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Effects of Sesame Oil Against After the Onset of Acetaminophen-Induced Acute Hepatic Injury in Rats

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Background: Acetaminophen (APAP) is a safe and effective analgesic and antipyretic when used at therapeutic levels. However, an acute or cumulative overdose can cause severe liver injury with the potential to progress to liver failure in humans and experimental animals. Much attention has been paid to the development of an antioxidant that protects against APAP-induced acute hepatic injury. Hence, we aimed to investigate the effect of sesame oil against after the onset of acute hepatic injury in APAP-overdosed rats. *Methods:* Male Wistar rats were first given 2 oral doses (1,000 mg/kg each) of APAP (at 0 and 24 hours) and then 1 oral dose of sesame oil (8 mL/kg at 24 hours). *Results:* After 48 hours, APAP increased aspartate and alanine aminotransferase levels in the rats' serum and centrilobular necrosis in liver tissue. In addition, APAP significantly decreased the rats' glutathione levels and mitochondrial aconitase activity, but increased superoxide anion, hydroxyl radical, and lipid peroxidation levels. Oral sesame oil (8 mL/kg, given at 24 hours) reversed all APAP-altered parameters and protected the rats against APAP-induced acute liver injury. *Conclusion:* We hypothesize that sesame oil acts as a useful agent that maintains intracellular glutathione levels and inhibits reactive oxygen species, thereby protecting rats against after the onset of APAP-induced acute oxidative liver injury. (*JPEN J Parenter Enteral Nutr.* 2010;34:567-573)

Keywords: acetaminophen; glutathione; aconitase; reactive oxygen species; sesame oil

Acetaminophen (APAP, N-[4-hydroxyphenyl]-acetamide) is widely used as an analgesic and antipyretic.[1] APAP has a good safety profile,[2,3] but an APAP overdose may lead to severe hepatic necrosis and fatal hepatic failure.[4] APAP is also the most common substance in self-poisoning in the United Kingdom and United States.[5,6] At therapeutic doses, it is conjugated with either a sulfate or a glucuronide and is safely excreted.[7] However, when taken in toxic doses, APAP is metabolized by the cytochrome P450 system to form highly electrophilic N-acetyl-p-benzoquinone imine (NAPQI).[8-10] Further, glutathione (GSH), the main component of the endogenous sulfhydryl pool, is a reducing agent and antioxidant important for scavenging free radicals and reducing oxidative stress.[11] When excessive amounts of APAP are ingested, there is more NAPQI in the liver, which depletes GSH and causes hepatic damage.[12]

Sesame oil (SO), derived from the plant species *Sesamum indicum* L., is an herbaceous annual in the family *Pedaliaceae*.[13] SO increases the hepatic detoxification of chemicals and reduces the incidence of chemically induced oxidative stress after the onset of endotoxication.[14] Prophylactic use of SO attenuates APAP-induced reactive oxygen species and lipid peroxidation, thereby preventing hepatic injury.[15] However, the effect of SO against hepatic injury after the onset of APAP poisoning is unclear. Therefore, we investigated the effect of SO against after the onset of acute hepatic injury in APAP-overdosed rats.

Materials and Methods

Chemicals

APAP, SO, and polyethylene glycol (PEG) were obtained from Sigma-Aldrich Co. (St Louis, MO). APAP was prepared in a suspension using 40% PEG.[15]

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Animals

Ten-week-old male albino Wistar rats (n = 30; 250–300 g), purchased from our Institutional Laboratory Animal Center were used in this study. They were given pellet feed (Richmond Standard; PMI Feeds, Inc, St Louis, MO) and water ad libitum. The animal facility had a 12-hour light/dark cycle and central air-conditioning (25°C, 70% humidity) throughout the experimental period. The animal care and experimental protocols were in accordance with nationally approved guidelines.

Experimental Design

Food was withheld from the rats for at least 12 hours before each experiment, and the rats were pair-fed after 3 hours of APAP and SO treatments. All experimental treatments were given orally. The doses of APAP and SO were selected based on our previous study.[15] The rats were divided into 5 groups (n = 6 per group): Group I, healthy, untreated controls (HC group); Group II (SO group) received SO (8 mL/kg) at 24 hours; Group III (PEG group) received PEG (3 mL/kg) at 0 hours and 24 hours; Group IV (APAP group), positive controls, received APAP (1,000 mg/kg) at 0 hours and 24 hours; and Group V (ASO group) first received APAP (1,000 mg/kg) at 0 hours and 24 hours and then SO (8 mL/kg) at 24 hours. After 48 hours, we collected rat serum to assess the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and we harvested a small piece of liver tissue from each rat for histopathologic examination. We also assessed the levels of GSH, superoxide anion, hydroxyl radical, and lipid peroxidation in the liver tissue. In addition, we also measured aconitase activity in isolated mitochondria.

Blood Collection

Blood was collected in serum separation tubes from a femoral vein via venipuncture while the rats were under mild ether anesthesia. The tubes were left to clot at room temperature for 30 minutes and then centrifuged at 1,000 *g* at 4°C for 10 minutes.

Assessment of Hepatic Injury

We assessed hepatic injury by measuring the levels of AST and ALT in serum using a biochemistry analyzer (Fujifilm Dri-Chem 3500s; Fujifilm, Kanagawa, Japan). Hepatic injury was further confirmed using histologic studies. A small piece of liver tissue from each rat was cut and placed in 4% phosphate-buffered formalin. The tissue pieces were dehydrated using a graded percentage of alcohol and then fixed in paraffin wax for 1 hour to form blocks. The blocks were trimmed and cut into 4-µm-thick sections, stained with hematoxylin and eosin, and then mounted using Depex Polystyrene dissolved in xylene mountant. The permanently mounted sections of liver tissue were examined under a microscope (Eclipse E 600; Nikon Instech Co Ltd, Kawasaki, Japan; 100× magnification) to assess hepatic injury.[16] Histopathologic score categories indicated the type of injury: necrosis, sinusoidal dilatation, and lymphocytic infiltration. The scoring system was scaled from 1 to 4 (1 = no abnormalities, 2 = mildly abnormal, 3 = moderately abnormal, and 4 = markedly abnormal).[17]

Determination of GSH Levels

A 10% liver-tissue homogenate (1 g in 10 mL of ice-cold 10% trichloroacetic acid) was used to determine GSH levels. In brief, tissue samples were homogenized and centrifuged (3,000 rpm for 10 minutes), and then 500 µL supernatant was added to 2 mL 0.3 M Na_2HPO_4 $2H_2O$ solution. Next, 200 µL dithiobis(2-nitrobenzoic acid) (1% sodium citrate, 0.4 mg/mL) was added, and the absorbance was immediately measured at 412 nm.[18]

Determination of Superoxide Anion and Hydroxyl Radical Levels in Liver Tissue

Liver tissue was homogenized (1:10; w/v) in Tris-sucrose buffer (0.24 M sucrose in 20 mM Tris-HCl buffer containing 1 mM ethylene-diaminetetraacetic acid [EDTA; pH 7.4]). The homogenates were centrifuged at 400 *g* at 4°C for 30 minutes. Superoxide anion and hydroxyl radical levels were measured using a high-performance chemiluminescence (CL) analyzer (CLA-2100; Tohoku Electronic Industrial Co Ltd, Rifu, Japan). In brief, 400 µL supernatant was mixed with 200 µL phosphate-buffered saline in a stainless dish, and then the background CL count was read for 60 seconds. After adjusting the baseline count, 100 µL lucigenin and indoxyl β-D-glucuronide (17 mM dissolved in phosphate-buffered saline, to determine superoxide anion and hydroxyl radical, respectively) were injected into the machine, and CL was counted for another 15 minutes at 10-second intervals. The data were analyzed using chemiluminescence analyzer data acquisition software CLA-DAS (Tohoku Electronic Industrial Co.).[14]

Isolation of Mitochondria From Liver Tissue

Liver tissue was washed once with ice-cold isolation buffer (10 mL Tris-MOPS [0.1 M; pH 7.4], 20 mL sucrose [1 M], and 1 mL Ethylene glycol-bis(2-aminoethylether)-N,N,N,N-tetraacetic acid-Tris buffer [0.1 M; pH 7.4]) and cut into small pieces; the buffer was discarded. Five milliliters of fresh isolation buffer was then added, and the mixture was homogenized. The homogenates were centrifuged at 600 *g* at 4°C for 10 minutes, and then the supernatants were centrifuged at 7,000 *g* at 4°C for 10 minutes, after which the supernatants were discarded.

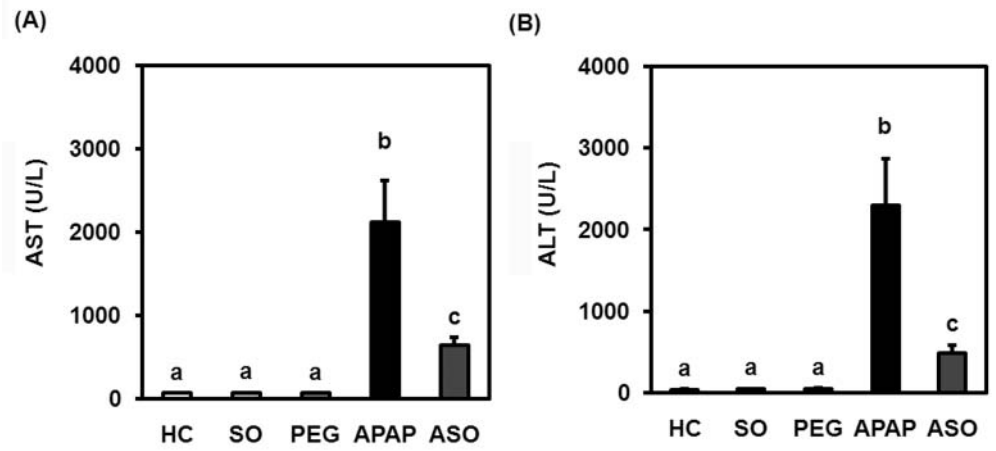

Figure 1. Effects of sesame oil against after the onset of acetaminophen (APAP)-induced hepatic injury. The rats were divided into 5 groups of 6. Group I (HC): healthy controls; Group II (SO): given oral sesame oil (8 mL/kg) at 24 hours; Group III (PEG): given oral polyethylene glycol (3 mL/kg) at 0 and 24 hours; Group IV (APAP): positive controls, given oral APAP (1,000 mg/kg) at 0 and 24 hours; and Group V (ASO): first given oral APAP (1,000 mg/kg) at 0 and 24 hours, and then oral sesame oil (8 mL/kg) at 24 hours. Aspartate transaminase (AST) and alanine transaminase (ALT) levels were assessed in rat serum 48 hours later. Data are given as mean ± standard error. Different letters (a,b, and c) indicate a significant difference between groups. ($P < .05$; one-way analysis of variance, and then the Tukey multiple-comparison test).

The pellets were washed once with isolation buffer, and the above centrifugation steps were repeated twice. After the 3-step centrifugation, the supernatants were discarded and the pellets were suspended in 1 mL isolation buffer and used for further analysis.[19]

Determination of Mitochondrial Aconitase Activity

Mitochondrial aconitase activity was determined using a commercial kit (Bioxytech Aconitase-340; Oxis Research, Foster City, CA) based on the molar extinction coefficient of nicotinamide adenine dinucleotide phosphate (6.220×10^{-3}) and the temperature coefficient (2.4435), and then read on a spectrophotometer (DU 640B; Beckman, Fullerton, CA) at 340 nm.

Measuring Lipid Peroxidation in Liver Tissue

Liver tissue was homogenized in Tris-HCl buffer (20 mM; pH 7.4) and then centrifuged at 2,500 *g* at 4°C for 10 minutes. Two hundred microliters of supernatant were analyzed for malondialdehyde (MDA) levels using a kit (Bioxytech MDA-586; Oxis Research) and then read on a spectrophotometer (Beckman) at 586 nm.

Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) and the Tukey multiple-comparison test to evaluate the significance between the treatment groups. Results are given as mean ± standard error. A P value $< .05$ was considered statistically significant.

Results

Effects of Sesame Oil After the Onset of APAP-induced Hepatic Injury

To examine the effects of SO after the onset of APAP-induced liver toxicity, we measured AST and ALT levels in serum. APAP significantly increased AST and ALT levels compared with those in the HC, SO, and PEG groups (all $P < .001$). SO significantly protected against after the onset of APAP-induced liver toxicity by preventing the rise of AST and ALT levels at 48 hours (Figure 1).

Effects of Sesame Oil on Liver Histopathology After the Onset of APAP-induced Liver Damage

We analyzed the histopathologic features of liver tissue to find out the effects of SO against after the onset of APAP-induced liver toxicity. APAP-treated liver tissue showed centrilobular necrosis, lymphocytic infiltration, and sinusoidal dilatation (Figure 2A). This necrotic effect was minimal in the ASO group compared with that in the APAP group. APAP significantly altered the normal architecture of the liver compared with that in the HC, SO, and PEG groups ($P < .001$; Figure 2B). Neither SO alone

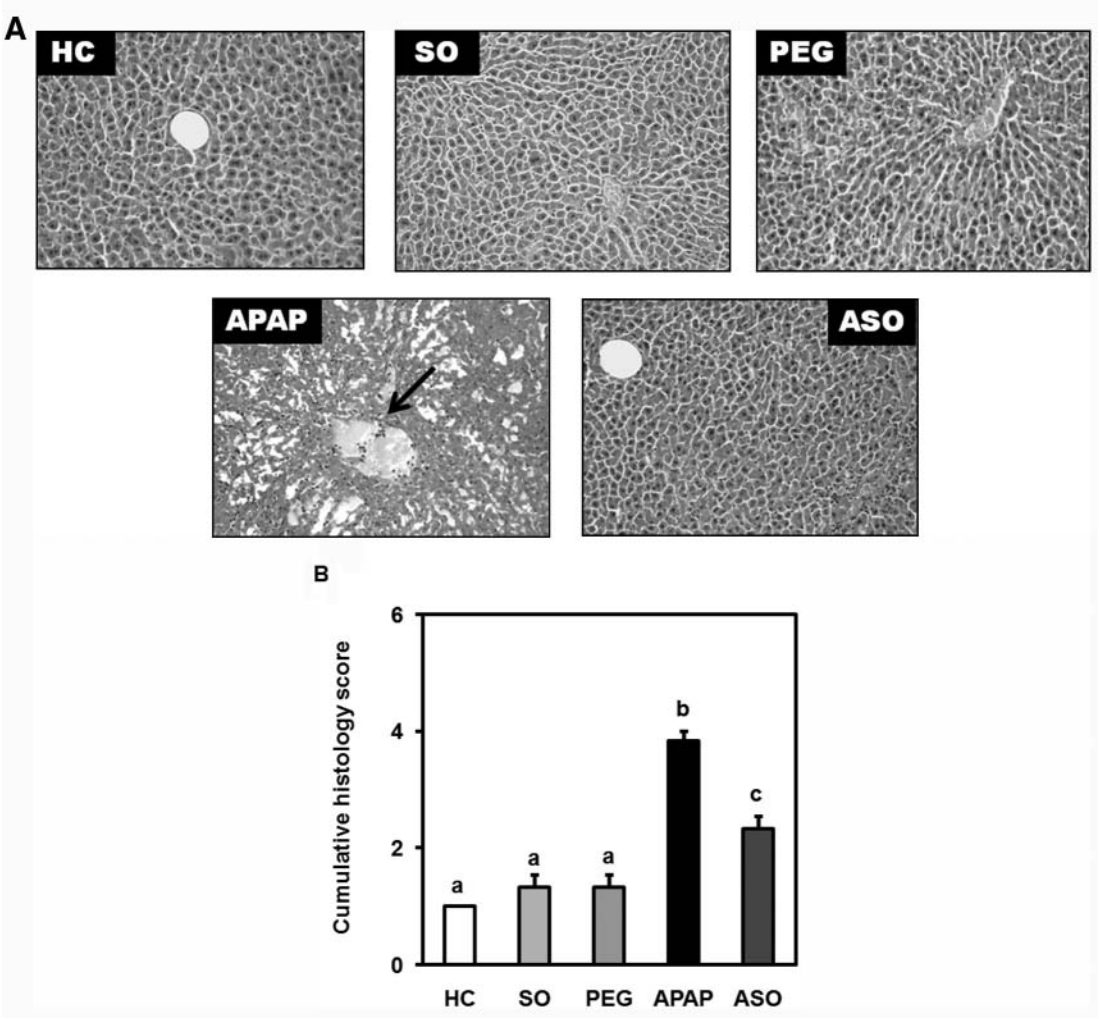

Figure 2. Effects of sesame oil against after the onset of acetaminophen (APAP)-induced hepatic histopathology. The rats were divided into 5 groups of 6. Group I (HC): healthy controls; Group II (SO): given oral sesame oil (8 mL/kg) at 24 hours; Group III (PEG): given oral polyethylene glycol (3 mL/kg) at 0 and 24 hours; Group IV (APAP): positive controls, given oral APAP (1,000 mg/kg) at 0 and 24 hours; and Group V (ASO): first given oral APAP (1,000 mg/kg) at 0 and 24 hours, and then oral sesame oil (8 mL/kg) at 24 hours. Hepatic histopathologic changes (A) and their respective scores (B) were observed 48 hours after the initial treatment. The arrow in the APAP image indicates the necrotic region (hematoxylin and eosin stain; 100× magnification). Data are given as mean ± standard error. Different letters (a,b, and c) indicate a significant difference between groups. ($P < .05$; one-way analysis of variance and then the Tukey multiple-comparison test).

nor PEG alone altered the normal architecture of liver tissue.

Effects of Sesame Oil Against After the Onset of APAP-induced GSH Levels, Superoxide Anion Generation, and Mitochondrial Aconitase Activity

APAP significantly ($P < .001$) decreased GSH (Figure 3A) levels and increased superoxide anion generation (Figure 3B) in liver tissue compared with that in the HC, SO, PEG, and ASO groups (all $P < .001$). In addition, APAP significantly ($P < .001$) decreased aconitase activity (Figure 3C) in rat liver mitochondria compared with that in the HC, SO, PEG, and ASO groups (all $P < .001$). We found no differences in those levels among the HC, SO, PEG, and ASO groups.

Effects of Sesame Oil Against After the Onset of APAP-induced Hydroxyl Radical and Lipid Peroxidation Levels

To study the effect of SO against after the onset of APAP-induced hydroxyl radical generation and lipid peroxidation, we measured hydroxyl radical and MDA levels in liver tissue. Hydroxyl radical and MDA levels

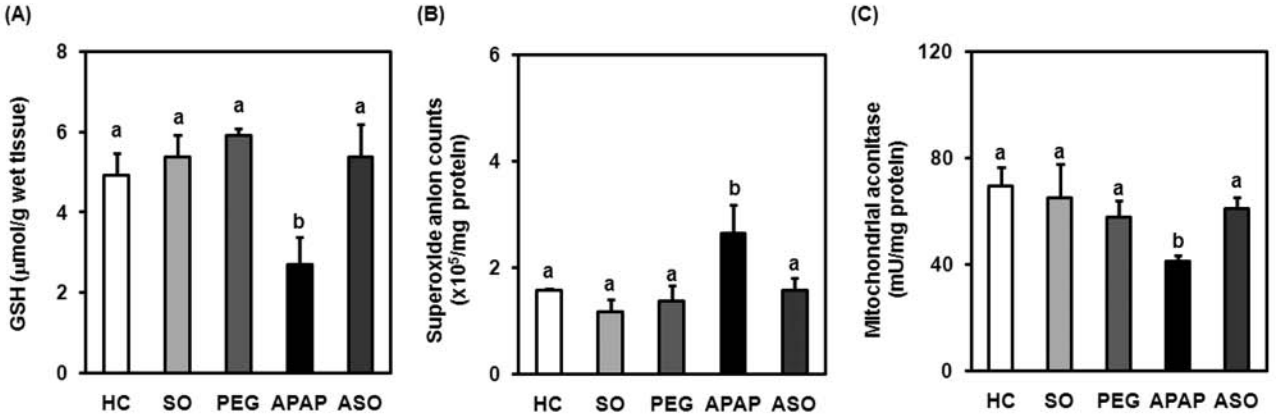

Figure 3. Effects of sesame oil on the levels of glutathione (GSH), superoxide anion generation in liver tissue, and mitochondrial aconitase activity after the onset of acetaminophen-induced hepatic injury. The rats were divided into 5 groups of 6. Group I (HC): healthy controls; Group II (SO): given oral sesame oil (8 mL/kg) at 24 hours; Group III (PEG): given oral polyethylene glycol (3 mL/kg) at 0 and 24 hours; Group IV (APAP): positive controls, given oral APAP (1,000 mg/kg) at 0 and 24 hours; and Group V (ASO): first given oral APAP (1,000 mg/kg) at 0 and 24 hours, and then oral sesame oil (8 mL/kg) at 24 hours. Forty-eight hours after the initial treatment, we assessed GSH and superoxide anion levels in liver tissue. In addition, aconitase activity was estimated in rat liver mitochondria. Data are given as mean ± standard error. Different letters (a and b) indicate a significant difference between groups. ($P < .05$; one-way analysis of variance and then the Tukey multiple-comparison test).

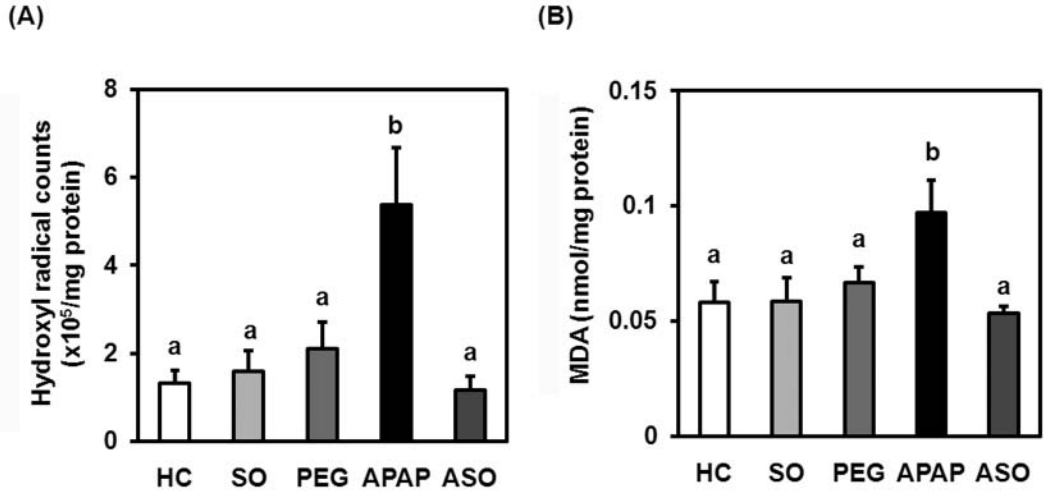

Figure 4. Effects of sesame oil on the levels of hydroxyl radical and lipid peroxidation after the onset of acetaminophen-induced hepatic injury. The rats were divided into 5 groups of 6. Group I (HC): healthy controls; Group II (SO): given oral sesame oil (8 mL/kg) at 24 hours; Group III (PEG): given oral polyethylene glycol (3 mL/kg) at 0 and 24 hours; Group IV (APAP): positive controls, given oral APAP (1,000 mg/kg) at 0 and 24 hours and Group V (ASO): first given oral APAP (1,000 mg/kg) at 0 and 24 hours, and then oral sesame oil (8 mL/kg) at 24 hours. Forty-eight hours after the initial treatment, we assessed hydroxyl radical and malondialdehyde (MDA) levels in liver tissue. Data are given as mean ± standard error. Different letters (a and b) indicate a significant difference between groups. ($P < .05$; one-way analysis of variance and then the Tukey multiple-comparison test).

in liver tissue increased significantly ($P < .001$) in the APAP group compared with those in the HC, SO, PEG, and ASO groups (all $P < .001$). The levels of hydroxyl radical and MDA were unaltered in the SO, PEG, and ASO groups (Figure 4).

Discussion

SO not only prevented[15] but also attenuated APAP-induced acute hepatic injury in rats. SO maintained the GSH levels in the liver tissue after the onset of

APAP-induced hepatic injury. GSH content has been inversely linked to APAP hepatotoxicity. GSH depletion induced either by chemicals or fasting has been shown to increase APAP toxicity.[20] GSH plays an important role in scavenging NAPQI, a toxic metabolite of APAP.[21] Excessive NAPQI formation starts to deplete GSH levels and initiates hepatic injury.[22] Reactive oxygen species formation starts immediately after the depletion of GSH,[23] which leads to cell death. In addition, GSH depletion increases the accumulation of superoxide anion in hepatocytes and triggers mitochondrial oxidative stress.[24] The mitochondrial aconitase enzyme acts as a 2-faced protein, enzyme, and iron-regulatory factor,[25] which can be affected by superoxide anion[26] during APAP-induced hepatic injury.[27] When superoxide anion inactivates mitochondrial aconitase activity, it may pose a significant oxidative burden because it provides equimolar amounts of hydrogen peroxide per mole of superoxide anion.[28] Inactive iron-sulfur centered aconitase enzyme releases free ferrous ion, which reacts with hydrogen peroxide to form potent hydroxyl radicals.[27] Hydroxyl radicals are highly reactive, which causes hepatic lipid peroxidation during APAP overdose.[15,27] Hence, we hypothesize that the effect of SO is associated with maintaining GSH levels and mitochondrial aconitase activity to inhibit the generation of superoxide, hydroxyl radicals, and lipid peroxidation leading to protection against after the onset of APAP-induced acute hepatic injury.

The value of SO against after the onset of APAP-induced acute hepatic injury might reduce the incidence of liver transplantation. APAP-induced acute hepatic injury or failure requiring liver transplantation is a major concern in developing and developed countries.[6, 29-31] It is important to provide an alternative to reduce the incidence of liver transplantation after APAP-induced acute hepatic injury or failure. SO, a natural nutrition supplement, includes various antioxidants such as tocopherol, sesamin, sesamolin, and sesaminol.[13,32-35] SO protects the liver from APAP-induced hepatic damage without affecting the absorption of APAP.[15] Thus, the protection against APAP-induced liver damage exhibited by SO suggests its high therapeutic value. However, further studies are warranted to confirm this hypothesis.

In summary, the effect of SO might be associated with maintaining GSH levels, trapping reactive oxygen species, decreasing lipid peroxidation, and maintaining the architecture of liver tissue after the onset of APAP poisoning in rats.

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