Protective Effect of a Fermented Substance from *Saccharomyces cerevisiae* on Liver Injury in Mice Caused by Acetaminophen

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The protective effect of a fermented substance from *Saccharomyces cerevisiae* (FSSC) on liver injury caused by acetaminophen (AAP) was studied in mice. Mice were pretreated with FSSC (0.5–2.0 g/kg, p.o.) for 4 d, and on the fourth day, the mice received an overdose of AAP (500 mg/kg, i.p.). Subsequently, they were sacrificed at 7 h, and blood was drawn from the abdominal vein and liver samples were collected. Histological and biochemical examinations revealed that the administration of AAP caused liver injury in the mice, including increases in plasma alanine aminotransferase and asparate aminotransferase activities and decreases in the hepatic reduced form of glutathione (GSH) content and antioxidant enzyme activities. Prior to AAP treatment, the mice pretreated with FSSC showed significantly reduced levels of alanine aminotransferase (ALT) and asparate aminotransferase (AST) activity. Liver histology in the FSSC-pretreated mice was significant. In these mice, pretreatment with FSSC also served to reduce hepatic GSH depletion and the inhibition of antioxidant enzyme activity caused by AAP overdose. In conclusion, oral administration of FSSC significantly reduced AAP-induced hepatic injury in the mice.

Key words: acetaminophen; glutathione; *Saccharomyces cerevisiae*

Acetaminophen (AAP) is a widely used over-the-counter drug known for its analgesic and antipyretic effects but it can cause fulminant liver failure on account of overdose or when used in chronic alcohol abuse cases.1) It is well known that the first step to AAP toxicity is metabolism to a reactive metabolite, which leads to the depletion of a reduced form of glutathione (GSH) and subsequent binding to cellular proteins.2) Protein binding initiates the injury process, which can be then propagated and amplified by mitrocondrial dys-

function and oxidative stress formation.2) *N*-acetylcysteine (NAC), a cysteine prodrug, is most widely used in clinical practice to treat AAP-induced liver injury.3) In addition to NAC, certain mineral elements (e.g., zinc and selenium) serve as antidotes in AAP overdose cases.4,5)

For several centuries, *Saccharomyces cerevisiae* has been used in the production of food and alcoholic beverages. Presently, this organism has numerous applications in the pharmaceutical industry. A well-established fermentation and processing technique available for large-scale production of *S. cerevisiae* make this organism suitable for several biotechnological purposes.6) Currently, it is used in the production of glutathione on an industrial scale.7) Beside glutathione, it also produces glutathione-related thiol compounds (e.g., γ-glutamylcysteine and l-cysteine), and certain mineral elements, e.g., zinc and selenium.8–11)

In our laboratory, *S. cerevisiae* YA03083, a strain as modified from the parent strain *S. cerevisiae* BCRC21727 to produce high levels of glutathione, was screened. We have established an optimal medium composition for *S. cerevisiae* YA03083 to achieve maximum glutathione yield. In the present study, we examined whether FSSC can protect mouse liver against AAP-induced injury.

Materials and Methods

*Preparation of FSSC.* *S. cerevisiae* 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 screened as modified 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 containing

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Abbreviations: AAP, acetaminophen; ALT, alanine aminotransferase; AST, asparate aminotransferase; FSSC, a fermented substance from *Saccharomyces cerevisiae*; GSH, a reduced form of glutathione; GSH-Px, glutathione peroxidase; GSH-Rd, glutathione reductase; GSH-T, glutathione transferase; GSSG, oxidized glutathione; LPO, lipid peroxidation; NAC, *N*-acetylcysteine; NAPQI, *N*-acetyl-α-benzoquinoneimine; ROS, reactive oxygen species; SOD, superoxide dismutase
sugarcane molasses (110 g/l), soybean hydrolyzate (30 g/l), and mineral components, (NH₄)₂SO₄, 4 g/l, KH₂PO₄, 6 g/l, and MgSO₄·7H₂O, 1 g/l was used as the main cultivation medium. The fermenter was progressively fed the feeding medium, which comprised glucose, sugarcane molasses, yeast powder, soybean powder, minerals, and amino acids. Originally, the experiments were carried out in a 2-liter fermenter to feed-batch cultivation, for which 1 liter 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.

Composition of FSSC. The analytical methods used in the determination of total protein, total carbohydrate, crude fat, ash, and moisture in FSSC followed the AOAC guidelines (976.06, 995.13, 948.22, 920.54, and 981.05 respectively). The fractions of total protein, total carbohydrate, crude fat, ash, and moisture in FSSC were 40.8%, 42.1%, 6.7%, 5.6% and 4.8% respectively. Similarly, the content of zinc (21.6 ppm) and selenium (1.76 ppm) in FSSC was measured according to the AOAC guidelines (999.11 and 986.15 respectively) by atomic absorptiometry (Perkin Elmer 6000, Norwalk, CT).

Simultaneously, the contents of glutathione and glutathione-related thiol compounds in FSSC were determined by HPLC. In the preparation of samples for HPLC analysis, 50 mg of FSSC was extracted using 1 ml of 0.1 M acetic acid. The suspension was indirectly heated with boiling water for 10 min to enhance glutathione release. Subsequent to the removal of solid material, 0.5 ml of the extracted supernatant was mixed with 0.1 ml of isoacetic acid (40 mM in H₂O) and 0.2 ml of NaHCO₃ (1 M in 1 M KOH) for derivatization in the dark at 37°C for 1 h. An aliquot (0.5 ml) of 1.5% 1-fluoro-2,4-dinitro-benzene in absolute ethanol was added, and the reaction was allowed to occur in the dark at 37°C overnight. The suspension was then centrifuged at 12,000 rpm for 5 min, and the clear solution was further filtrated using a 0.45-μm membrane filter prior to HPLC measurements. The HPLC analysis conditions were as follows: for the mobile phase, buffer A was 80% methanol and buffer B a mixture of 200 ml sodium acetate (prepared from 272 g of sodium acetate trihydrate in 122 ml of H₂O and 378 ml of glacial acetic acid) and 800 ml of buffer A. Chromatography was performed on a LiChrosorb-NH₂ column using the mobile phase at a flow rate of 1 ml/min. Detection was performed by UV absorbance at 365 nm. The L-cysteine, GSH, oxidized glutathione (GSSG), and γ-glutamyl-cysteine peaks were identified by comparison of retention times with standards. The standards for L-cysteine, GSH, GSSG, and γ-glutamyl-cysteine were obtained from Merck KGaA (Darmstadt, Germany). The respective peak areas were used in quantitative analysis. HPLC analysis showed that the L-cysteine, GSH, GSSG, and γ-glutamyl-cysteine fractions of FSSC were approximately 3, 35, 1.9, and 20 mg/g respectively.

In addition, free amino acids of FSSC were also determined by the method of AOAC (979.20). The results were as follows: o-phosphoserine, 0.42 mg/g; o-phosphoethanolamine, 0.40 mg/g; L-glutamic acid, 0.08 mg/g; glycine, 1.41 mg/g; L-citrulline, 0.25 mg/g; L-valine, 0.10 mg/g; L(-)-cystine, 0.36 mg/100 g; L-methionine, 0.60 mg/g; L-isoleucine, 8.03 mg/g; L-leucine, 3.52 mg/g; L-tyrosine, 2.50 mg/g; α-t-l-allo-δ-hydroxylysinine, 0.10 mg/g; L-anserine, 1.34 mg/g; and L(-)-proline, 1.33 mg/g.

Animals. BALB/c male mice were obtained from the National Laboratory Animal Breeding and Research Center, National Science Council, Taiwan. The experimental animals received humane care, and the study protocols complied well with the institutional guidelines of China Medical University for the use of laboratory animals. The mice were housed in an air-conditioned room (21–24°C) under 12 h of light (7:00–19:00), and were allowed free access to food pellets and water throughout the study. Mice that weighed 26–30 g were used in the experiments. One day before the administration of the test substance, the mice were randomly allocated into six groups of 10 mice each.

AAP-Induced hepatotoxicity. FSSC (0, 0.5, 1, and 2 g/kg) or NAC (1.0 g/kg) was administered orally to the mice for 4 d. On the fourth day, FSSC or NAC was administered at 9 A.M., and 40 min thereafter, AAP (500 mg/kg dissolved in propylene glycol/water, i.p.) was injected. Seven h after AAP injection the mice were sacrificed and blood was drawn from the abdominal vein. Liver samples were also collected for histological and biochemical examination.

Subsequent to specimen collection, mouse blood was centrifuged at 3,000 rpm at 4°C for 10 min to extract plasma. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using clinical kits (Roche Diagnostics, Mannheim, Germany) and a spectrophotometric system (Cobas Mira; Roche, Rotkreuz, Switzerland).

Assay of liver lipid peroxidation (LPO), GSH, and GSSG. Liver specimens were homogenized in 9 volumes of ice-cold 0.15 M KCl and 1.9 mM EDTA. The homogenate was used in the determination of LPO levels by the method of Ohkawa et al., using 2-thiobarbituric acid. LPO was expressed as the amount of malondialdehyde per mg of protein. Protein was measured by the method of Lowry et al., with bovine serum albumin as the standard. GSH and GSSG in liver homogenates were assayed by measuring the fluorescence produced by 0-phthaldehyde at 420 nm, by the method of Hissin and Hilf.
Measurement of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), glutathione transferase (GSH-T), and catalase activities. SOD activity was measured using a Ransod kit (Randox Laboratories, Crumlin, UK). This method is based on the formation of red formazan by the reaction of 2-(4-iodophenyl)-3-(4-nitrophenoxy)-5-phenyltetrazolium chloride and the superoxide radical (produced in the incubation medium by xanthine oxidase reaction), which is assayed in a spectrophotometer at 505 nm. Inhibition of the chromogen produced was proportional to the activity of SOD present in the sample. One unit (U) of SOD was defined as the quantity of enzyme sufficient to inhibit the reduction of formazan by 50%, and the results were expressed as U/mg protein.

GSH-Px activity was measured using a Ransel kit (Randox Laboratories). GSH-Px catalyzed the oxidation of GSH by cumene hydroperoxide. In the presence of GSH reductase and NADPH, oxidized GSH was converted to a reduced form, with concomitant oxidation of NADPH to NADP⁺. The decrease in absorbency was measured at 340 nm. The enzyme activity required to convert 1 μmol of NADPH to NADP in 1 min was defined as 1 U of GSH-Px, and the results were expressed in U/mg protein.

GSH-Rd was assayed with a commercially available kit (Randox Laboratories). GSH-Rd activity was determined by monitoring GSH-dependent oxidation of NADPH at 340 nm. One unit of GSH-Rd activity was defined as the amount of enzyme required to oxidize 1 μmol of NADPH per min. The result were expressed in U/mg protein.

GSH-T activity measurement was based on the spectrophotometric (340 nm) determination of 1-chloro-2,4-dinitrobenzene conjugate formed in a GSH-coupled reaction (Cayman Chemical, Ann Arbor, MI). One unit of enzyme activity was defined as the amount of enzyme required to catalyze the formation of 1 μmol of S,2,4-dinitrophenylgluthathione per min with 1 mm of GSH and 1-chloro-2,4-dinitrobenzene. The result were expressed in U/mg protein.

Catalase activity was assayed by the method of Aebi. The rate of reduction was the measure of catalase activity. One unit of enzyme activity was defined as the amount of enzyme giving K = 1, where K is the rate constant of the enzyme. The expressed as U/mg protein.

Histopathological observation. After the blood drained, sections from each lobe of the liver were taken. The tissue was fixed in 10% neutral formalin, dehydrated with various ethanol solutions ranging from 50 to 100%, and embedded in paraffin; subsequently, it was cut into sections of 4–5 μm thickness and stained with hematoxylin and eosin. The sections were examined by light microscopy and graded for the presence and intensity of lesions using a scale from 0 to 5 (no lesions = 0; minimal lesions involving a single or few necrotic cells = 1; mild lesions, i.e., 10–25% necrotic cells or mild diffuse degenerative changes = 2; moderate lesions, i.e., 25–40% necrotic or degenerative cells = 3; marked lesions, i.e., 40–50% necrotic or degenerative cells = 4; severe lesions, i.e., more than 50% necrotic or degenerative cells = 5). Conventionally, sections with a score higher than 2 can be considered to exhibit significant liver injury.

Statistical analysis. The results were expressed as means ± SD. All other experimental data, except pathological findings, were analyzed by one-way analysis of variance by Dunn’s test. Liver histopathological examination data were analyzed by the Kruskall-Wallis non-parametric test, followed by a Mann-Whitney U-test. A P-value < 0.05 was considered statistically significant.

Results

Plasma ALT and AST activities

As shown in Table 1, AAP resulted in a marked increase in plasma AST and ALT activities as compared to the control group. Pretreatment with FSSC (0.5–2.0 g/kg) and with NAC (1.0 g/kg) significantly reduced the AAP-induced increase in both AST and ALT activities.

Hepatic LPO and glutathione

The hepatic malondialdehyde concentrations remained unaffected under AAP treatment. Similarly, pretreatment with FSSC did not affect the LPO levels in mice treated with AAP (Fig. 1).

After AAP treatment, the GSH content in the liver decreased by as much as 3.4% relative to the controls (Fig. 1). The proportion of GSSG to GSH + GSSG was significantly higher after AAP treatment. GSSG made up 55% upon treatment with AAP (Fig. 1). Pretreatment with FSSC (0.5–2.0 g/kg) and with NAC (1 g/kg) treatment reduced the depletion in hepatic GSH content and decreased the proportion of GSSG in total glutathione induced by AAP treatment (Fig. 1).

Table 1. Effects of FSSC on the Activities of Plasma AST, and ALT in AAP-Treated Mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (g/kg)</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + H₂O</td>
<td>—</td>
<td>67.5 ± 5.0</td>
<td>32.5 ± 5.0</td>
</tr>
<tr>
<td>AAP + H₂O</td>
<td>—</td>
<td>3315.0 ± 514.1†††</td>
<td>5948.3 ± 800.9†††</td>
</tr>
<tr>
<td>AAP + FSSC</td>
<td>0.5</td>
<td>1563.8 ± 1238.2***</td>
<td>2083.8 ± 1794.3***</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>478.8 ± 482.3***</td>
<td>541.3 ± 542.4***</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>312.5 ± 190.6***</td>
<td>300.0 ± 168.9***</td>
</tr>
<tr>
<td></td>
<td>+ NAC</td>
<td>280.0 ± 225.3***</td>
<td>287.5 ± 277.9***</td>
</tr>
</tbody>
</table>

All values are means ± SD (n = 10).
†††P < 0.001 compared with the vehicle + H₂O group.
***P < 0.001 compared with the AAP + H₂O group.
Fig. 1. Effects of FSSC on the Hepatic Contents of Malondialdehyde (MDA), GSH and GSSG/(GSH + GSSG) Ratio in AAP-Treated Mice.

All values are means ± SD (n = 10). ***P < 0.001 compared with the vehicle (VEH) + H₂O group. **P < 0.01 compared with the AAP + H₂O group.

Hepatic SOD, catalase GSH-Px, GSH-Rd, and GSH-T activities

AAP administered to H₂O-treated mice resulted in decreases in hepatic SOD, catalase, GSH-Px, GSH-Rd, and GSH-T activities, which were significantly different from those of the control group (Tables 2 and 3). In the AAP-treated mice, FSSC prevented the depletion of hepatic SOD, catalase, GSH-Px, GSH-Rd, and GSH-T activities in a dose-dependent manner. NAC (1 g/kg) also prevented a decrease in enzyme activities.

Pathological changes

Diffuse areas of necrosis, especially in the centrilobular area, sinusoidal congestion, inflammatory cell infiltration around the central vein, and loss of cell boundaries, were observed after administration of AAP (Fig. 2B). Treatment of animals with FSSC (1 and 2 g/kg) and with NAC (1 g/kg) reduced the extent of necrosis and inflammatory cell infiltration around the central vein (Fig. 2D–F and Table 4).

Discussion

In agreement with previous studies, AAP administered intraperitoneally to mice at an overdose (500 mg/kg) caused centrilobular hepatic necrosis, as evidenced by histological and biochemical changes. It is also evident that pretreatment with FSSC, which produced significant quantities of glutathione-related thiol compounds and mineral elements (e.g., selenium and zinc), suppressed AAP-induced liver injury. Marked elevations in plasma

Table 2. Effects of FSSC on the Hepatic Activities of SOD and Catalase in AAP-Treated Mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (g/kg)</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + H₂O</td>
<td>—</td>
<td>7.7 ± 0.3</td>
<td>29.2 ± 4.5</td>
</tr>
<tr>
<td>AAP + H₂O</td>
<td>—</td>
<td>5.2 ± 0.7**</td>
<td>18.3 ± 5.1***</td>
</tr>
<tr>
<td>AAP + FSSC</td>
<td>0.5</td>
<td>6.1 ± 0.8</td>
<td>19.7 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.8 ± 0.8***</td>
<td>20.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>7.1 ± 0.5***</td>
<td>24.0 ± 3.5**</td>
</tr>
<tr>
<td>+ NAC</td>
<td>1.0</td>
<td>7.4 ± 0.8***</td>
<td>23.3 ± 1.9***</td>
</tr>
</tbody>
</table>

All values are means ± SD (n = 10). ***P < 0.001 compared with the vehicle + H₂O group. **P < 0.01, ***P < 0.001 compared with the AAP + H₂O group.

Table 3. Effects of FSSC on the Hepatic Activities of GSH-Px, GSH-Rd, and GSH-T in AAP-Treated Mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (g/kg)</th>
<th>GSH-Px (mU/mg protein)</th>
<th>GSH-Rd (U/mg protein)</th>
<th>GSH-T (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + H₂O</td>
<td>—</td>
<td>1803.9 ± 455.6</td>
<td>64.3 ± 2.7</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>AAP + H₂O</td>
<td>—</td>
<td>804.4 ± 153.1**</td>
<td>41.2 ± 6.7***</td>
<td>2.9 ± 0.3**</td>
</tr>
<tr>
<td>AAP + FSSC</td>
<td>0.5</td>
<td>1497.0 ± 433.4**</td>
<td>50.3 ± 5.9*</td>
<td>3.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1661.5 ± 206.8***</td>
<td>53.8 ± 6.3**</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1746.6 ± 350.5***</td>
<td>54.7 ± 6.2**</td>
<td>4.0 ± 0.4*</td>
</tr>
<tr>
<td>+ NAC</td>
<td>1.0</td>
<td>1775.5 ± 385.4***</td>
<td>55.2 ± 7.4**</td>
<td>4.0 ± 0.7*</td>
</tr>
</tbody>
</table>

All values are means ± S.D (n = 10). *P < 0.05, **P < 0.01, ***P < 0.001 compared with the AAP + H₂O group.
AST and ALT activities associated with AAP toxicity were reduced by FSSC in a dose-dependent manner. The hepatotoxicity of AAP was due to the formation of a toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P4502E1, which induced a dose-dependent depletion of intracellular GSH. The lack of GSSG formation acts as an indicator for reactive-oxygen generation during the metabolism of AAP in rats and mice. Presently, NAC is the treatment option because it swiftly increases GSH levels in the liver and detoxifies the highly reactive and cytotoxic NAPQI. NAC acts as a precursor of GSH and it can penetrate the cells easily, and is subsequently deacylated to form cysteine. Improvement in GSH levels is considered the most significant mechanism of NAC against AAP toxicity. Our data also indicate enhanced baseline GSH levels and decreased GSSG/(GSH + GSSG) ratios as a result of NAC pretreatment, which resulted in reduced liver injury subsequent to AAP treatment.

Much of the ingested glutathione is converted into its constituent amino acids in the intestinal lumen. The constituent amino acids (glutamate, cysteine, and glycine) are absorbed, and their levels in the portal plasma increase significantly. This results in an increased availability of precursors of GSH in hepatic cells. Oral glutathione, thus, may be considered a cellular GSH precursor. Sugimura and Yamamoto also found that oral administration of glutathione acts against AAP-induced liver injury in rats. Similarly to NAC, FSSC contains glutathione-related thiol compounds that replenish AAP-induced depletion of hepatic GSH, presumably by increasing the availability of precursors for GSH synthesis. However, the total content of cysteine in FSSC was far lower than that in NAC, but the restoration of GSH levels was similar in both. Therefore, there was another mechanism involved in restoring the GSH level by FSSC, besides the supplementing of precursors for GSH synthesis.

There is much evidence that protective preparation has no capability to enhance GSH levels; however, when administered with a toxin, e.g., lipoic acid and cisplatin or lycopene and T-2 toxin, it can substantially reduce GSH depletion. These observations can be partly explained on the basis of the regulatory mechanisms of GSH synthesis. Thus a decrease in the GSH concentration caused by toxin treatment creates conditions for enhanced synthesis, which can be additionally stimulated by the protective substance. According to this hypothesis, FSSC restores the hepatic GSH levels in mice after AAP treatment, and this partly explains how it can be that FSSC enhances GSH synthesis.

Another hypothesis proposed as the mechanism of AAP hepatotoxicity is oxidative stress. Both in vitro and in vivo studies have shown that hepatic reactive oxygen species (ROS) can be produced after AAP challenge. LPO is generally considered a product of ROS. Consistently with previously reported data, AAP did not increase hepatic LPO levels in this study. It is hypothesized that the first step in AAP toxicity is metabolism of NAPQI, leading to the depletion of GSH and covalent adduct formation. Subsequently, induction of NO synthesis and superoxide generation occur, leading

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**Table 4. Effects of FSSC on Hepatic Damage Induced by AAP in Mice**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (g/kg)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>% &gt; 2</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + H2O</td>
<td>—</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AAP + H2O</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AAP + FSSC</td>
<td>0.5</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>2.7</td>
</tr>
<tr>
<td>AAP + FSSC</td>
<td>1.0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>40</td>
<td>2.2*</td>
</tr>
<tr>
<td>AAP + FSSC</td>
<td>2.0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>30</td>
<td>2.1*</td>
</tr>
<tr>
<td>AAP + NAC</td>
<td>1.0</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>30</td>
<td>2.0*</td>
</tr>
</tbody>
</table>

Livers were graded from 0 to 5 as described in Materials and Methods. Scores higher than 2 are indicative of significant necrosis. Each value is the number of animals with grading changes. *P < 0.05 compared with the AAP + H2O group.

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**Fig. 2.** Light Microscopic Comparison of Liver Sections (hematoxylin and eosin stained) Obtained from Mice Treated with AAP.

A, Vehicle + H2O group; B, AAP + H2O; C, AAP + FSSC (0.5 g/kg); D, AAP + FSSC (1 g/kg); E, AAP + FSSC (2 g/kg); F, AAP + NAC (1 g/kg).
to the production of peroxynitrite, which causes tissue injury.\textsuperscript{31} Thus superoxide can be scavenged by NO by forming peroxynitrite.\textsuperscript{32} Therefore, hepatic LPO levels were not increased by AAP intoxication. Since AAP did not increase hepatic LPO in mice, administration of FSSC also did not affect the hepatic level of LPO.

The reactions of peroxynitrite with proteins lead to modification of proteins. Several enzymes, including SOD, GSH-Px, GSH-Rd, GSH-T, and catalase, have been reported to be inactivated by peroxynitrite.\textsuperscript{34,35} In the present study, the activities of hepatic antioxidant enzymes and GSH-T were inhibited by AAP intoxication. These results might be related to peroxynitrite production by AAP. Similar results were reported by Lores Arnaiz \textit{et al.}, who observed decreases in GSH-Px and catalase activity in mice administered AAP,\textsuperscript{30} and by Kumar \textit{et al.}, who reported decreased GSH-Rd activity in AAP-treated rats.\textsuperscript{36} GSH can scavenge peroxynitrite and prevent protein nitration.\textsuperscript{37} Except for GSH conjugated with NAPQI for excretion, the mechanisms of GSH-attenuating hepatic necrosis induced by AAP were mediated by scavenging of peroxynitrite. In this study, FSSC restored the activities of antioxidant enzymes and GSH-T in AAP-treated mice. This might also be explained by the fact that FSSC can increase depleted hepatic GSH levels following AAP treatment.

There is evidence indicating that mineral elements, such as zinc and selenium, might be involved in hepatoprotective action. Chengelis \textit{et al.} reported that zinc protected against AAP-induced hepatotoxicity in mice.\textsuperscript{5} Peterson \textit{et al.} found that acute AAP lethality is potentiated by selenium deficiency in mice.\textsuperscript{6} The protective actions of zinc and selenium might be due to their known antioxidant effects.\textsuperscript{38,39} Hence, it is clear that FSSC protected against AAP-induced hepatotoxicity partly via its antioxidant activities. It is, however, unclear whether antioxidants can enhance GSH synthesis. Some reports indicate that antioxidants, such as \textae\-tocopherol and ginkgo biloba, can reverse the decreased hepatic levels of GSH due to AAP in rats and mice.\textsuperscript{40,41}

In conclusion, our results clearly indicate that FSSC attenuated the effects of AAP-induced hepatotoxicity. It may be expected that FSSC has preventive potential in AAP-induced hepatotoxicity.

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References


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