic effects, such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity (Weber et al., 2003). Histological injury score of liver exhibited by the pre-treatment with tea seed oil (150 g/kg diet) group is similar to silymarin treatment (Table 3). Furthermore, through the examination of the activities of enzymes, including AST and ALT in the plasma, we can discern the index of liver injury. It is well known that chemical agents produce hepatic injury, causing both large increases in ALT and AST activity (Weber et al., 2003).

In this study, CCl_4 treatments could modify liver function, since the activities of AST and ALT were significantly higher than those of normal values as compared with the control group (Table 4). It was more important to confirm whether there is any difference between the treatment with or without tea seed oil under the damage of CCl_4. The results showed that pre-treatment of rats with tea seed oil effectively protected the animals against CCl_4-induced hepatic destruction, as evidenced by decreased serum AST, ALT, and LDH activities. In the histological examination, pre-treatment with a tea seed oil diet or silymarin suppressed the acute hepatic damage and was consistent with improvement of the serum biological parameters for hepatotoxicity.

Acute CCl_4 damage could significantly decrease the expression of antioxidant enzymes in liver, including GPx, GRd and GST. GSH-related enzymes play detoxifying and antioxidant roles in metabolizing xenobiotics through the conjugation with glutathione or reduction of free radicals. GRd is responsible for the re-generation of GSH, and GPx worked together with GSH in disintegrating hydrogen peroxide or other organic hydroperoxides. The results showed that the activities of GPx and GRd were significantly reduced during acute CCl_4 damage when compared with the control group (Table 5). The GSH-dependent antioxidant enzymes (GPx, GRd and GST) in silymarin group were significantly increased as compared to the group of CCl_4-treated only ($p < 0.05$). High dose tea seed oil (150 g/kg of diet) treatment could significantly recuperate the activities of GPx and GRd when compared with the CCl_4-treated only group ($p < 0.05$). Pre-treatment with tea seed oil showed an improved effect on the expression of antioxidant enzymes examined as compared to the CCl_4 damaged group. CCl_4 could exhaust the activities of antioxidant enzymes, and tea seed oil may enhance the innate mechanisms of the antioxidant system or provide its antioxidant capacity against CCl_4-induced oxidative stress. Sheweita et al. (2001) suggested that antioxidants could provide protective effects on carbon tetrachloride-induced damage and retard the index of hepatitis. Tea seed oil showed improved effects on antioxidant capacity against chemical toxicants.

Sulfhydryl compounds such as glutathione (GSH) are well known to be a antioxidant substance in organisms, playing a critical role against CCl_4-induced injury by covalently binding to $CCl_3^{\cdot}$. This is considered as the initial reactant in the chain reaction of oxidation, and then result in the lipid peroxidation and the cell membrane disruption (Brattin et al., 1985). Pre-treatment with tea seed oil (50–150 g/kg of diet) resulted in elevating the content of liver GSH compared with the control group. The GSH level in pre-treatment with tea seed oil (50–150 g/kg of diet) groups is similar to silymarin treatment. Several diseases have been associated with changes in GSH levels, and reduce the resistance to oxidation stress. The levels of GSH and the activities of the GPx and GRd were used to monitor the balance of oxidative stress and chemopreventive ability (Hatono et al., 1996). In our study, the tea seed oil exhibited protective effects against liver damage from $CCl_3^{\cdot}$. Furthermore, the GSH-related antioxidant system has also been improved.

Sesamin and compound B were identified in tea seed oil and showed antioxidant effects against oxidative stress in a cell model (Lee and Yen, 2006). Because lipid accumulation in the liver is a major symptom of CCl_4 damage, sesamin has been investigated for its ability to be absorbed and detected in bile, and improve liver β-oxidation effects, while simultaneously inhibiting the synthesis of fatty acids (Kiso, 2004). Elevation of β-oxidation is beneficial to decrease fatty degeneration, which exhibits the protective effect of sesamin.

Lipid peroxidation is one of the major characteristics that can be included as an oxidative damage marker. The biochemical mechanisms involved in the development of CCl_4-mediated hepatotoxicity have long been investigated. MDA is widely used as marker of lipid peroxidation (Mansour, 2000). Rats treated with CCl_4 showed significantly increased levels of MDA compared with the control group ($p < 0.05$). Pre-treatment with different doses of tea seed oil (50–150 g/kg of diet) showed 10.13%, 9.22% and 21.27% reduction, respectively, in CCl_4-induced elevations of MDA. High dose tea seed oil (150 g/kg of diet) significantly inhibited the formation of MDA in the liver during acute CCl_4 damage. Diet antioxidants could provide protective effects against CCl_4-induced liver damage (Sheweita et al., 2001). In the present study, tea seed oil could enhance antioxidant capacity in the liver, and decrease the damage mediated by $CCl_3^{\cdot}$, through blocking the oxidative chain reaction and suppressing the formation of lipid peroxidant products.

GSH is the frontline of the antioxidant system, but it seems not to be sufficient to prevent the cytotoxicity of ROS. Dietary phytochemicals, such as polyphenols and flavonoids, could provide potent antioxidant effects (Masella et al., 2005). Tea seed oil shows antioxidant effects against radical-induced damage. Biocompounds of tea seed oil could provide antioxidant benefits in hepatoprotection. Recently, sesamin, identified from sesame, shows potent antioxidant ability both *in vitro* and *in vivo* (Nakai et al., 2003; Kiso, 2004). Epidemiology studies in Europe have shown that the source of dietary fat in the Mediterranean area was mainly olive oil, which contains high ratios of monounsaturated fatty acid (Stark and Madar, 2002). The ratio of monounsaturated fatty acids is an important factor in stabilizing the integrity of the cell membrane (Lopez-Miranda et al., 2000). Tea seed oil is similarly rich in phytochemicals and exhibits antioxidant capacity against oxidative stress. All evidence, including GSH level, damage markers, and pathological histology show that a tea seed oil diet could decrease CCl_4-induced oxidative stress.

In conclusion, pre-treatment with tea seed oil from *Camellia oleifera* could reduce damage induced by CCl_4. The mechanisms of protection include the inhibition of lipid peroxidation, increasing the content of GSH, elevating the expression of antioxidant enzymes, all of which result in the recuperation of biological parameters and the integrity of the tissue.

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