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Original Article

The protective effects of a fermented substance from *Saccharomyces cerevisiae* on carbon tetrachloride-induced liver damage in rats

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SUMMARY

Background & aims: The aim of this study was to investigate the effect of a fermented substance from *Saccharomyces cerevisiae* (FSSC) on the liver fibrosis induced by chronic carbon tetrachloride (CCl_4) administration in rats.

Methods: Rats were divided randomly into four groups: control, CCl_4, and two FSSC groups. Except for rats in the control group, all rats were orally administered CCl_4 twice a week for 8 weeks. Rats in the FSSC groups were treated daily with FSSC (0.5 or 1.5 g/kg) through gastrogavage for the entire experimental period.

Results: CCl_4 caused liver damage, as characterized by increases in levels of plasma transaminase, hepatic malondialdehyde, and hydroxyproline, in addition to increases in spleen and liver weights and decreases in plasma albumin levels. Compared with CCl_4 group, FSSC (1.5 g/kg) treatment significantly decreased the spleen ($P < 0.01$) and liver ($P < 0.01$) weights, the activities of transaminase ($P < 0.05$), and levels of hepatic malondialdehyde ($P < 0.05$) and hydroxyproline ($P < 0.01$); however, the treatment increased plasma albumin level ($P < 0.05$). The pathological results also showed that FSSC (1.5 g/kg) suppressed hepatic inflammation, steatosis and necrosis. Data for hepatic fibrosis were expressed as the mean percentage of the total hepatic area in the tissue sections. FSSC (1.5 g/kg) treatment significantly decreased the hepatic fibrosis (12.8 ± 1.2 and 6.4 ± 0.7 in CCl_4 and FSSC group, respectively, $P < 0.001$). RT-PCR analysis showed that FSSC (1.5 g/kg) treatment decreased the expression of *methionine adenosyltransferase 2A* ($P < 0.01$), *collagen (α1)(I)* (3.15 ± 0.05 and 1.52 ± 0.04 in CCl_4 and FSSC groups, respectively, $P < 0.001$), and *transforming growth factor-β1* (2.50 ± 0.05 and 1.21 ± 0.04 in CCl_4 and FSSC groups, respectively, $P < 0.001$), apart from increasing the expression of *methionine adenosyltransferase 1A* ($P < 0.05$).

Conclusion: These results showed that FSSC protects the liver against CCl_4 damage in rats.

1. Introduction

The tripeptide glutathione (GSH) is the chief antioxidant and redox regulator and thus an important preventive agent for many diseases, including liver disease.[1] Cirrhotic patients show a significant decrease in hepatic GSH content and levels of plasma thiol-related compounds, including GSH and cysteine.[2,3] In animal experiments, administration of L-cysteine has been shown to prevent liver fibrosis.[4] Some studies also show that GSH and γ-glutamylcysteine, which is a precursor of GSH, can prevent the liver injury induced by carbon tetrachloride.[5,6] These indicate that thio-compounds are important regulators in the process of liver fibrosis development.

Oxidative stress has been implicated in the process of liver fibrogenesis and many etiological agents of fibrogenesis stimulate free-radical reactions. Consequently, antioxidants have emerged as potent antifibrotic agents.[7] There is evidence indicating that mineral elements, such as Zn and Se, are decreased in patients with cirrhosis.[8] Some studies also show that Zn and Se have hepatoprotective activities.[9,10]

Saccharomyces cerevisiae is a very important cell factory. The well-established fermentation process technology for large-scale production using *S. cerevisiae* makes this organism an attractive

Abbreviations: CCl_4, carbon tetrachloride; GSH, glutathione; GSH-Px, glutathione peroxidase; FSSC, fermented substance from *Saccharomyces cerevisiae*; LPO, lipid peroxidation; MAT1A, methionine adenosyltransferase 1A; MAT2A, methionine adenosyltransferase 2A; MDA, malondialdehyde; PCR, polymerase chain reaction; SOD, superoxide dismutase; TGF-β1, transforming growth factor-β1.

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resource for several biotechnological purposes. *S. cerevisiae* has been used to produce GSH on an industrial scale.[11] In addition to GSH, *S. cerevisiae* also produces GSH-related thiol compounds (e.g., γ-glutamylcysteine, L-cysteine), antioxidants, and some mineral elements, such as Zn and Se.[12–16]

In the laboratory of the authors, *S. cerevisiae* YA03083, a strain modified from the parent strain *S. cerevisiae* BCRC21727 to produce high levels of GSH, has been screened. As mentioned earlier, the fermented material from *S. cerevisiae* YA03083 (FSSC) contained, γ-glutamylcysteine, L-cysteine, Zn, Se, and antioxidants, in addition to GSH. In a previous report by the authors, FSSC has been shown to inhibit the acute hepatitis induced by acetaminophen.[17] In the present study, it has been examined whether FSSC can ameliorate CCl_4-induced chronic liver fibrosis in rats.

2. Materials and methods

2.1. Preparation of FSSC

The yeast strain was provided by the Food Industry Research and Development Institute (Hsinchu, Taiwan). The baker's yeast used in this culture was *S. cerevisiae* YA03083, a high GSH-producing strain that was selected after modification from the parent strain *S. cerevisiae* BCRC21727. Yeast-malt agar and broth were used as the stock culture and seed-culture medium, respectively. A solution that contained sugarcane molasses (110 g/l), soybean hydrolyzate (30 g/l), and mineral components $(NH_4)_2SO_4$, 4 g/l, KH_2PO_4, 6 g/l, and $MgSO_4 \cdot 7H_2O$, 1 g/l, was used as the main cultivation medium. The fermenter was progressively fed with the feeding medium, which contained glucose, sugarcane molasses, yeast powder, soybean powder, a few minerals, and amino acids. Originally, the experiments were carried out in a 5-l fermenter with a 2-l working volume in feed-batch cultivation. For this experiment, 1 l of feeding medium was prepared. Following a 50-h incubation, the cells were harvested by centrifugation and disrupted with a bead-mill agitator. Ground cell powder (FSSC) was obtained after spray-drying. The FSSC was stored at 4 °C for further study.

2.2. Composition of FSSC

The content of GSH and GSH-related thiol compounds, such as L-cysteine, oxidized glutathione and γ-glutmylcysteine, in FSSC was determined using HPLC. The HPLC conditions were the same as in the previous study.[17] The L-cysteine, GSH, oxidized glutathione, and γ-glutamylcysteine peaks were identified by a comparison of the retention times with a standard. The standards of L-cysteine, GSH, oxidized glutathione, and γ-glutamylcysteine were obtained from Merck KGaA (Darmstadt, Germany). The respective peak areas were used for quantitative estimation. HPLC analysis showed that the L-cysteine, GSH, oxidized glutathione, and γ-glutamylcysteine fractions of FSSC were approximately 3, 35, 1.9, and 20 mg/g, respectively.

The superoxide dismutase-resembling activity of FSSC was assayed according to the method of Robak and Gryglewski.[18] In brief, 1 ml methanol extract of FSSC was mixed with 1 ml of 120 μM phenazine methosulfate, 1 ml of 936 μM dihydronicotinamide adenine dinucleotide, and 1 ml of 300 μM nitro blue tetrazolium solution. After mixing and storing statically for 5 min, the optical density at 560 nm was determined. The percentage of superoxide scavenging by gallic acid (1 mg/ml; positive control) and FSSC (1 mg/ml) was 84.7 and 50.1, respectively.

The contents of Zn (21.66 ppm) and Se (1.76 ppm) in FSSC were measured according to the AOAC guidelines (999.11 and 986.15, respectively) using atomic absorptiometry (Perkin Elmer 6000, Norwalk, CT, USA).

2.3. Animals

Male Wistar rats were obtained from the National Laboratory Animal Breeding and Research Center, National Science Council (Taiwan), and fed with a standard laboratory diet and water ad libitum. The experimental animals were housed in an air-conditioned room at 22 ± 2 °C with a 12-h light/dark cycle. When the rats attained a weight of 240–260 g, 40 rats were divided randomly into four groups (containing 10 rats per group), such as control, model, and two FSSC treatment groups, according to their body weight 1 day before the administration of the test substance. All the animal experiments were conducted in accordance with the guidelines established by the Animal Care and Use Committee of China Medical University and were approved by this committee.

2.4. CCl_4-induced liver fibrosis

Liver fibrosis was induced by the oral administration of 2 ml/kg of CCl_4 (20%; diluted in olive oil) twice a week for 8 weeks. Animals received CCl_4 with distilled water (10 ml/kg, p.o., daily) and CCl_4 with FSSC (0.5 and 1.5 g/kg, p.o., daily) throughout the whole experimental period. During CCl_4 administration, the time-interval between the administrations of CCl_4 and FSSC was at least 5 h to avoid disturbance of the absorption of each substance. Rats in all groups were killed 8 weeks after CCl_4 treatment. Rats were sacrificed under ether anesthesia and blood was withdrawn from the abdominal artery. The liver was quickly removed, washed with cold normal saline, blotted dry, and weighed. The livers were divided into four parts, which were subsequently treated as follows: (i) submerged in 10% neutral formalin for preparation of sections for pathological studies; (ii) after weighing, completely dried at 100 °C for the determination of collagen content; (iii) kept in liquid nitrogen for RT-PCR analyses; (iv) stored at −80 °C in reserve.

2.5. Assessment of liver functions

Blood samples were centrifuged at 4700 rpm at 4 °C for 15 min to separate plasma. The plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin were assayed using clinical test kits (Roche Diagnostics, Mannheim, Germany) for spectrophotometric determination (Cobas Mira Plus, Roche, Rotkreuz, Switzerland).

2.6. Assays of hepatic lipid peroxidation and GSH content

Liver specimens were homogenized in nine volumes of ice-cold 0.15 M KCl and 1.9 mM EDTA. The homogenate was used for the determination of lipid peroxidation (LPO) levels, according to the method of Ohkawa et al., using 2-thiobarbituric acid.[19] LPO was expressed as the amount of malondialdehyde (MDA) per milligram of protein. Protein was measured by the method of Lowry et al., with bovine serum albumin as the standard.[20] The GSH in liver homogenates was assayed by measuring the fluorescence produced by O-phthaldehyde at 420 nm, following the method of Hissin and Hilf.[21]

2.7. Assay of hepatic hydroxyproline

Hydroxyproline determination was carried out according to a method designed by Neuman and Logan.[22] Dried liver tissue, after hydrolysis with 6 N HCl, was oxidized with H_2O_2 and the colored product obtained by reaction with p-dimethylaminobenzoaldehyde was estimated by its absorbance at 540 nm.

2.8. Measurement of hepatic superoxide dismutase, GSH peroxidase, and catalase activities

Livers were homogenized in nine volumes of ice-cold buffer (0.32 mol/l sucrose, 1 mmol/l EDTA, and 10 nmol/l Tris–HCl, pH 7.4).

The liver homogenate was centrifuged at 13,600 × g for 30 min at 4 °C. The supernatant was used for the assay of activities of superoxide dismutase (SOD), GSH peroxidase (GSH-Px), and catalase.

SOD activity was determined by using xanthine and xanthine oxidase to generate superoxide radicals, which subsequently reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. SOD activity was measured by the degree of inhibition of this reaction (Ransod; Randox Laboratory, Crumlin, UK).

GSH-Px activity was measured using commercially available kits (Ransel; Randox Laboratory). GSH-Px catalyzes the oxidation of GSH by cumene hydroperoxide. In the presence of GSH-reductase and NADPH, oxidized GSH is converted to its reduced form, with the concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance was measured at 340 nm.

Catalase activity was assayed by the method of Aebi.[23] After 0.2 ml of the homogenate was added to 1.2 ml of 50 mM phosphate buffer (pH = 7.0), the reaction was started by the addition of 1.0 ml of 30 mM H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30-s intervals for 3 min. One unit (U) of this enzyme activity is defined as the amount of the enzyme yielding a value of $K = 1$, where K is the rate constant of the enzyme. Activity is expressed as U per mg of protein (U/mg protein).

2.9. RT-PCR analysis

Total RNA was isolated from rat livers using the acid guanidinium thiocyanate phenol–chloroform extraction method, as described by Chomczynski and Sacchi.[24] Total RNA (5 μg) from each liver sample was subjected to reverse transcription using moloney murine leukemia virus reverse transcriptase in a 50-μl reaction volume. Aliquots of the reverse transcription mix were used for amplification of fragments specific to *methionine adenosyltransferase 1A* (*MAT1A*), *MAT2A*, *collagen (α1)(I),* and *transforming growth factor-β1* (*TGF-β1*) by the polymerase chain reaction (PCR) using the primer pairs listed in Table 1. The levels of expression of all the transcripts were normalized to that of *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) mRNA in the same tissue samples. The primer pairs were designed using the Primer select program (Primer 3). The identities of the resulting PCR products were confirmed by sequence analysis. The PCR products were run on a 2% agarose gel, recorded on Polaroid film, and the bands quantitated by densitometry.

2.10. Pathological examinations

After formalin fixation, the tissue samples were sliced, embedded in a standard manner, and stained with hematoxylin–eosin or Sirius Red. Fibrosis was quantified by a computerized image-analysis system (Image-Pro Plus version 5.1; Media Cybernetics, MD, USA). Data for fibrosis were expressed as the mean percentage of the total hepatic area in the tissue sections.

Table 1
Primer sequences for PCR amplification.

mRNA	Primer sequence	Length (bp)
MAT1A	Sense 5′ CGG TAG GAG AAG GGC ATC C 3′ Antisense 5′ GGG ACT GTT GCT CCA GAG CC 3′	174
MAT2A	Sense 5′ GCA TCT GCG CCC TCC GCA GT 3′ Antisense 5′ GTG ACT GTT GTT CCA AGG CA 3′	420
Collagen α1(I)	Sense 5′ GGT CCC AAA GGT GCT GAT GG 3′ Antisense 5′ GAC CAG CCT CAC CAC GGT CT 3′	182
TGF-β1	Sense 5′ TAT AGC AAC AAT TCC TGG CG 3′ Antisense 5′ TGC TGT CAC AGG AGC AGTG 3′	162
GAPDH	Sense 5′ TGT GTC CGT CGT GGA TCT GA 3′ Antisense 5′ CCT GCT TCA CCA CCT TCT TGA 3	76

Table 2
Effect of FSSC on body weight and weight of liver and spleen in CCl_4-treated rats.

Treatments	Doses (g/kg)	Body weight (g)	Liver (g)	Spleen (g)
Control	–	455.1 ± 10.2	14.9 ± 0.5	1.0 ± 0.0
$CCl_4 + H_2O$	–	407.1 ± 10.7$^{###}$	23.1 ± 0.8$^{###}$	2.0 ± 0.2$^{###}$
CCl_4 + FSSC	1.5	401.3 ± 9.5	19.5 ± 1.3*	1.4 ± 0.1**
	0.5	401.0 ± 7.6	20.8 ± 0.4	1.7 ± 0.1

All values are means ± S.E. ($n = 10$). $^{###}P < 0.001$ compared with the control group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the $CCl_4 + H_2O$ group.

2.11. Statistical analysis

Results are expressed as the mean ± SE. All experimental data were analyzed by the one-way analysis of variance using Dunnett's test. A P- value < 0.05 was considered as statistically significant.

3. Results

3.1. Body weight and weights of liver and spleen

Treatment with CCl_4 caused a significant decrease in the body weight of treated rats, compared with control rats. There were no differences in the body weights of rats in the [$CCl_4 + H_2O$] and [CCl_4 + FSSC] groups (Table 2).

CCl_4 treatment obviously caused both hepatomegaly and splenomegaly in the rats, with liver and spleen weights in the [$CCl_4 + H_2O$] group being approximately about 155% and 200% in comparison to the control group, respectively (Table 2). In contrast, FSSC (1.5 g/kg) significantly reduced the increase in weights of liver and spleen that had been induced by CCl_4 (Table 2).

3.2. Effects of FSSC on biochemical parameters

As shown in Table 3, CCl_4 treatment resulted in a significant increase in the plasma AST and ALT activities, compared to the control group. Oral administration of FSSC (1.5 g/kg) significantly reduced the CCl_4-induced increase in AST and ALT activities. The plasma albumin concentration in the [$CCl_4 + H_2O$] group was significantly lower than that in the control group. The CCl_4-induced decrease in plasma albumin concentration was significantly reversed following the administration of FSSC (1.5 g/kg) (Table 3).

3.2.1. Hepatic GSH, MDA, and hydroxyproline concentrations

After CCl_4 treatment, the GSH content in liver decreased by as much as 48.4% relative to the controls (Table 4). FSSC (1.5 mg/kg) treatment reduced the depletion in hepatic GSH content induced by CCl_4 (Table 4).

CCl_4 produced liver fibrosis in rats, accompanied by an approximately 223% and 177% increase of hepatic MDA and

Table 3
Effect of FSSC on the activities of plasma AST, ALT and albumin concentration in CCl_4-treated rats.

Drugs	Doses (g/kg)	AST (U/L)	ALT (U/L)	Albumin (g/dL)
Control	–	69 ± 4	40 ± 3	3.5 ± 0.0
$CCl_4 + H_2O$	–	1857 ± 238$^{###}$	1866 ± 247$^{###}$	2.5 ± 0.1$^{###}$
CCl_4 + FSSC	1.5	1194 ± 114*	1195 ± 104*	2.9 ± 0.1*
	0.5	1459 ± 110	13639 ± 143	2.7 ± 0.1

All values are means ± S.E. ($n = 10$). $^{###}P < 0.001$ compared with the control group. $^{*}P < 0.05$ compared with the $CCl_4 + H_2O$ group.
AST, aspartate aminotransferase; ALT, alanine aminotransferase

Table 4
Effect of FSSC on hepatic glutathione, malondialdehyde and hydroxyproline contents in CCl_4-treated rats.

Treatments	Doses (g/kg)	GSH (nmol/mg protein)	MDA (nmol/mg protein)	Hydroxyproline (μg/g tissue)
Control	–	15.7 ± 2.9	1.7 ± 0.1	590.5 ± 38.4
$CCl_4 + H_2O$	–	$8.1 \pm 1.1^{\#}$	$3.8 \pm 0.5^{\#\#\#}$	$1048.0 \pm 80.6^{\#\#\#}$
CCl_4 + FSSC	15	$14.6 \pm 1.3^{*}$	$2.6 \pm 0.1^{*}$	$799.8 \pm 51.6^{**}$
	0.5	11.0 ± 1.1	2.8 ± 0.2	865.8 ± 41.8

All values are means ± S.E. (n = 10). $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$compared with control group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with $CCl_4 + H_2O$ group.
GSH, glutathione; MDA, malondialdehyde;

hydroxyproline, respectively, compared to controls. Administration of FSSC (1.5 g/kg) significantly decreased the hepatic levels of MDA and hydroxyproline, down to 68% and 76%, respectively, compared to the [$CCl_4 + H_2O$] group (Table 4).

3.2.2. Hepatic SOD, GSH-Px, and catalase activities

Hepatic SOD, GSH-Px, and catalase activities were significantly lower in the [$CCl_4 + H_2O$] group than in the control group. In CCl_4-treated rats, FSSC treatment did not affect the activities of SOD, GSH-Px, and catalase. The activities of the enzymes in the control and [$CCl_4 + H_2O$] groups were as follows: 26.2 ± 0.4 and 3.6 ± 0.4 U/mg protein for SOD ($P < 0.001$); 984.7 ± 41.6 and 719.9 ± 26.7 U/mg protein for GSH-Px ($P < 0.001$), and 25.9 ± 1.2 and 21.1 ± 1.4 U/mg protein for catalase ($P < 0.01$), respectively.

3.3. RT-PCR analysis of liver tissue

Fragments specific to *MAT1A* and *MAT2A* (Fig. 1A) were amplified by RT-PCR. Results of the densitometric analysis, after normalization against the corresponding *GAPDH* transcript, are reported as *MAT1A/GAPDH* (Fig. 1B) and *MAT2A/GAPDH* (Fig. 1C). *MAT1A* was expressed in livers of control rats. The *MAT1A/GAPDH* ratio in the [$CCl_4 + H_2O$] group was 20% lower than that in the control group. Treatment with FSSC (1.5 and 0.5 g/kg) caused 138% and 125% increase in the *MAT1A* expression compared to the [$CCl_4 + H_2O$] groups, respectively. *MAT2A* was not expressed in the livers of control rats. The [$CCl_4 + H_2O$] group had a 350% higher *MAT2A/GAPDH* ratio compared to the control group. Treatment with FSSC (1.5 g/kg) effectively decreased *MAT2A* levels to 68% of the mean ratio for the [$CCl_4 + H_2O$] group.

Fragments specific to *collagen (α1)(I)* and *TGF-β1* (Fig. 2A) were amplified by RT-PCR. Results of the densitometric analysis, after normalization against the corresponding *GAPDH* transcript, are reported as *collagen (α1)(I)/GAPDH* (Fig. 2B) and *TGF-β1/GAPDH* (Fig. 2C). Very low levels of expression of both *collagen (α1)(I)* and *TGF-β1* were detected in the control rats. The *collagen (α1)(I)/GAPDH* and *TGF-β1/GAPDH* ratios in the [$CCl_4 + H_2O$] group were 310% and 250% higher than in the control group, respectively. Treatment with FSSC (1.5 g/kg) caused a 51% and 52% decrease of *collagen (α1)(I)* and *TGF-β1* expression, respectively.

3.4. Pathological changes

CCl_4 administration caused liver morphological changes, evidenced by marked inflammation, fatty deposition, and necrosis (Fig. 3B). FSSC (1.5 g/kg) significantly reduced the CCl_4-induced steatosis, inflammation, and necrosis (Fig. 3C). As shown in Fig. 4, CCl_4 produced liver fibrosis in rats; Sirius Red staining showed clear nodular fibrosis (Fig. 4B). Treatment with FSSC (1.5 g/kg) showed a marked improvement in the pathological alterations to these tissues (Fig. 4C and 4E).

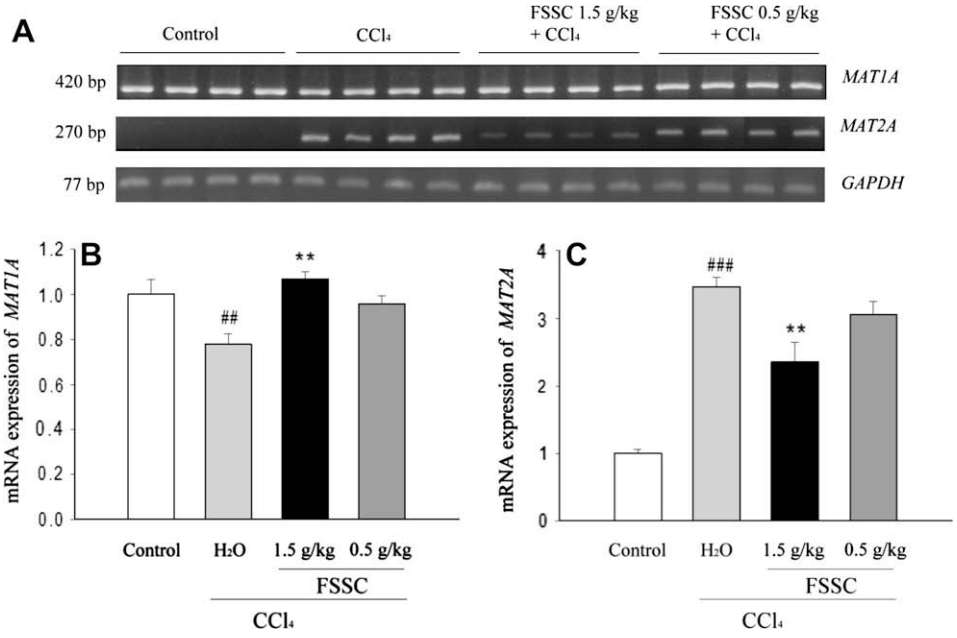

Fig. 1. Effect of FSSC on the hepatic mRNA expressions of *methionine adenosyltransferase 1A* (*MAT1A*) and *methionine adenosyltransferase 2A* (*MAT2A*) in CCl_4-treated rats. (A) RT-PCR analysis of *MAT1A* and *MAT2A* expression in control rats, in CCl_4-treated rats, and in rats treated with CCl_4 + FSSC (1.5 or 0.5 g/kg). Expression of the amplified fragment corresponding to GAPDH is shown for comparison. (B) Densitometric analyses of *MAT1A* expression after normalization against *GAPDH*. (C) Densitometric analyses of *MAT2A* expression after normalization against *GAPDH*. Data are expressed as the mean of ratio ± S.E. ($n = 10$). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the control group. $^{**}P < 0.01$ compared with the $CCl_4 + H_2O$ group.

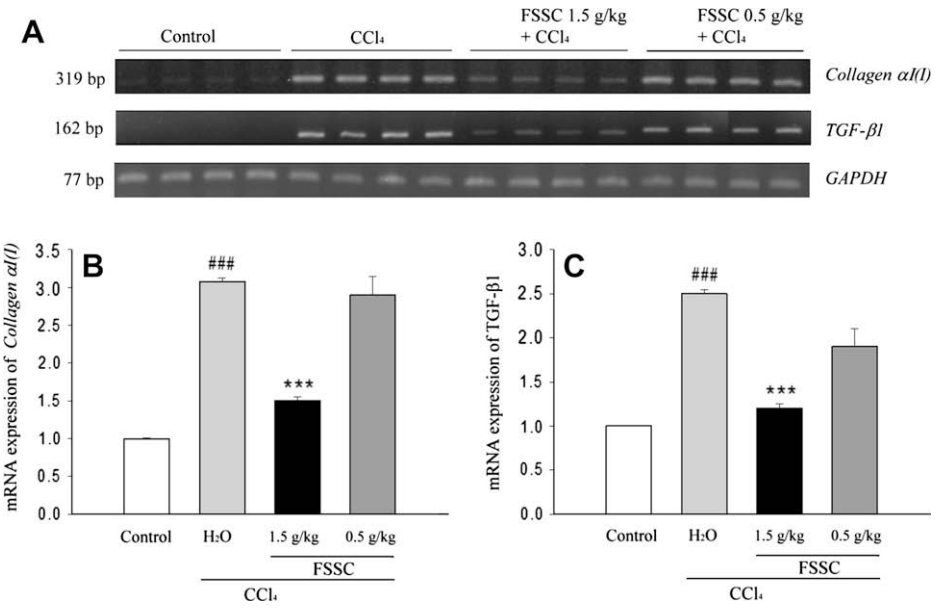

Fig. 2. Effect of FSSC on the hepatic mRNA expressions of *collagen (α1)(1)* and *transforming growth factor-β1* (*TGF-β1*) in CCl_4-treated rats. (A) RT-PCR analysis of the expression of *Collagen α1(I)* and *TGF-β1* in control rats, in CCl_4-treated rats, and in rats treated with CCl_4 + FSSC (1.5 or 0.5 g/kg). Expression of the amplified fragment corresponding to *GAPDH* is shown for comparison. (B) Densitometric analyses of *Collagen α1(I)* expression after normalization against *GAPDH*. (C) Densitometric analyses of *TGF-β1* expression after normalization against *GAPDH*. Data are expressed as mean of ratio ± S.E. ($n = 10$). $^{###}P < 0.001$ compared with the control group. $^{***}P < 0.01$ compared with $CCl_4 + H_2O$ group.

4. Discussion

In this study, FSSC was shown to effectively reduce chronic CCl_4-induced liver damage in rats. Plasma biochemical parameters were assayed and liver sections were examined. RT-PCR was carried out to analyze the expressions of various mRNAs involved in hepatoprotection and hepatic fibrosis. The data obtained consistently showed that FSSC reduced the effects of continuing toxic liver injury.

Plasma activities of ALT and AST are the most commonly used biochemical markers of liver injury. In the present examination of the progress of liver injury by repeated administrations of CCl_4, activities of both plasma ALT and AST markedly increased. FSSC could clearly reduce the increase in plasma ALT and AST activities caused by CCl_4; thus, it showed significant action in reducing the liver damages induced by CCl_4.

Two genes (*MAT1A* and *MAT2A*) encode the essential enzyme MAT, which catalyzes the biosynthesis of S-adenosylmethionine,

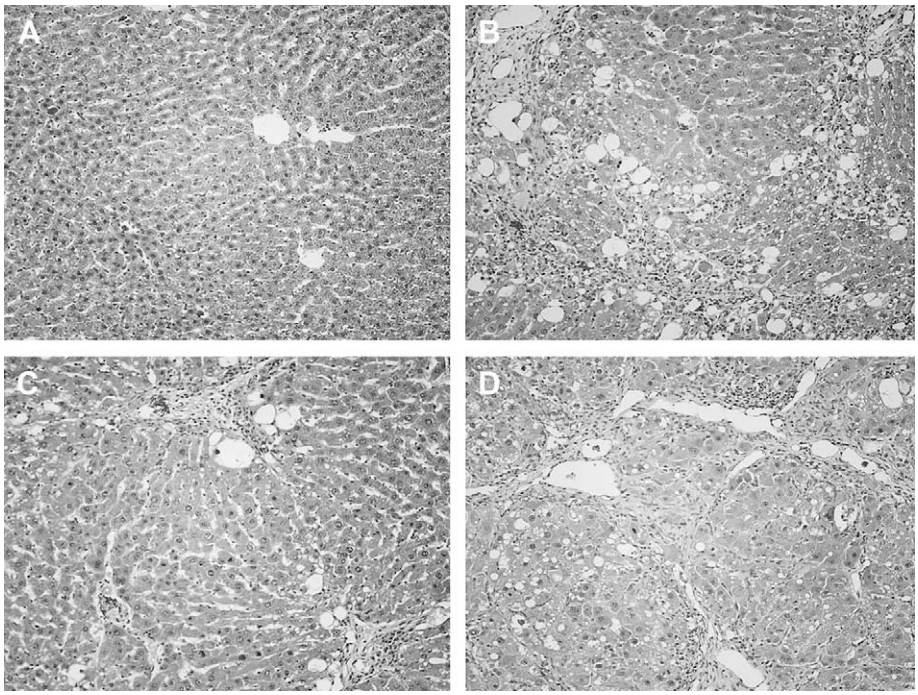

Fig. 3. Histologic analysis of liver section (hematoxylin–eosin stain). A, control group; B, $CCl_4 + H_2O$ group, showing fatty change, inflammatory cell infiltration and necrosis. C, CCl_4 + FSSC (1.5 g/kg) group, showing a marked reduction in fatty changes, inflammatory cell infiltration and necrosis. D, CCl_4 + FSSC (0.5 g/kg) group.

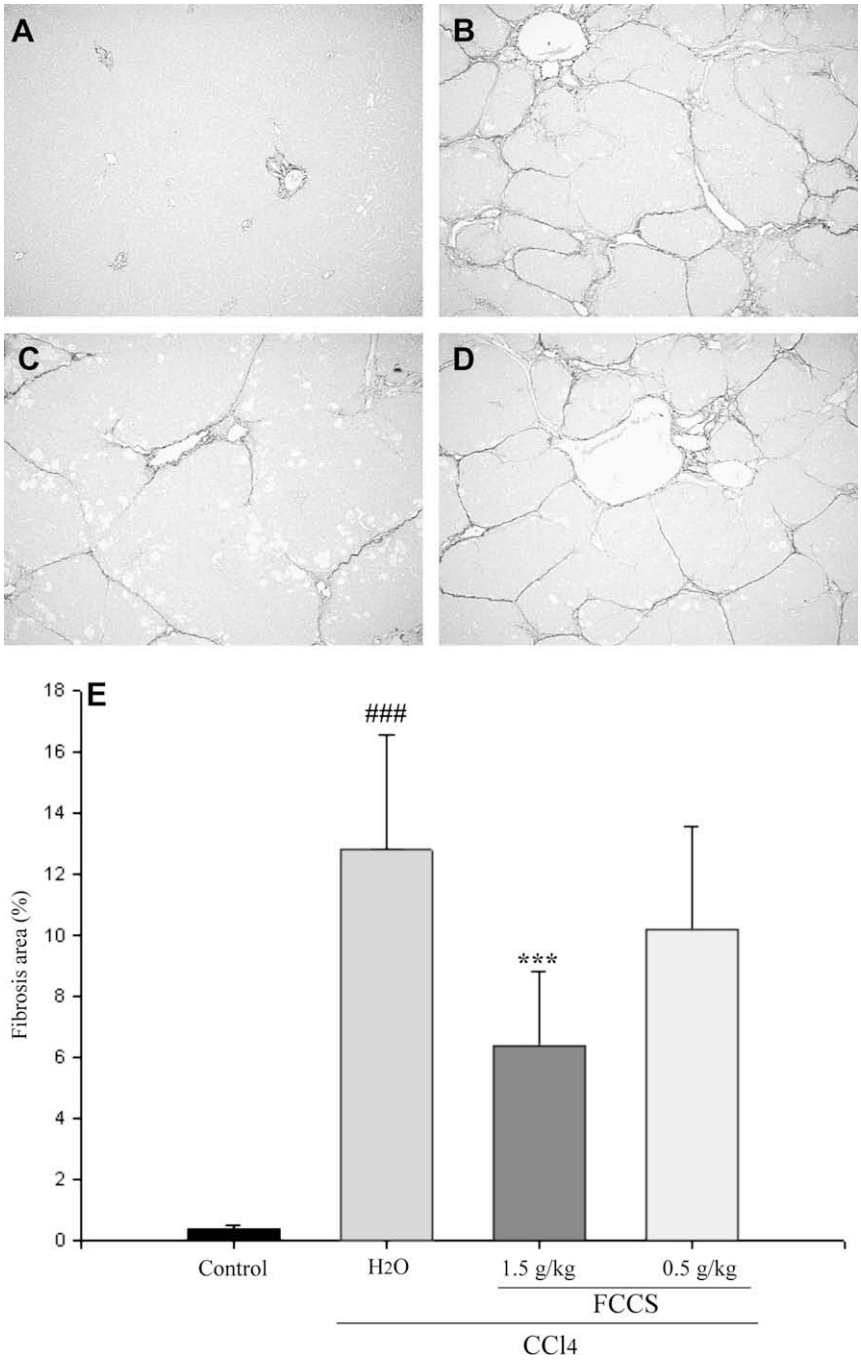

Fig. 4. Histologic analysis of liver fibrosis (Sirus red staining). A. control group; B, $CCl_4 + H_2O$ group, showing micronodular formation and complete interconnection of septa with each other. C, CCl_4 + FSSC (1.5 g/kg) group showing a marked reduction in fiber deposition. D, CCl_4 + FSSC (0.5 g/kg) group. E. Histogram representing image-quantitation of the mean percentage collagen fibers/total slide area. $^{###}P < 0.001$ compared with control group. $^{***}P < 0.001$ compared with $CCl_4 + H_2O$ group.

the principal methyl donor and a precursor of GSH in the liver.[25] *MAT1A* is primarily restricted to adult liver.[25] *MAT2A* is found in large amounts in fetal liver, decays at birth to negligible levels, and increases in the adult liver during regeneration after partial hepatectomy.[25] Thus, in response to liver injury, *MAT1A* expression is switched off and *MAT2A* expression is switched on. Consistent with this, the expression of *MAT1A* was found to be reduced in the livers of rats with chronic CCl_4 injury, whereas the expression of *MAT2A* increased.[26] FSSC treatment reduced *MAT2A* expression which was turned on by CCl_4 exposure, whereas *MAT1A* expression was actually restored to normal values by FSSC treatment. These results further support the fact that FSSC possesses a hepatoprotective effect. Histopathological evaluation of the rat livers also established that FSSC reduced the liver necrosis induced by CCl_4.

Hepatomegaly, splenomegaly, and hypoalbuminemia are the most common complications of liver fibrosis.[27] In the present study, FSSC could improve the hepatomegaly, splenomegaly, and hypoalbuminemia induced by chronic CCl_4 administration in rats, indicating that FSSC can retard the progression of liver fibrosis

It is well known that liver fibrosis is the result of increased collagen synthesis and that hydroxyproline is the characteristic

compound present in collagen.[28] The amount of collagen can be reflected by the hydroxyproline content, which can be used to express the extent of fibrosis.[28] When CCl_4 was administered to induce liver fibrosis in the present study, hydroxyproline levels in liver obviously increased. FSSC reduced the hydroxyproline content in liver, indicating that it can reduce hepatic fibrosis. Quantitative histopathological results support these findings. Many studies have shown that type I collagen increases predominantly in liver fibrosis.[29] Therefore, the effect of FSSC on the mRNA expression of *Collagen α1(I)* was also investigated. Treatment with FSSC was effective in reducing the amount of *Collagen α1(I)* mRNA expression. These results further confirm that FSSC can reduce hepatic fibrosis.

Matrix production by activated stellate cells is markedly increased through the action of TGF-β1.[30] Various inhibitors of TGF-β1 were developed and are now being investigated as potential drug candidates in experimental models of hepatic injury.[30] In this study, CCl_4 treatment increased, whereas FSSC significantly reduced *TGF-β1* mRNA expression. This result suggests that FSSC ameliorated liver fibrosis by reducing TGF-β1 secretion.

Much of the ingested GSH is converted into its constituent amino acids in the intestinal lumen.[31] The constituent amino acids (i.e., glutamate, cysteine, and glycine) are absorbed, and their levels in the portal plasma increase significantly. This will result in an increased availability of the precursors of GSH in hepatic cells.[31] Oral GSH-related thiol compounds, therefore, may be considered as cellular GSH precursors. In the previous works by the current authors, FSSC was shown to inhibit acetaminophen-induced hepatitis in mice probably through (a) supplementation of precursors for GSH synthesis and (b) enhancing GSH synthesis. Results of this study also indicate that FSSC increased the level of hepatic GSH in CCl_4-treated rats.

Increased free-radical production and LPO have been proposed as major cellular mechanisms involved in CCl_4 hepatotoxicity.[32] Furthermore, a close relationship has been reported between LPO and fibrogenesis in rats, in which fibrosis was induced by CCl_4 administration.[32] FSSC attenuated the oxidative stress, expressed by the reduction in the level of LPO in the CCl_4 rat model. Except for an elevation of the hepatic GSH level, other mechanisms using mineral elements and antioxidants, which may be involved in the antioxidant activity of FSSC, cannot be excluded.

Hepatic cells possess a number of enzymatic defenses against reactive free radicals. There are three classes of enzymes known to provide protection against reactive oxygen species: SOD, GSH-Px, and catalase. In this study, CCl_4 treatment markedly decreased the hepatic activities of SOD, GSH-Px, and catalase. These were in accordance with the results of previous reports by the other studies.[33,34] The present study showed that FSSC administration did not improve the activities of SOD, GSH-Px, and catalase, indicating that the antifibrotic action of FSSC did not include modulation of the activities of hepatic antioxidant enzymes.

This study showed that FSSC protects the rat liver from CCl_4-caused injury and fibrogenesis by suppressing hepatic oxidative stress. It may thus be stated that FSSC has the potential to be exploited as a preventive for liver fibrosis.

Conflict of interest

None declared.

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