Food Sci. Biotechnol. Vol. 16, No. 6, pp. 967 ~ 974 (2007)

Food Science and Biotechnology

Protective Effect of Administrated Glutathione-enriched *Saccharomyces cerevisiae* FF-8 Against Carbon Tetrachloride (CCl_4)-induced Hepatotoxicity and Oxidative Stress in Rats

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Abstract The present work is aimed to evaluate the protective effect of glutathione-enriched *Saccharomyces cerevisiae* FF-8 strain on carbon tetrachloride (CCl_4)-induced hepatotoxicity and oxidative stress in rats. The activities of liver markers (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase), lipid peroxidative index (thiobarbituric acid-reactive substances), and the antioxidant status (reduced glutathione) were used to monitor those protective roles of FF-8 strain. The liver marker enzymes in plasma and the lipid peroxidation in the liver were increased when CCl_4 was treated but these were significantly decreased by FF-8 strain treatment. The hepatic concentration of glutathione in the current glutathione-enriched FF-8 strain fed animal was approximately twice as high as the normal, but this was slightly increased in response to CCl_4 plus glutathione-enriched FF-8 strain. The increased liver triglyceride concentration due to the CCl_4 treatment was significantly decreased by FF-8 strain and the reduced level reached to that of normal group. Administration of FF-8 strain in normal rat did not show any signs of harmful effects. Therefore, the current findings suggest that FF-8 strain could be an effective antioxidant with no or negligible side-effects and it might be useful for the purpose of protection treatment of hepatotoxicity and oxidative stress in CCl_4-treatment in rat.

Key words: *Saccharomyces cerevisiae* FF-8, glutathione, carbon tetrachloride (CCl_4), hepatotoxicity, rat

Introduction

Liver is one of the largest organs in the body. Those functions of liver, such like filtration of circulating blood and removal and breakdown of toxic substances, are essential and play critical roles in many metabolic processes to maintain organisms alive (1). Various toxigenic materials, such as viruses, chemicals (carbon tetrachloride, D-galactosamine, and orotic acid), alcohol, drugs, microbiological agents, and other xenobiotics, which may contribute to long term tissues damage, can cause and induce liver diseases (2-5). Carbon tetrachloride (CCl_4), especially, is a classical hepatotoxicoid and is able to cause acute- and reversible liver injuries concomitant with centrilobular hydropic degeneration and necrosis (2). CCl_4 is generally metabolized into a reactive metabolite trichloro-methyl radical (CCl_3) by the liver microsomal cytochrome P450 system and it incorporates with O_2 to form trichloromethylperoxy radical (CCl_3O_2) then withdraws allylic hydrogens from polyunsaturated fatty acids to initiate lipid peroxidation (2). Therefore, CCl_4-treated animal, as an animal model of liver injury, would intimately be linked to oxidant stress and cytokine production.

Glutathione in reduced form is a tripeptide containing L-glutamate, L-cysteine, and glycine. Intracellular glutathione is widely involved in oxidation and reduction processes in organisms. Glutathione has widely been used as medicine for the treatment of liver injury and as additives in functional health food and cosmetic industry, thus its commercial demand has been expending (6). Several yeasts contain bio-active compounds, especially, such like glutathione and S-adenosylmethionine has been found to protect hepatic injuries which being induced by alcohol and D-galactosamine (7, 8). Recent studies have well demonstrated that glutathione plays a major role in the detoxification of xenobiotics in animal hepatocytes (6, 9). Sugiyama and Yamamoto (6) reported that the reduced form of glutathione-enriched yeast extract (i.e., 2% oxidized form plus 10.9% reduced form) showed the dose-dependent hepatoprotective effects, but this could not be observed with the low levels of glutathione (only 0.5% oxidized form) contained bread yeast extract. However, some species of yeast strains, such as *Saccharomyces cerevisiae* and *Candida utilis*, contain high concentrations of glutathione (10-12). A previous *in vitro* study with the intercellular glutathione-containing cell free extracts from *S. cerevisiae* FF-8 (13) has also observed antioxidative effects in both YM basal medium and optimal medium.

However, the hepatoprotective effects of the intercellular glutathione-enriched *S. cerevisiae* FF-8 in Korean traditional rice wine on CCl_4-induced hepatotoxicity and oxidative stress in rats are not certain yet. Therefore, the current study has investigated the possible protective effects of orally administrated glutathione-enriched *S. cerevisiae* FF-8 on acute CCl_4-induced hepatotoxicity and oxidative stress model in rats.

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Received May 14, 2007; accepted June 18, 2007

Materials and Methods

Materials Carbon tetrachloride (CCl_4) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of the best commercial grade availability.

Yeast strain and culture conditions Yeast strain used in this study was *S. cerevisiae* FF-8 (a laboratory yeast strain). *S. cerevisiae* FF-8, a high glutathione producing yeast strain, was aerobically cultured in a 5-L flask containing 1 L of the YM optimal medium (3.0% glucose as carbon source, 3.0% yeast extract as nitrogen source, 0.06% KH_2PO_4 as salt source, and 0.06% L-cysteine as precursor amino acid of glutathione; initial pH 6.0) at 30°C under the agitation at 100 rpm for 72 hr. The culture was centrifuged at 7,000×g for 15 min after the incubation then the supernatant was removed and the yeast cells were washed 3 times with distilled water. The harvested yeast cells were lyophilized to prepare experimental diet.

Animal and experimental design Seven-week old male Sprague-Dawley rats were obtained from the Hyochang Science Animals Co. (Daegu, Korea). Animal was housed individually in the suspended wire-mesh stainless steel cage under room temperature between 21 to 24°C and lighting between 08:00 and 20:00. Animals were allowed to access freely to semi-purified basal diet for 2 weeks before the experiment. Animals were then randomly divided into 4 experimental groups (normal group, normal rats fed with basal diet; FF-8 group, normal rats fed with 5% glutathione-enriched *S. cerevisiae* FF-8 contained basal diet; CCl_4 group, CCl_4 treatment rats fed with basal diet; CCl_4 plus FF-8 group, CCl_4 treatment rats fed with 5% glutathione-enriched *S. cerevisiae* FF-8 contained basal diet). The glutathione-enriched *S. cerevisiae* FF-8 was prepared as described above, and an equal amount of FF-8 was replaced with casein when this was added into the diets for FF-8 treatment (Table 1).

Those FF-8 and CCl_4 plus FF-8 groups were pre-treated with 5%(w/w) FF-8 for 6 weeks before the CCl_4 injection. CCl_4 was intraperitoneally injected twice with at 20 μL/kg body weight of each dose during the final 2 days. The rats were killed 12 hr after the final injection. The time interval was determined from the basis of preliminary observations that to be optimum for the assessment of liver injury. Olive oil, instead of CCl_4, was injected to non-CCl_4 treated rats. The rats were not fasted during before and after the injection of CCl_4 or olive oil. Animal care was followed by the National Institute of Health guidelines on the care and use of laboratory animals.

Table 1. Compositions of experimental diets (%)

Group	Normal	FF-8[1)]	CCl_4[2)]	CCl_4 plus FF-8
Casein	20	15	20	15
Corn starch	15	15	15	15
Sucrose	55	55	55	55
Cellulose	5	5	5	5
Corn oil	10	10	10	10
Mineral mixture[3)]	3.5	3.5	3.5	3.5
Vitamine mixture[3)]	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2
DL-Methionine	0.3	0.3	0.3	0.3
FF-8	0	5	0	5

[1)]Glutathione-enriched *Saccharomyces cereviciae* FF-8 strain.
[2)]Carbon tetrachloride.
[3)]AIN 93 G-MX mineral mix, 18 MP Biomedicals, German.

Analytical procedure At the end of the experimental period, the rats were sacrificed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia. The concentrations of total lipid, triglyceride, total-cholesterol, high density lipoprotein (HDL)-cholesterol, and nonesterified fatty acid (NEFA) and, alanine amino-transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in serum were measured by Chemiclinical Chemistry Analyzer in Neodin Medicinal Institute (Seoul, Korea). The hepatic lipids were extracted using the procedure developed by Folch *et al.* (14). The dried lipid residues were dissolved in 1 mL of ethanol for triglyceride assay. The hepatic triglyceride was enzymatically measured by using the commercial kit from Sigma Chemical Co., a modification of the lipase-glycerol phosphate oxidase method (15).

Preparation of liver homogenate fractions The liver was quickly removed, weighed, and eventually used for the estimation of lipid peroxidation. The liver was homogenized in ice-cold 0.25 M sucrose solution containing 10 mM Tris-HCl buffer (pH 7.4) and 1 mM ethylenediamine tetraacetate (EDTA) using with Ika-Ultra-TURRAX T25 basic homogenizer (KG; Ika-Werke Gmbh & Co., Staufen, Germany) as described previously. Protein concentration was measured by the method of Lowry *et al.* (16) and bovine serum albumin was used as protein standard.

Determination of lipid peroxidation (TBARS) The concentrations of lipid peroxidation in hepatic and its subcellular fractions were determined by measuring thiobarbituric acid reactive substances (TBARS) (17). The reaction mixture, containing hepatic homogenate solution, subcellular fractions, and thiobarbituric acid (TBA), was incubated under boiling water for 30 min. After the centrifugation at 1,000×g for 10 min, the light absorbance of the upper layer was measured at 532 nm. The concentrations of TBARS were expressed as nmole of malondialdehyde (MDA) per g liver.

Determination of glutathione concentrations The concentration of glutathione was determined by the method of Beutler *et al.* (18). A 0.2 mL of liver homogenate was mixed well with 1.8 mL of EDTA solution then a 3.0 mL of the precipitating reagent (1.67 g of metaphosphoric acid, 0.2 g of EDTA disodium salt, 30 g NaCl in 1 L of distilled water) was added and mixed thoroughly then stood the mixture at 4°C for 5 min. The mixture was centrifuged at 3,000×g for 5 min and 2 mL of the supernatant was mixed with 4 mL of 0.3 M disodium hydrogen phosphate solution and 0.1 mL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) reagent then the concentration of glutathione was spectrophotometrically determined at

412 nm. Total glutathione concentrations were expressed as nmol per g liver.

Liver histopathological examination Liver was carefully removed and small fragments fixations for histomorphology were prepared with 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.4). The chemically fixed sample was embedded in paraffin then sliced at an approximate 6 μm thick for standard Hematoxylin & Eosin staining as described previously (19). The morphology of any lesions observed was classified and registered at the Anatomy Laboratory in the College of Medicine, Dong-A University, Busan, Korea.

Amino acid analysis Freeze-dried yeast sample (10 mg) was placed into a hydrolysis tube and amino acid composition of the hydrolysate was determined by using amino acid analyzer (model 3A30; Carlo Erba-Fisons, Rodano, Milan, Italy).

Statistical analysis The data from animal experiments are presented as the mean±SEM, and were analyzed using one way analysis of variance (ANOVA), with the differences analyzed using the Duncan's new multiple-range test (20). A p value <0.05 was accepted as being a statistical significance of difference.

Results and Discussion

Body weight and liver weight changes Body weight changes of experimental rats are shown in Table 2. The final body weights were not affected by with glutathione-enriched *S. cerevisiae* FF-8 strain for 6 weeks. Liver is a target organ for CCl_4 toxicity, and its exposure increased liver weight in a dose dependent manner (21). CCl_4 treatment resulted in a significant increase of total liver weight and the ratio between liver and whole body weight (Table 3). Similar results were found in liver weight change as reported by Jeon and Park (22) and suggested that the increase of liver weight was due to the accumulation of lipids in the liver by CCl_4 treatment. The increase was slightly decreased numerically (5.8%) by glutathione-enriched *S. cerevisiae* FF-8 strain but this was not statistically different (Table 3).

Total lipid concentration in serum The concentration of serum total lipid showed a tendency to decrease slightly by glutathione-enriched *S. cerevisiae* FF-8 strain or CCl_4 alone treatment and this was significantly decreased by pretreatment with glutathione-enriched *S. cerevisiae* FF-8 strain in CCl_4-treated rats in comparison to normal rats (Fig. 1). The reduced total lipid might be due to the reduced the levels of triglyceride, total cholesterol, and free fatty acid by the pretreatment of glutathione-enriched *S. cerevisiae* FF-8 strain.

Table 3. Concentrations of total cholesterol, HDL-cholesterol, and free fatty acid of serum in CCl_4 and FF-8 treated rats[1)]

Group	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	Free fatty acid (μEq/L)
Normal	99.83±4.22[a]	24.83±1.17[ab]	591.00±43.65[a]
FF-8[2)]	85.83±4.77[a]	28.67±1.41[a]	366.17±14.83[b]
CCl_4[3)]	87.00±4.95[a]	20.50±1.69[b]	761.00±29.51[c]
CCl_4 plus FF-8	61.50±7.37[b]	20.67±2.54[b]	530.20±23.13[a]

[1)]Values with different letters are significantly different at $p<0.05$ (mean ±SE, n=6).
[2)]The dried powder of glutathione-enriched *S. cereviciae* FF-8 strain.
[3)]Carbon tetrachloride.

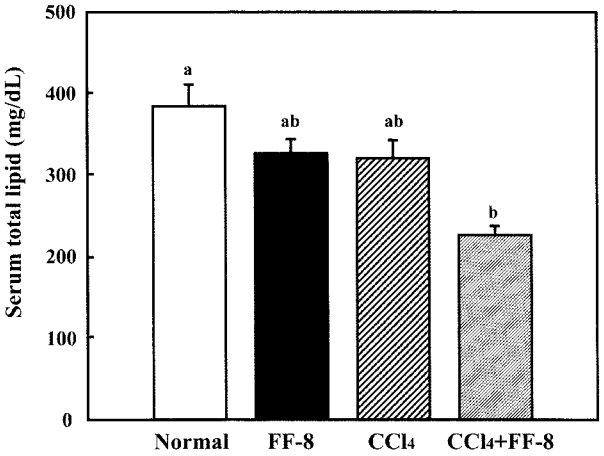

Fig. 1. Concentrations of total lipids of serum in CCl_4 and FF-8 strain treated rats. Values with different letters are significantly different at $p<0.05$ (mean±SE, n=6).

Triglyceride concentrations in serum and liver The concentrations of triglyceride in the serum and liver are shown in Fig. 2. The current study showed that CCl_4 treatment significantly increased the concentration of liver triglyceride and this is in agreement with those findings

Table 2. Body weight changes of CCl_4 and FF-8 treated rats[1)]

Group	Body weight (g)			Liver weight (g)	
	Initial	Final	Changes (6 weeks)	Absolute (g)	Relative (%)
Normal	381.8±9.3	436.4±21.8	54.6±14.40	10.58±0.26	2.45±0.11[a]
FF-8[2)]	381.5±11.8	426.8±13.9	45.4±6.22	10.65±0.46	2.49±0.07[a]
CCl_4[3)]	388.6±7.4	428.0±7.8	39.4±3.02	13.40±0.34	3.15±0.07[b]
CCl_4 plus FF-8	389.9±13.1	432.8±15.9	42.8±4.41	12.77±0.81	2.94±0.10[b]

[1)]Relative liver weight means the % of the liver weight in the body weight; Values with different letters are significantly different at $p<0.05$ (mean ±SE, n=6).
[2)]The dried powder of glutathione-enriched *S. cereviciae* FF-8 strain.
[3)]Carbon tetrachloride.

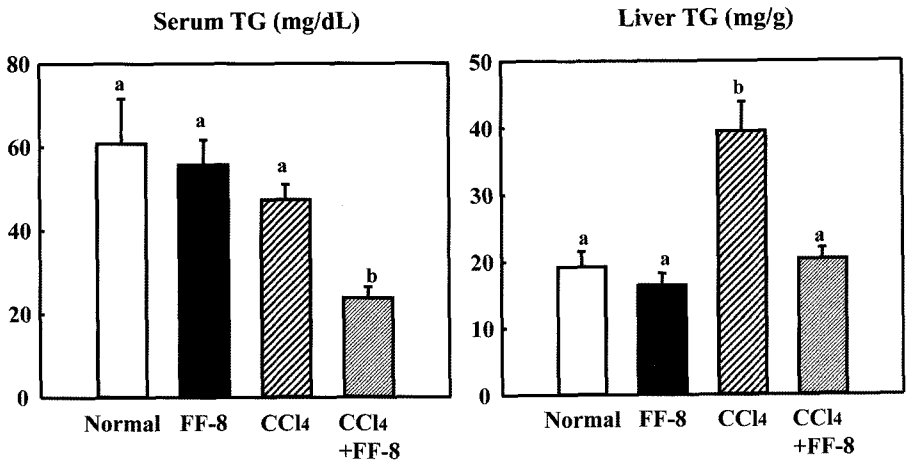

Fig. 2. Concentrations of triglyceride of serum and liver in CCl_4 and FF-8 strain treated rats. Values with different letters are significantly different at $p<0.05$ (mean±SE, n=6).

that CCl_4-induced triglyceride accumulation in the liver resulted in fatty liver (22, 23). However, the increased liver triglyceride concentration due to the CCl_4 treatment was significantly decreased by glutathione-enriched *S. cerevisiae* FF-8 and the reduced level reached to that of normal group. Apparently, somehow, the dietary provision of glutathione-enriched *S. cerevisiae* FF-8 had a suppressive effect on the liver triglyceride accumulation which was induced by CCl_4.

Hepatic triglyceride accumulation is a major complication associated with obesity, insulin resistance, and alcoholic and nonalcoholic fatty liver disease (5, 24). Liver has been generally known to be involved in many lipids and lipoprotein metabolism, and serum triglyceride level has also been known as its close associations with hepatic triglyceride metabolism (24, 25). Hepatic triglyceride level is commonly regulated by such biosynthesis of triglyceride and free fatty acids, the secretion of lipoprotein into the blood stream, and the degradations of fatty acids by β-oxidation (25, 26). Many reports indicate that CCl_4 treatment decreased plasma triglyceride-rich lipoproteins and induces triglyceride accumulation in the liver by apoB-lipoprotein production and microsomal triglyceride transfer protein (MTP) activity (27, 28).

However, the hepatotoxicants-induced lipid accumulations in rat liver were prevented by those antioxidants such as vitamin E (29) and herbal products (22). The present study also demonstrated that high-glutathione in *S. cerevisiae* FF-8 strain, as a member of antioxidants (13), markedly reduced the CCl_4-induced accumulation of liver triglyceride in rats (Fig. 2). The data suggest that triglyceride levels may be reduced to that of normal group with administration of glutathione-enriched *S. cerevisiae* FF-8 strain and may imply a beneficial effect of glutathione-enriched yeast strain.

Treatment of CCl_4 did not influence the concentration of serum triglyceride (Fig. 2) and this is in agreement with the results in the previous study (30). Moreover, the administration of glutathione-enriched *S. cerevisiae* FF-8 strain alone did not also alter the serum triglyceride level compared to the normal rats (Fig. 2). The current study demonstrated a possibility that glutathione-enriched *S. cerevisiae* FF-8 strain would be able to decrease the serum triglyceride concentration by the simultaneous addition of CCl_4.

Cholesterol and free fatty acid concentrations in serum Dietary glutathione-enriched *S. cerevisiae* FF-8 strain did not have any significant effect on serum concentrations of total cholesterol or HDL-cholesterol, but these in CCl_4-treated rats were significantly decreased by glutathione-enriched *S. cerevisiae* FF-8 strain (Table 4). Indeed, likely the FF-8 strain, sole treatment of CCl_4 did also not alter those total cholesterol and HDL-cholesterol levels in the serum and this might be due to the enhanced

Table 4. Concentrations of compositional amino acid of *Saccharomyces cerevisiae* FF-8 strain[1)]

Compositional amino acid	% in sample
Aspartic acid	5.46
Threonine	2.32
Serine	2.46
Glutamic acid	11.52
Proline	1.86
Glycine	2.93
Alanine	3.59
Cystine	-
Valine	5.95
Methionine	-
Isoleucine	3.02
Leucine	4.09
Tyrosine	1.92
Phenylalanine	2.42
Histidine	1.51
Lysine	4.58
Arginine	3.29

[1)]The compositional amino acids in FF-8 yeast strain cells were measured with a amino acid analyzer by using the acid hydrolysis and ninhydrin procedure. Cultivated yeast cells were washed 3 times with sterilized distilled water and then analyzed.

lipid accumulation in the liver and/or other tissues by CCl_4. Any clear evidence for the possibility was not observed in the current experiment but those numerical changes in comparison to the normal group, although this was not statistically significant, would suggest the idea. If the putative possibility was correct, those decreased total- and HDL-cholesterol levels in the serum in response to the simultaneous treatment of FF-8 and CCl_4 might be reasonable because CCl_4 was able to reduce lipids release from the liver and FF-8 was able to decrease lipid accumulations in the liver and tissues.

The concentration of serum free fatty acid was remarkably elevated by CCl_4 treatment in comparison to the normal, but this was draw back to the normal level by the pre-treatment of glutathione-enriched *S. cerevisiae* FF-8 strain although CCl_4 was treated (Table 4). The decreased serum fatty acids level by FF-8 in the CCl_4-treated rat was not lower than that in the sole FF-8-treated animal ($p<0.05$) and this may indicate that FF-8 itself has an effect to reduce serum fatty acid level (Table 4).

ALT and AST activities CCl_4-induced hepatic injury is a common method to screen hepato-protective medicine and functional health foods effects (22, 23). Previous reports (30, 32) found that CCl_4 increased those activities of ALT, AST, ALP, and LDH in the serum significantly in both dose- and time-dependent manners. These enzymes are generally well-documented as indicators of hepatic dysfunction, likely these increased AST and ALT activities reflect impaired liver functions (31), thus several hepatic enzymes in the serum can be used as the biochemical markers of early acute hepatic damage. The current study observed that marker enzymes of ALT, AST, and LDH in the serum were increased in the CCl_4-treated groups, but ALT activity was significantly decreased as glutathione-enriched *S. cerevisiae* FF-8 strain was treated (Fig. 3). The results of present study demonstrated that pretreatment of rats with glutathione-enriched *S. cerevisiae* FF-8 strain had a markedly protective effect against CCl_4-induced hepatotoxicity, as evidenced by decreased serum ALT.

Sugiyama and Yamamoto (6) reported that the treatment with reduced glutathione-enriched extracts from *S. cerevisiae* showed dose-dependent hepatoprotective effects which were associated with decreased in serum AST and ALT activities and recovery to the liver glutathione levels in a model of acute hepatotoxicity induced by a high intraperitoneal dose of acetaminophen in rats, but hepatoprotective effect was not obtained using bread yeast extract that containing only low levels of glutathione. These observations indicated that the glutathione-enriched *S. cerevisiae* FF-8 strain possibly produced hepatoprotective substance, glutathione, against acute hepatotoxicity induced chemicals such as CCl_4 and acetaminophen.

LDH and ALP activities CCl_4-induced hepatic injury was also characterized by the increases of serum LDH and ALP activities (32). Several studies have also indicated the elevation of LDH and ALP levels in the serum by CCl_4 would accurately be reflected as hepatic injury (22, 33).

The LDH activity was significantly increased in response to the CCl_4 treatment but this was significantly decreased by glutathione-enriched *S. cerevisiae* FF-8 strain, although the reduced level was not as low as the level of normal rat (Fig. 4). There was no statistical difference of LDH activity between the normal and the sole glutathione-enriched *S. cerevisiae* FF-8 strain fed rats. This indicates that glutathione-enriched *S. cerevisiae* FF-8 strain may have a powerful hepatoprotective activity, at least, especially on the elevated LDH level.

However, there was no significant difference in those serum ALP activities among the experimental groups except glutathione-enriched *S. cerevisiae* FF-8 strain showed a slightly decreased tendency of serum ALP activity.

Liver TBARS and glutathione concentrations CCl_4 has been used extensively to induce rapid liver damage and oxidative stress of experimental animal model (34). TBARS determination in tissues has been used as an assay of liver peroxidides. Zhu and Fung (35) found the TBARS level in the liver homogenate was significantly increased by CCl_4 in dose- and time-dependent manners. The level of TBARS in liver homogenate, likely those hepatic enzymes such as ALT and AST in the serum, was

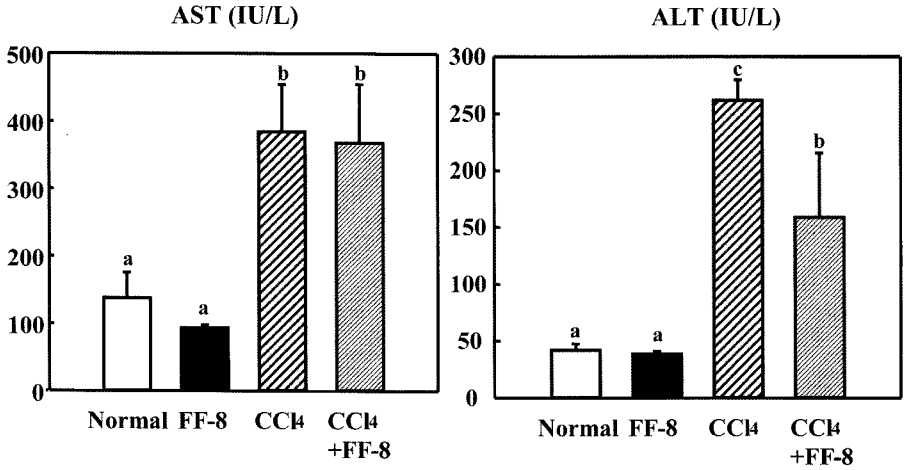

Fig. 3. Activities of serum AST and ALT in CCl_4 and FF-8 strain treated rats. Values with different letters are significantly different at $p<0.05$ (mean±SE, n=6).

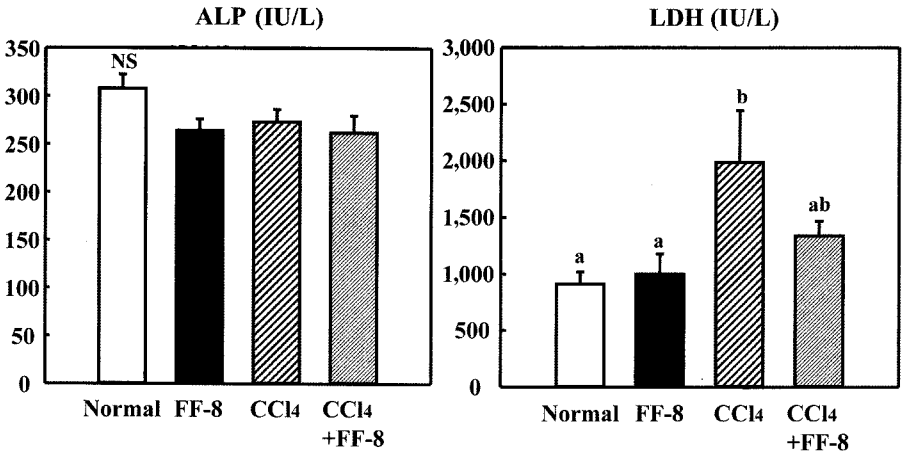

Fig. 4. Activities of serum ALP and LDH in CCl_4 and FF-8 strain treated rats. Values with different letters are significantly different at $p<0.05$ (mean±SE, n=6).

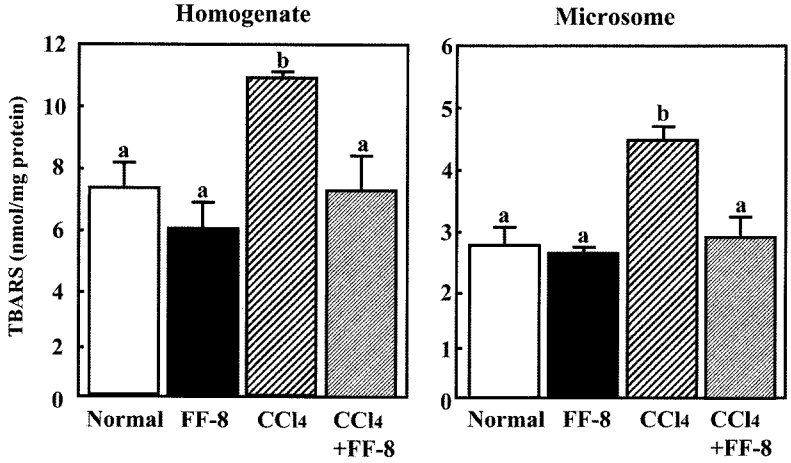

Fig. 5. Concentrations of TBARS of liver homogenate and microsomal fractions in CCl_4 and FF-8 strain treated rats. Values with different letters are significantly different at $p<0.05$ (mean±SE, n=6).

used as the biochemical markers for early acute hepatic damage (35, 36). The current study observed significant elevations of the thiobarbituric acid reactive sbustances (TBARS), as the lipid peroxidation of the liver homogenate fractions, in the CCl_4 treated rats compared with normal rats (Fig. 5). Administration of high-glutathione *S. cerevisiae* FF-8 significantly inhibited lipid peroxidation of liver homogenate fractions in the CCl_4 treatment rats. The result suggest that high-glutathione *S. cerevisiae* FF-8 would be useful for the treatment of hepatotoxicity and oxidative stress of which induced by CCl_4-treatment in rat.

Many studies have found glutathione is an antioxidant and high concentration of glutathione is contained in yeast strains (10, 37). Glutathione from microorganisms have been found to inhibit those chemicals (i.e., ethanol and acetaminophen)-induced oxidant stresses *in vitro* and *in vivo* (6, 13, 38). It have also been observed that glutathione level is closely associated with antioxidant system to prevent cells from those toxic effects of lipid peroxidation, reactive free radicals, and other oxidant species (7, 39). The hepatic concentration of glutathione in the current glutathione-enriched *S. cerevisiae* FF-8 strain fed animal was approximately twice as high as the normal

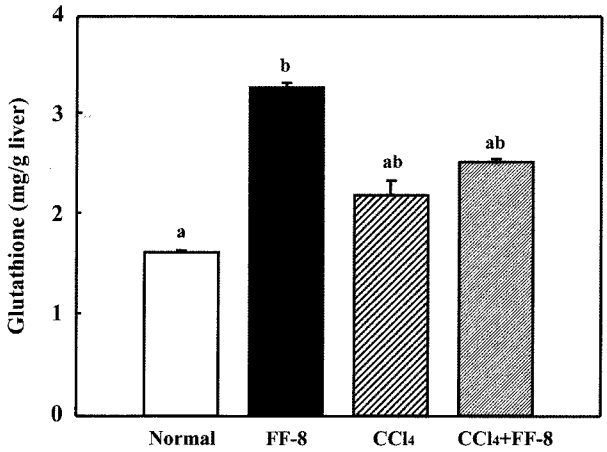

Fig. 6. Concentrations of liver glutathione in CCl_4 and FF-8 strain treated rats. Values with different letters are significantly different at $p<0.05$ (mean±SE, n=6).

(Fig. 6).

A study with rats caused hepatic damage by

acetaminophen found that glutathione-enriched yeast extracts to be effective for the protection of liver from hepatic damage dose-dependently and suggested the provision of precursors for glutathione biosynthesis in the liver would derive hepatoprotective effects (6). The CCl_4 did not reduce the hepatic glutathione level in the current study (Fig. 6). However, the hepatic glutathione level in CCl_4+FF-8 group was slightly higher than that of CCl_4 group without statistically significant. The liver protected effects of FF-8 may also be due to the level of its contents like amino acids. Recent reports have indicated that methionine or cysteine as precursors of glutathione metabolism in the liver played key roles in the intercellular glutathione synthesis (40, 41), and the administration of cysteine and cysteine-containing compounds increased glutathione concentrations in the liver and kidney (42), thus glutathione production in the liver might be regulated by the availability of cysteine or methionine.

A recent study (43) with silk fibroin showed liver protective effects from the alcohol-induced hepatotoxicity and there were 42% of glycine and 32% of alanine in the silk fibroin. Some kinds of dietary amino acids, such as serine, glycine, aspargine, histidine, and tyrosine were also effective to protect the galactosamine- and alcohol-induced liver injury (8, 44, 45). The compositions of free- and constitutional-amino acids in the current *S. cerevisiae* FF-8 strain were mainly glutamic acid (11.51%), aspartic acid (5.46%), arginine (3.20%), alanine (3.59%), and glycine (2.93%) based on dry matter (Table 4), and this suggested that high concentration of these amino acids in *S. cerevisiae* FF-8 strain would be able to inhibit the CCl_4-induced oxidant stress.

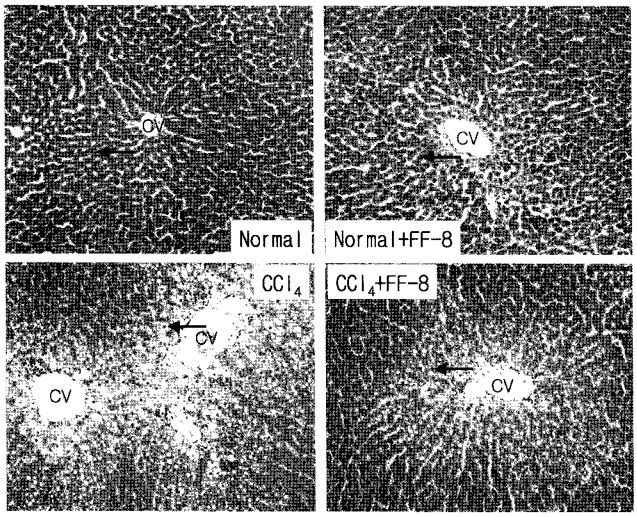

Fig. 7. Hepatic histopathologic changes in the normal rats treated with olive oil alone (normal group), the glutathione-enriched *S. cerevisiae* FF-8 strain feeding rats treated with olive oil alone (FF-8 group), the rats treated CCl_4 dissolved within olive oil (CCl_4 group) and glutathione-enriched *S. cerevisiae* FF-8 strain in CCl_4 treatment rats (CCl_4+FF-8 group).

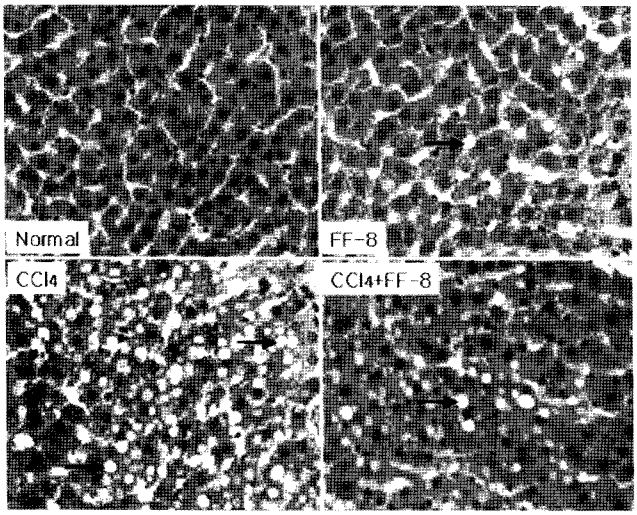

Fig. 8. Effect of glutathione-enriched *S. cerevisiae* FF-8 strain on hepatic fatty changes in CCl_4 treatment rats (hematoxylin and eosin stain, right 200× and left 400×). FF-8, glutathione-enriched *S. cerevisiae* FF-8 strain; CCl_4, carbon tetrachloride; CCl_4+FF-8, CCl_4 +glutathione-enriched *Saccharomyces cerevisiae* FF-8 strain. Arrow: fatty drops.

Liver histopathological examination CCl_4 has been widely used as one of hepatic toxicate to induce hepatic fibrosis and cirrhosis in laboratory animal models, and the morphologic and pathophysiologic results showed high similarities in human fibrosis (21). The current observations demonstrated that glutathione-enriched *S. cerevisiae* FF-8 strain effectively protected liver from the CCl_4-induced hepatotoxicity by decreasing those serum ALT and AST activities and lipid peroxidation. The evidential histological observations are also represented in Fig. 7 and 8. CCl_4 induced the marked hepatocytes necrosis with mild degeneration and inflammation around the central veins, and the steatosis with the formulation of lipidic intercytoplasmic vascuoles (Fig. 7). The lipid droplets in the hepatocytes were increased in numbers and volumes as the fatty liver progressions (5) and this led the hepatomegaly accompanied with liver weight increase. The lipid droplets observed in the current study with CCl_4 treatment (Fig. 7) was in agreement with the previous reports (28). However, the current dietary glutathione-enriched *S. cerevisiae* FF-8 administration attenuated the CCl_4-induced hepatocyte necrosis and fatty accumulation in the liver (Fig. 8).

In summary, this study shows that the glutathione-enriched *S. cerevisiae* FF-8 isolated from Korea traditional rice wine prevents CCl_4-induced hepatotoxicity and oxidative stress in rats.

Acknowledgments

This study was supported by the Dong-A University research fund in 2007. We acknowledge the financial support of the Brain Korea 21 Project, Dong-A University, in the form of scholarships to Sang-Hyun Park.

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