Scientific Research Publishing

Food and Nutrition Sciences, 2020, 11, 1053-1069
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Hepatic Protective Effects of *S*-Allyl-L-Cysteine (SAC) in Rats with Carbon Tetrachloride (CCl_4) Induced Liver Injury

Soo-Nyun Choi[1], H. M. Arif Ullah[1,2], Il-Hwa Hong[3], Jin-Kyu Park[1], SunYoung Park[1,2], Myung-Jin Chung[1,2], Ji-Yoon Son[1,2], Hyun-Ho Yun[1,2], Jae-Hyuk Yim[1,2], Seung-Jun Jung[1,2] Hae-Young Chung[4], Kyu-Shik Jeong[1,2*]

[1]Department of Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea
[2]Stem Cell Therapeutic Research Institute, Kyungpook National University, Daegu, Republic of Korea
[3]Department of Pathology, College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea
[4]Department of Pharmacy, College of Pharmacy, Pusan National University, Busan, Republic of Korea
Email: *jeongks@knu.ac.kr

How to cite this paper: Choi, S.-N., Ullah, H.M.A., Hong,I.-H., Park, J.-K., Park, S.Y., Chung, M.-J., Son, J.-Y., Yun, H.-H., Yim, J.-H., Jung, S.-J., Chung, H.-Y. and Jeong, H.-S. (2020) Hepatic Protective Effects of *S*-Allyl-L-Cysteine (SAC) in Rats with Carbon Tetrachloride (CCl_4) Induced Liver Injury. *Food and Nutrition Sciences*, **11**, 1053-1069.
https://doi.org/10.4236/fns.2020.1112074

Received: January 26, 2020
Accepted: December 12, 2020
Published: December 15, 2020

Abstract

S-allyl-L-cysteine (SAC) is an organosulfur compound derived from aged garlic extract (AGE). Studies have reported that AGE possesses bioprotective capacity, including antidiabetic, antimicrobial, antioxidant, and antitumor effects. The present study examined the protective effects of SAC against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. Ten male Wistar rats aged 11 - 12 weeks were randomly divided into two groups (five rats/group) as control and SAC groups. All rats had ad libitum access to water, and the SAC group received water containing SAC intragastrically (200 mg/kg) once daily for five consecutive weeks. In the fifth experimental week, 50% CCl_4 in olive oil (1 mL/kg) was administered intraperitoneally three times a week to induce liver injury in both groups. Rats were sacrificed at 24 hours after the last CCl_4 injection, and liver tissues were excised for histopathological, immunohistochemical and antioxidant analyses. The rats in the SAC group did not show abnormal behavior, such as decreased water intake or food consumption, during the experimental period. Body weights in all groups did not change significantly over the experimental period. Histopathological analysis showed that the percentage of hepatic steatosis was lower in the SAC group at 12.75% ± 3.74% compared to 24.64% ± 5.29% in the control group ($p < 0.05$). The percentage of cytochrome P4502E1 (CYP2E1) distribution area in the SAC group was also lower at 19.61% ± 6.18% compared with 25.22% ± 6.21% in the control group ($p < 0.05$). These results suggest that SAC can alleviate CCl_4-induced liver damage by decreasing hepatic steatosis and reducing CYP2E1 expression in rats.

Keywords

S-Allyl-L-Cysteine (SAC), Garlic, Hepatic Protective Effects, Carbon Tetrachloride, Rats, Hepatic Steatosis, Cytochrome P4502E1

1. Introduction

Oxidative damages to hepatic cells are common sequelae of many liver diseases, including hepatic steatosis, chronic hepatitis, cholestasis, and liver cirrhosis [1]. Oxidative stress, a consequence of an imbalance between pro and antioxidants in cells and tissues, is generally defined as excess formation or insufficient removal of damaging molecules, such as reactive oxygen species (ROS). ROS have been implicated in pathophysiological changes in the liver, particularly in viral infections, cholestasis, and alcohol abuse [2]. Excessive alcohol consumption generates ROS in the liver where alcohol is metabolized, disrupting the liver's cellular antioxidant system [3] [4]. ROS can bind to cellular macromolecules, including proteins, lipids, and DNA, leading to physiological dysfunction.

Carbon tetrachloride (CCl_4) has been frequently used to produce xenobiotic induced and free radical mediated hepatotoxicity in animal models [5]. Its toxicity requires bioactivation to produce reactive metabolic intermediates using a trichloromethyl radical like $CCl_3^{\bullet}$ or peroxy trichloromethyl radicals ($CCl_3OO^{\bullet}$) by mixed function oxidase cytochrome P450 (CYP450) in hepatic microsomes [6]. These free radicals can bind to polyunsaturated fatty acids (PUFAs) and form alkoxy ($R^{\bullet}$) and peroxy radicals ($ROO^{\bullet}$), which serially generate lipid peroxide, damage cell membranes, and disrupt antioxidant enzyme activity and antioxidative substrate content, resulting in hepatic injury [7]. Although several isoforms of CYP450 can metabolize CCl_4, the focus has been on catalase and cytochrome P4502E1 (CYP2E1), which are ethanol inducible [8] [9]. The histopathological features of CCl_4 induced hepatotoxicity are fatty liver, vacuolar degeneration, inflammation, necrosis, fibrosis, and cirrhosis [10] [11]. Mechanisms for preventing hepatotoxicity induced by oxidative stress involve blocking the chain reaction of oxidation, improving phase II antioxidant enzyme activity, and increasing levels of antioxidant substances.

Garlic (*Allium sativum* Lynn.) has been used as a spice and as a folk medicine for centuries [12]. In recent years, studies have reported that garlic possesses bioprotective attributes, such as antidiabetic, antimicrobial, antioxidant, antithrombotic, anticancer, and cholesterol lowering effects [13] [14] [15] [16]. The health benefits of garlic derive from a diversity of its constituents, and it is administered in different preparations depending on use. Among its many components, *S*-allyl-L-cysteine (SAC) is a naturally occurring, water soluble, nontoxic, and odorless organosulfur compound made from aged garlic extract (AGE). AGE is produced by aging for up to 20 months to reduce garlic's harsh taste and odor [17]. Pharmacokinetic studies of SAC, the most abundant organosulfur compound in AGE (6.1 ± 2.7 mg/g dry extract), have revealed that SAC

is easily absorbed in the rat gastrointestinal tract and is distributed to the plasma, liver, kidneys, lungs, and heart with a bioavailability of 98% [17]. Furthermore, SAC is 30 fold less toxic than other garlic components, such as allicin and diallyl disulfide, and relatively inexpensive and easy to synthesize [17]. SAC has been reported to prevent amyloidogenesis, atherosclerosis, glycation, and cholesterol synthesis and to reduce mortality via a decreased incidence of stroke [18] [19] [20] [21]. SAC was also found to exert dose dependent inhibition of nuclear factor kappa B (NF-KB) activation in human T lymphocytes (Jurkat cells) induced by tumor necrosis factor α (TNF-α) and hydrogen peroxide (H_2O_2) [22]. SAC also mediates chemopreventive effects in carcinogenesis [23] and has been shown to be the active compound responsible for mitigating oxidative stress [24] [25] [26] [27] [28]. However, evidence of potential hepato-protective effects of SAC remains insufficient. In the present study, we examined the hepatoprotective properties of SAC against CCl_4 induced hepatotoxicity in a rat model.

2. Materials and Methods

2.1. Chemicals

SAC was obtained from Tokyo Chemical Industry Co., Ltd. (Japan) at a purity of more than 98.0%, as shown by gas chromatography. The water soluble test compound SAC was dissolved in tap water. CCl_4 (Sigma-Aldrich Co., USA) was diluted in olive oil (Sigma-Aldrich Co., USA) to produce 50% CCl_4. All other chemicals and solvents were of the highest grade commercially available.

2.2. Experimental Animals

Male Wistar rats (11 - 12 weeks old) weighing 353.06 ± 22.09 g were housed in conventional cages with ad libitum access to water and a standard rodent chow diet. Animals were housed in a controlled environment at a temperature of 22˚C ± 3˚C and a relative humidity of 50% ± 10% with a 12/12h light/dark cycle. Animals were randomly divided into two groups: the control group received 50% CCl_4 in olive oil (1 mL/kg) intraperitoneally three times in the fifth week; the SAC group was treated intragastrically with water containing SAC (200 mg/kg) once daily for the first four weeks and the same amount of CCl_4 in olive oil was administered at the same frequency in the fifth (**Figure 1**). Animals were sacrificed at 24 hours after the last CCl_4 dose and liver tissues were excised for histopathological, immunohistochemical and antioxidant analyses. All experimental procedures were performed in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

2.3. Determination of Antioxidant Enzymes

Superoxide dismutase (SOD) activity was measured according to the method described by Beauchamp and Fridovich [29]. An adequate amount of liver supernatant was mixed with the reaction mixtures containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 25 mM nitroblue tetrazolium (NBT), 0.1 mM

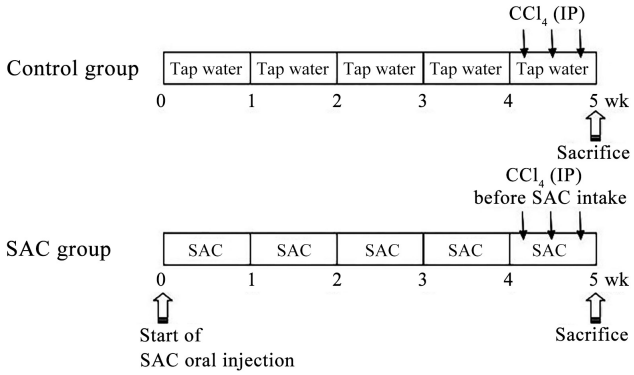

Figure 1. Experimental design of the study.

xanthine, 50 mM sodium carbonate buffer (pH 10.2), and distilled water to a final volume of 3 mL. The reaction was initiated by the addition of 2 mU/mL xanthine oxidase and maintained under two 40 W lamps at 25˚C. After 15 min, the inhibition rate of NBT reduction was spectrophotometrically determined at 560 nm. One unit of SOD was defined as the amount of enzyme required to reduce NBT by 50%. The specific activity of SOD is expressed as unit/mg for each supernatant. Catalase activity was measured according to the method described by Aebi [30]. One unit of catalase was defined as the amount of enzyme required to decompose 1.0 μM of H_2O_2 in 1 min. The reaction was initiated by the addition of 1.0 mL of freshly prepared 20 mM H_2O_2. The decomposition rate of H_2O_2 was measured spectrophotometrically at 240 nm at 1 min. Enzyme activity is expressed as unit/mg.

2.4. Determination of Glutathione Levels

Glutathione (GSH) and glutathione disulfide (GSSG) were prepared in 0.1 M sodium phosphate containing 0.005 M EDTA buffer (pH 8.0) and kept on ice until used. *O*-phthalaldehyde (OPT) solution was prepared in reagent-grade absolute methanol just prior to use. Liver samples were homogenized on ice with a homogenizer in a solution containing 3.75 mL of phosphate EDTA buffer and 1 mL of 25% H_3PO_4 for protein precipitation. Total homogenate was centrifuged at 4˚C at 100,000 *g* for 20 min, and the supernatant was then decanted to assess GSH and GSSG levels. Determination of GSH values was performed as described by Hissin and Hilf [31]. An aliquot of 4.5 mL of phosphate-EDTA buffer (pH 8.0) was added to 0.5 mL of the supernatant for a final assay mixture of 2.0 mL containing 100 μL of diluted tissue supernatant, 1.8 mL of phosphate EDTA buffer, and 100 μL of OPT solution containing 100 μg of OPT. After mixing and incubation at room temperature for 15 min, the solution was transferred to a cuvette. Fluorescence at 420 nm was assessed, with activation at 350 nm. In the GSSG assay, a 0.5 mL portion of the supernatant generated above was incubated at room temperature with 200 μL of 0.04 M *N*-ethylmaleimida (NEM) for 30 min to interact with GSH in the tissues. An aliquot of 4.3 mL of 0.1 N NaOH was added to the mixture, and a 100 μL portion thereof was used to measure

GSSG as described above, using 0.1 M NaOH as a diluent rather than phosphate-EDTA buffer. Results are expressed as nM/mg.

2.5. Determination of Lipid Peroxidation

Liver homogenate was assayed for lipid peroxidation level using a lipid peroxidation (LPO)-586 assay kit (OxisResearch, Portland, OR, USA) according to the manufacturer's instructions. This assay is based on the reaction of a chromogenic reagent, *N*-methyl-2-phenyl indole, with malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) at 45˚C. One molecule of either MDA or HAE reacts with two molecules of chromogenic reagent to yield a stable chromophore with maximal absorbance at 586 nm. Aliquots of 200 µL of samples were mixed with 650 µL of chromogenic reagent and 150 µL of methanesulfonic acid. Preparations were incubated at 45˚C for 60 min and then centrifuged at 15,000 *g* for 10 min. The supernatant was transferred to a cuvette and its absorbance was measured at 586 nm. MDA and MDA + HAE values were calculated using an MDA standard curve. The detection limit for the assay was 0.1 nmol/mL in the final reaction medium. Results are expressed as nM/mg.

2.6. Determination of ROS Generation

Intracellular production of ROS was measured with 2',7'-dichlorofluorescein diacetate (H_2DCF-DA) (Molecular Probes, Eugene, OR, USA). This nonpolar compound is converted to the polar, membrane-impermeable derivative H_2DCF by esterases upon cellular uptake. H_2DCF is nonfluorescent but is rapidly oxidized to the highly fluorescent DCF by intracellular H_2O_2 and other peroxides [32] [33]. Stocks of H_2DCF-DA were made in absolute ethanol at a concentration of 12.5 mM and stored at −80˚C in the dark under argon. The 25 µM H_2DCF-DA was added to the liver homogenate at a final concentration of 250 µM. After 30 min incubation, the homogenate was collected in a microcentrifuge, and the supernatant was removed and diluted 50-fold. Fluorescence was measured with excitation and emission wavelengths of 485 nm and 535 nm, respectively. Results are expressed as fluorescence/min/mg.

2.7. Histopathology

Liver tissue blocks were fixed in 10% neutral buffered formalin and processed routinely. Paraffin embedded liver sections were cut into 4 - 5 µm slices. Liver tissue blocks were then stained with hematoxylin and eosin (H&E) for histopathological analysis.

2.8. Immunohistochemistry

Liver sections were deparaffinized in xylene and toluene, rehydrated in a series of graded alcohol concentrations, incubated in 3% hydrogen peroxide (H_2O_2) in methanol for 30 min and microwaved at 750 W for 10 min in 10 mmol/L citrate buffer (pH 6.0). Tissue sections were washed with PBS. After blocking with rabbit serum (Vector Laboratories, Burlingame, CA, USA) for 1 h at room temper-

ature, sections were immunostained using anti-catalase and anti-cytochrome P4502E1 (CYP2E1) antibodies (Chemicon International, Inc., USA) at a dilution of 1:800. Antigen antibody complexes were visualized with an avidin biotin peroxidase complex solution using an ABC kit (Vector Laboratories, Burlingame, CA, USA). The sections were subsequently rinsed in distilled water and counterstained with Mayer's hematoxylin.

2.9. Histomorphometry

Histomorphometry was performed with a computerized analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD). Areas of the tissue sections to be measured were captured by a microscope connected to the system. Calibration was performed according to the instructions accompanying the software. The area of hepatic steatosis on H&E slides and the distribution area of CYP2E1 in immunohistochemistry were measured. Results are expressed as percentages of stained area.

2.10. Statistical Analysis

All data are presented as mean ± SD. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by *Student's t*-test. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Body Weight of Experimental Rats

The rats in the SAC group did not show abnormal behavior, such as decreased water intake or food consumption, and their body weights did not change significantly ($p < 0.05$) from the control group prior to CCl_4 treatment. After CCl_4 dosing, rats in the control group showed a slight reduction in body weight, although this reduction was not significantly different ($p > 0.05$) from the SAC group (Figure 2).

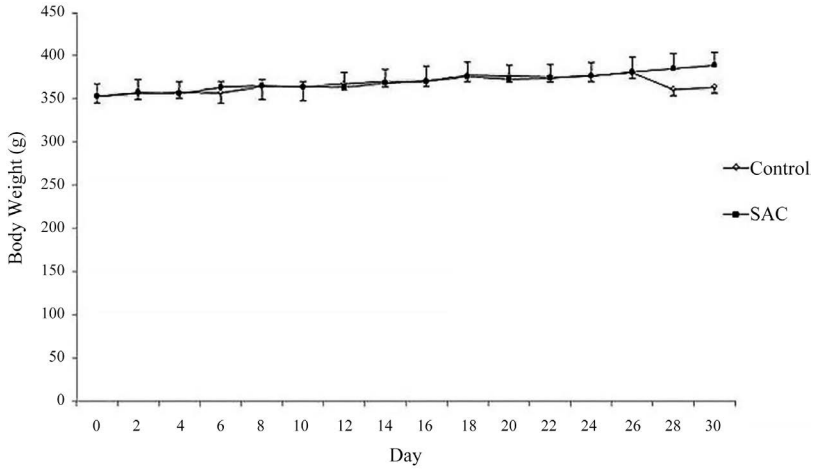

Figure 2. Body weight measurements of experimental animals; bars represent mean values ± SD of 5 animals.

3.2. SAC Effects on Antioxidant Enzymes

We measured superoxide dismutase (SOD) and catalase activities as indicators of oxidative stress. Hepatic SOD activity was 0.41 ± 0.09 unit/mg in the control group, while that in the SAC group increased to 0.46 ± 0.10 unit/mg. Catalase activity in liver tissue in the control group was 1.71 ± 0.52 unit/mg, but treatment with SAC resulted in an increase thereof to 3.69 ± 0.26 unit/mg ($p < 0.05$) over the control group (Figure 3).

3.3. SAC Effects on Glutathione Levels

Glutathione is the first line of defense against free radicals. Levels of glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG ratio, and total liver glutathione (GSH + GSSG) are shown in Figure 4. GSH in the control group was 1.37 ± 0.24 nM/mg, while that in the SAC group increased to 2.14 ± 0.38 nM/mg ($p < 0.05$). GSSG was 0.94 ± 0.18 nM/mg in controls and decreased to 0.84 ± 0.10 nM/mg in the SAC group ($p < 0.05$). The GSH/GSSG ratio of 1.83 ± 0.59 in the control group was lower than the 2.75 ± 0.56 in the SAC group. Total glutathione (GSH + GSSG) in the control group was 2.31 ± 0.20 nM/mg, while that in the SAC group increased to 2.98 ± 0.35 nM/mg ($p < 0.05$) (Figure 4).

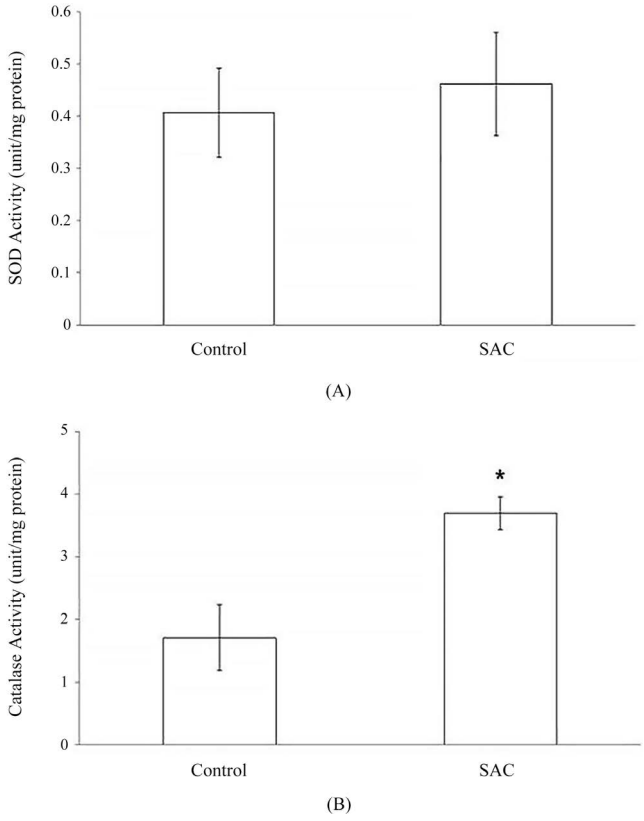

Figure 3. Effects of SAC on hepatic superoxide dismutase (SOD) and catalase activity in CCl_4 induced hepatotoxicity in rats: (**A**) SOD activity in control and SAC groups (mean ± SD) was 0.41 ± 0.09 and 0.46 ± 0.10 unit/mg, respectively. (**B**) Catalase activity was 1.71 ± 0.52 and 3.69 ± 0.26 unit/mg in control and SAC groups (mean ± SD), respectively. Bars represent mean values ± SD of 5 animals. *$P < 0.05$.

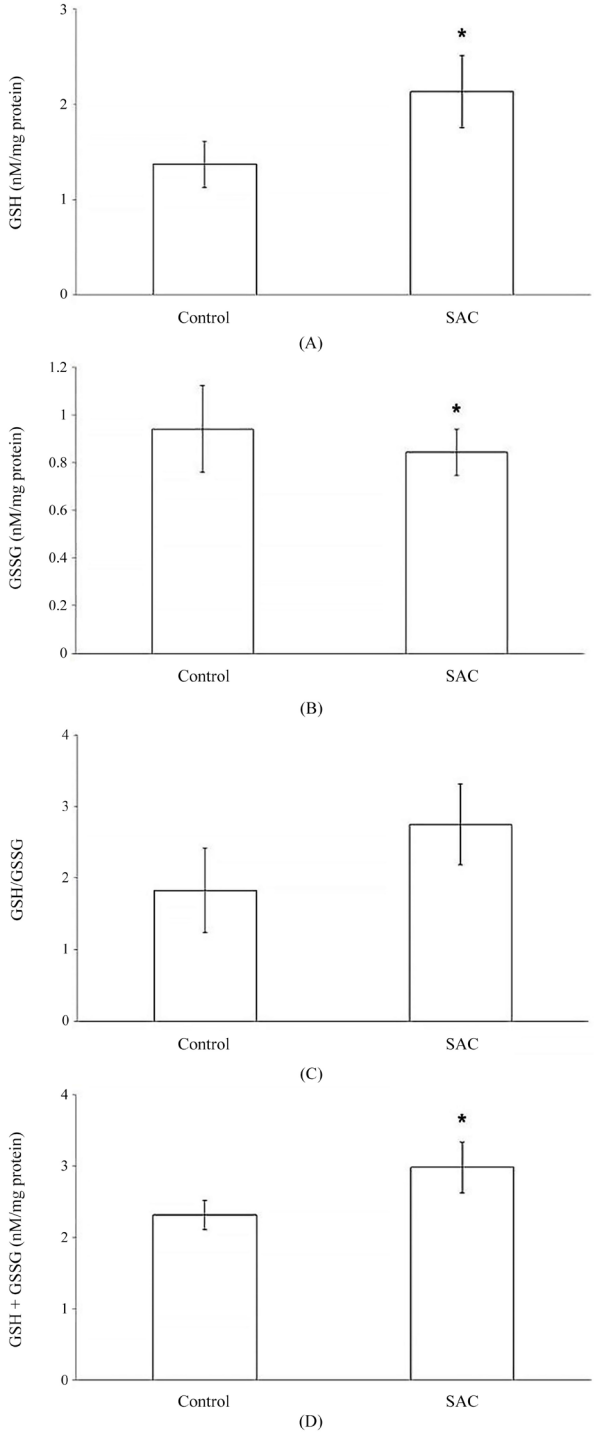

Figure 4. Effects of SAC on reduced hepatic glutathione (GSH) concentration, oxidized glutathione (GSSG) concentration, hepatic GSH/GSSG ratio, and total glutathione (GSH + GSSG) following CCl_4-induced hepatotoxicity in rats: (**A**) GSH concentrations were 1.37 ± 0.24 and 2.14 ± 0.38 nM/mg in control and SAC groups (mean ± SD), respectively. (**B**) GSSG concentrations in control and SAC groups (mean ± SD) were 0.94 ± 0.18 and 0.84 ± 0.10 nM/mg, respectively. (**C**) The GSSH/GSSG ratios in control and SAC groups (mean ± SD) were 1.83 ± 0.59 and 2.75 ± 0.56 nM/mg, respectively. (**D**) The GSH + GSSG levels in control and SAC groups (mean ± SD) were 2.31 ± 0.20 and 2.98 ± 0.35 nM/mg, respectively. Bars represent mean values ± SD of 5 animals. *$P < 0.05$.

3.4. SAC Effects on Lipid Peroxidation and ROS Generation

Lipid peroxidation is critically implicated in CCl_4-induced hepatotoxicity. Malondialdehyde (MDA) and 4-hydroxyalkenals (HAE), end products of lipid peroxidation, are common markers thereof. MDA and MDA + HAE contents in the SAC group were lower than those in the control group (Figure 5), suggesting that CCl_4-induced oxidative stress was slightly mitigated by SAC treatment. ROS

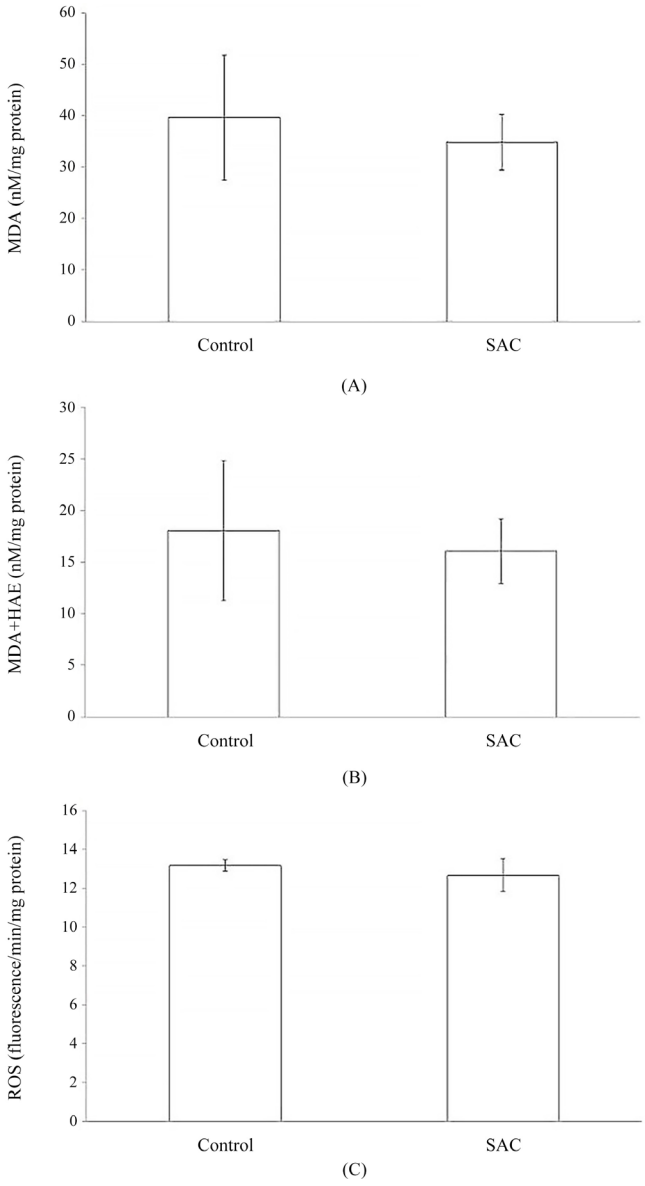

Figure 5. Effects of SAC on hepatic malondialdehyde (MDA) concentration, MDA + 4-hydroxyalkenals (HAE) concentration, and hepatic reactive oxygen species (ROS) production in CCl_4-induced hepatotoxicity in rats: (**A**) MDA production by liver tissue in control and SAC groups (mean ± SD) was 39.7 ± 12.2 and 34.9 ± 5.38 nM/mg, respectively. (**B**) MDA + HAE production by liver tissue was 18.1 ± 6.76 (control) and 16.1 ± 3.11 nM/mg (SAC). (**C**) ROS production in control and SAC groups (mean ± SD) was 13.2 ± 0.31 and 12.7 ± 0.85 fluorescence/min/mg, respectively. Bars represent mean values ± SD of 5 animals.

generation was measured to examine SAC effects on radical scavenging during hepatic CCl_4 toxicity. Figure 5 shows the amount of fluorescent dichlorofluorescein (DCF) in the liver tissues of rats from the SAC and control groups as an index of ROS formation. DCF content in the SAC group was slightly diminished compared to the control group, indicating that SAC attenuated CCl_4 induced free radicals.

3.5. Histopathological Change after SAC Treatment

Histopathological changes in liver sections after CCl_4 administration were assessed in control and SAC groups (Figure 6). Typical intense fatty hepatocytes surrounding central veins were observed in CCl_4-treated rats (Figure 6(A) and Figure 6(C)). Centrilobular necrosis, inflammatory cell infiltration, and ballooning degeneration were also observed (Figure 6(A) and Figure 6(C)). These hepatic histological changes were markedly reduced by treatment with SAC (Figure 6(B) and Figure 6(D)). SAC administration also significantly decreased lipid droplets and areas of lipid laden cells.

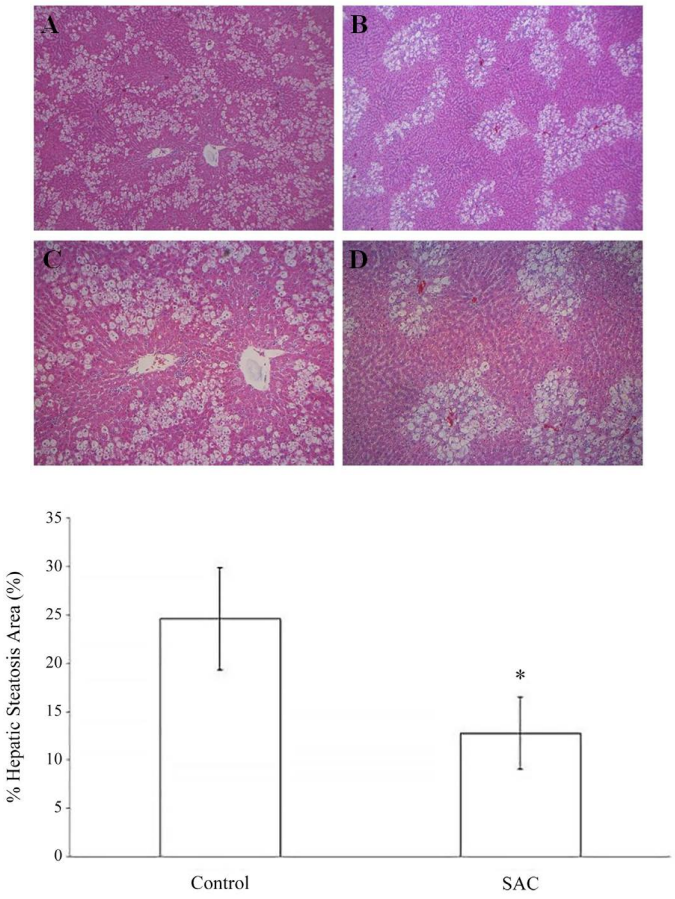

Figure 6. Accumulation of fat droplets and percentage of hepatic steatosis in the rat liver: (**A** and **C**) hepatic steatosis in control group and (**B** and **D**) hepatic steatosis reduced in SAC group. The average hepatic steatosis area percentages were 24.64 ± 5.29 (control) and 12.75 ± 3.74 (SAC) (mean ± SD). *$P < 0.05$. Hematoxylin & eosin (H&E) staining. Bars represent mean values ± SD of 5 animals. Magnification: (A, B) 50X; (C, D) 100X.

3.6. Immunohistochemical Changes after SAC Treatment

Immunohistochemical changes to CYP2E1 levels were observed after CCl_4 administration in liver sections in both control and SAC groups. CYP2E1 expression in the control group was found at the injured hepatocytes around central veins in a pattern similar to hepatic steatosis (Figure 7(A) and Figure 7(C)), demonstrating that histological expression of CYP2E1 in the SAC group was repressed in CCl_4 induced hepatocyte damage (Figure 7(B) and Figure 7(D)).

3.7. Hepatic Histomorphometry after SAC Treatment

The percentages of hepatic steatosis area and CYP2E1 distribution area are shown in Figure 6 and Figure 7. The SAC group showed less hepatic steatosis and less hepatic CYP2E1 than the control group ($p < 0.05$, both) (Figure 6 and Figure 7).

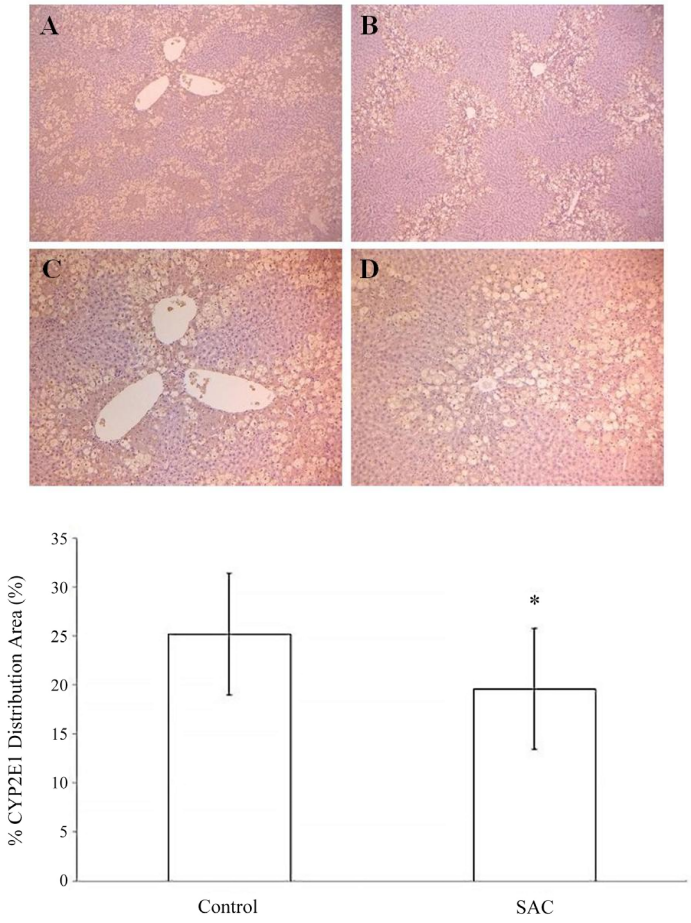

Figure 7. Immunohistochemical expression and percentage of cytochrome P4502E1 (CYP2E1) distribution area in a CCl_4-induced hepatotoxic rat liver: (**A** and **C**) immunohistochemical assay of CYP2E1 in control group and (**B** and **D**) immunohistochemical assay of CYP2E1 significantly reduced numbers of injured hepatocytes around the central vein in SAC group. The average CYP2E1 distribution area percentages in control and SAC groups (mean ± SD) were 25.22 ± 6.21 and 19.61 ± 6.18, respectively.*$P < 0.05$. Original magnification: (A, B) 50X, (C, D) 100X.

4. Discussion

Studies have shown that various components of garlic preparations exhibit a wide range of physiological, biochemical, and pharmacological effects [13] [16]. Garlic (*Allium sativum* Lynn.) has long been considered a valuable spice by many cultures and is still used today for its medicinal properties. Aged garlic extract (AGE) is produced by aging for up to 20 months, reducing garlic's harsh taste and odor [17]. SAC is a major water soluble organosulfur compound derived from AGE whose bioprotective capacity has been described in many reports. Its bioavailability has been well established in animal models as it has been shown to be easily absorbed and evenly distributed [17].

CCl_4 treatment has been shown to cause high levels of oxidative damage by significant increases in lipid peroxidation products, ROS generation, and hepatotoxic features, and by significant decreases in GSH concentration and antioxidant enzyme activity. In this study, treatment with SAC was associated with a significant protective effect against CCl_4 induced acute hepatic injury in rats. Superoxide dismutase (SOD) and catalase are major antioxidant enzymes that catalyze ROS in most cells; these enzymes are critical for the elimination of ROS via reduction of xenobiotics in liver tissue and are easily inactivated by lipid peroxide or ROS. SOD is an extremely effective antioxidant enzyme responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) [34]. SOD is most abundant in the liver and catalyzes H_2O_2 to oxygen (O_2) and water (H_2O) [35]. In this study, SOD and catalase activities were significantly increased in the SAC treated group (**Figure 3**), suggesting that SAC could restore or activate SOD and catalase enzymes in CCl_4 damaged liver tissue.

GSH (γ-glutamylcysteinylglycine) is an intracellular and extracellular nonenzymatic antioxidant that acts in conjunction with enzymatic processes to reduce H_2O_2 and lipid hydroperoxides [36] [37]. It has also been theorized to contribute to the conservation of normal cell structure and function via reduction and detoxification reactions. The reduced form of GSH helps detoxify reactive, toxic metabolites of CCl_4 [5]. In the present study, treatment with SAC was shown to significantly ameliorate CCl_4 induced depletion of reduced GSH and elevation of GSSG, an oxidized form of GSH, in rat livers (**Figure 4**). Total GSH + GSSG levels were significantly elevated and GSH/GSSG ratios were increased in the SAC treated group compared with controls (**Figure 4**). Glutathione results pointed to both the restoration of GSH and elimination of GSSG by antioxidant SAC effects in CCl_4 treated rats. Specially, thiol group-containing agents such as cysteine (Cys), thioproline, 2-mercaptopropionylglycine, captopril, and *N*-acetylcysteine have been reported to possess antioxidant capacity based on an association with increased amounts of reduced GSH [38]-[44]. SAC possesses a Cys structure, but without the SH residue, and may exert its protective activity by supplying Cys to hepatocytes.

Lipid peroxidation is an ROS mediated mechanism implicated in the pathogenesis of liver injuries in experimental animals and humans [45] [46]. MDA

and HAE are major reactive aldehydes that present during the decomposition of PUFAs in biological membranes and are reliable markers of lipid peroxidation [6]. Hepatic contents of MDA and HAE are often assessed as indicators of liver tissue damage involving a series of chain reactions. Lipid peroxidation of hepatocyte membranes is a principal cause of CCl_4 induced hepatotoxicity that is mediated by the production of free radical derivatives of CCl_4 [5] [29]. In this study, amounts of hepatic MDA and MDA + HAE in the SAC group were reduced compared with the control group (**Figure 5**), suggesting that treatment with SAC could reduce hepatic lipid peroxidation induced by CCl_4 in rats. ROS production was examined to evaluate the scavenging of free radicals by SAC and decreased concentrations of hepatic ROS in the SAC group indicate that SAC could scavenge free radicals produced by CCl_4 in rats (**Figure 5**).

Hepatocellular steatosis, inflammation, necrosis, and vacuolar degeneration induced by CCl_4 were more pronounced in the control group compared with the SAC group (**Figure 6**). Liver tissue damaged by CCl_4 was accompanied by CYP2E1 expression, as evidenced by immunohistochemistry, in a pattern similar to hepatic fatty changes around central veins in the control group; this pattern was less prevalent in the SAC treated group (**Figure 7**) and could indicate antioxidant action by SAC. Histomorphometry of liver sections with H&E staining and immunostained for CYP2E1 revealed that SAC treatment significantly reduced the average percentages of steatosis and CYP2E1 distribution areas (**Figure 6** and **Figure 7**). Although a previous study found that SAC did not inhibit hepatic CYP2E1 protein expression nor CYP2E1 mRNA levels [47], in this study, SAC treatment was associated with significantly reduced immunohistochemical CYP2E1 expression in liver tissue compared to controls (**Figure 6** and **Figure 7**). This activity suggests SAC as a potential inhibitor of P4502E1.

5. Conclusion

In conclusion, this study demonstrated that SAC treatment was associated with the alleviation of CCl_4 induced oxidative liver injury, accompanied by an elevation in antioxidant enzyme expression and action, and increased levels of reduced glutathione (GSH) together with decreased oxidized glutathione (GSSG) content, inhibition of lipid peroxidation, scavenging of free radicals, and CYP2E1 mediated hepatic steatosis induced by CCl_4. Taken together, our findings indicate that SAC could be an effective hepatoprotective dietary supplement. Future studies are needed, however, to explore the potential of SAC to reduce hepatotoxicity in humans.

Acknowledgements

This research supported by the National Research Foundation of Korea (NRF-2017R1E1A1A01072781).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Gong, Z., Ye, H., Huo, Y., Wang, L., Huang, Y., Huang, M. and Yuan, X. (2018) *S*-Allyl-Cysteine Attenuates Carbon Tetrachloride-Induced Liver Fibrosis in Rats by Targeting STAT3/SMAD3 Pathway. *American Journal of Translational Research*, **10**, 1337.

[2] Kodai, S., Takemura, S., Minamiyama, Y., Hai, S., Yamamoto. S., Kubo, S., Yoshida, Y., Niki, E., Okada, S., Hirohashi, K. and Suehiro, S. (2007) *S*-Allyl Cysteine Prevents CCl_4-Induced Acute Liver Injury in Rats. *Free Radical Research*, **41**, 489-497. https://doi.org/10.1080/10715760601118361

[3] Chen, P., Hu, M., Liu, F., Yu, H. and Chen, C. (2019) S-Allyl-L-Cysteine (SAC) Protects Hepatocytes from Alcohol-Induced Apoptosis. *FEBS Open Bio*, **9**, 1327-1336. https://doi.org/10.1002/2211-5463.12684

[4] Sid, B., Verrax, J. and Calderon, P.B. (2013) Role of Oxidative Stress in the Pathogenesis of Alcohol-Induced Liver Disease. *Free Radical Research*, **47**, 894-904. https://doi.org/10.3109/10715762.2013.819428

[5] Recknagel, R.O., Glende Jr., E.A., Dolak, J.A. and Waller, R.L. (1989) Mechanisms of Carbon Tetrachloride Toxicity. *Pharmacology & Therapeutics*, **43**, 139-154. https://doi.org/10.1016/0163-7258(89)90050-8

[6] Manibusan, M.K., Odin, M. and Eastmond, D.A. (2007) Postulated Carbon Tetrachloride Mode of Action: A Review. *Journal of Environmental Science and Health, Part C*, **25**, 185-209. https://doi.org/10.1080/10590500701569398

[7] McCay, P.B., Lai, E.K., Poyer, J.L., DuBose, C.M. and Janzen, E.G. (1984) Oxygen- and Carbon-Centered Free Radical Formation during Carbon Tetrachloride Metabolism. Observation of Lipid Radicals *in Vivo* and *in Vitro*. *The Journal of Biological Chemistry*, **259**, 2135-2143.

[8] Cederbaum, A.I. (2017) Role of Cytochrome P450 and Oxidative Stress in Alcohol-Induced Liver Injury. *Reactive Oxygen Species*, **4**, 303-319. https://doi.org/10.20455/ros.2017.851

[9] Zangar, R.C., Benson, J.M., Burnett, V.L. and Springer, D.L. (2000) Cytochrome P4502E1 Is the Primary Enzyme Responsible for Low-Dose Carbon Tetra Chloride Metabolism in Human Liver Microsomes. *Chemico-Biological Interactions*, **125**, 233-243. https://doi.org/10.1016/S0009-2797(00)00149-6

[10] Brunt, E.M. (2007) Pathology of Fatty Liver Disease. *Modern Pathology*, **20**, S40-S48. https://doi.org/10.1038/modpathol.3800680

[11] Lee, C.P., Shih, P.H., Hsu, C.L. and Yen, G.C. (2007) Hepatoprotection of Tea Seed oil (*Camellia oleifera* Abel.) against CCl_4-Induced Oxidative Damage in Rats. *Food and Chemical Toxicology*, **45**, 888-895. https://doi.org/10.1016/j.fct.2006.11.007

[12] Ayaz, E. and Alpsoy, H.C. (2007) Garlic (*Allium sativum*) and Traditional Medicine. *Türkiye Parazitoloji Dergisi*, **31**, 145-149.

[13] Ahmad, M.S. and Ahmed, N. (2006) Antiglycation Properties of Aged Garlic Extract: Possible Role in Prevention of Diabetic Complications. *The Journal of Nutrition*, **136**, 796S-799S. https://doi.org/10.1093/jn/136.3.796S

[14] Ariga, T. and Seki, T. (2006) Antithrombotic and Anticancer Effects of Garlic-Derived Sulfur Compounds: A Review. *BioFactors*, **26**, 93-103. https://doi.org/10.1002/biof.5520260201

[15] Kodera, Y., Ushijima, M., Amano, H., Suzuki, J.I. and Matsutomo, T. (2017) Chemical and Biological Properties of S-1-Propenyl-L-Cysteine in Aged Garlic Extract. *Molecules*, **22**, 570. https://doi.org/10.3390/molecules22040570

[16] Colín-González, A.L., Santana, R.A., Silva-Islas, C.A., Chánez-Cárdenas, M.E., Santamaría, A. and Maldonado, P.D. (2012) The Antioxidant Mechanisms Underlying the Aged Garlic Extract and *S*-Allyl-Cysteine-Induced Protection. *Oxidative Medicine and Cellular Longevity*, **2012**, Article ID: 907162. https://doi.org/10.1155/2012/907162

[17] Amagase, H., Petesch, B.L., Matsuura, H., Kasuga, S. and Itakura, Y. (2001) Intake of Garlic and Its Bioactive Components. *The Journal of Nutrition*, **131**, 955S-962S. https://doi.org/10.1093/jn/131.3.955S

[18] Ahmad, M.S., Pischetsrieder, M. and Ahmed, N. (2007) Aged Garlic Extract and *S*-Allyl Cysteine Prevent Formation of Advanced Glycation Endproducts. *European Journal of Pharmacology*, **561**, 32-38. https://doi.org/10.1016/j.ejphar.2007.01.041

[19] E, Abdel. Moneim. A. (2015) Oxidant/Antioxidant Imbalance and the Risk of Alzheimer's Disease. *Current Alzheimer Research*, **12**, 335-349. https://doi.org/10.2174/1567205012666150325182702

[20] Kim, J.M., Chang, N., Kim, W.K. and Chun, H.S. (2006) Dietary *S*-Allyl-L-Cysteine Reduces Mortality with Decreased Incidence of Stroke and Behavioral Changes in Stroke-Prone Spontaneously Hypertensive Rats. *Bioscience, Biotechnology, and Biochemistry*, **70**, 1969-1971. https://doi.org/10.1271/bbb.50697

[21] Ried, K., Toben, C. and Fakler, P. (2013) Effect of Garlic on Serum Lipids: An Updated Meta-Analysis. *Nutrition Reviews*, **71**, 282-299. https://doi.org/10.1111/nure.12012

[22] Geng, Z., Rong, Y. and Lau, B.H. (1997) *S*-Allyl Cysteine Inhibits Activation of Nuclear Factor Kappa B in Human T Cells. *Free Radical Biology and Medicine*, **23**, 345-350. https://doi.org/10.1016/S0891-5849(97)00006-3

[23] Sundaresan, S. and Subramanian, P. (2003) S-Allylcysteine Inhibits Circulatory Lipid Peroxidation and Promotes Antioxidants in N-Nitrosodiethylamine-Induced Carcinogenesis. *Polish Journal of Pharmacology*, **55**, 37-42.

[24] Hsu, C.C., Lin, C.C., Liao, T.S. and Yin, M.C. (2006) Protective Effect of *S*-Allyl Cysteine and *S*-Propyl Cysteine on Acetaminophen-Induced Hepatotoxicity in Mice. *Food and Chemical Toxicology*, **44**, 393-397. https://doi.org/10.1016/j.fct.2005.08.012

[25] Casanova, A.G., Vicente-Vicente, L., Hernández-Sánchez, M.T., Pescador, M., Prieto, M., Martínez-Salgado, C., Morales, A.I. and López-Hernández, F.J. (2017) Key Role of Oxidative Stress in Animal Models of Aminoglycoside Nephrotoxicity Revealed by a Systematic Analysis of the Antioxidant-to-Nephroprotective Correlation. *Toxicology*, **385**, 10-17. https://doi.org/10.1016/j.tox.2017.04.015

[26] Padmanabhan, M. and Prince, P.S. (2006) Preventive Effect of *S*-Allyl-Cysteine on Lipid Peroxides and Antioxidants in Normal and Isoproterenol-Induced Cardiotoxicity in Rats: A Histopathological Study. *Toxicology*, **224**, 128-137. https://doi.org/10.1016/j.tox.2006.04.039

[27] Baradaran, A. and Rafieian-Kopaei, M. (2013) Histopathological Study of the Combination of Metformin and Garlic Juice for the Attenuation of Gentamicin Renal Toxicity in Rats. *Journal of Renal Injury Prevention*, **2**, 15.

[28] Sundaresan, S. and Subramanian, P. (2008) Prevention of *N*-Nitrosodiethylamine-Induced Hepatocarcinogenesis by S-Allylcysteine. *Molecular and Cellular Biochemistry*, **310**, 209-214.https://doi.org/10.1007/s11010-007-9682-4

[29] Beauchamp, C. and Fridovich, I. (1971) Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Analytical Biochemistry*, **44**, 276-287. https://doi.org/10.1016/0003-2697(71)90370-8

[30] Aebi, H. (1984) Catalase *in Vitro*. *Methods in Enzymology*, **105**, 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

[31] Hissin, P.J. and Hilf, R. (1976) A Fluorometric Method for Determination of Oxidized and Reduced Glutathione in Tissues. *Analytical Biochemistry*, **74**, 214-226. https://doi.org/10.1016/0003-2697(76)90326-2

[32] Gerber, I.B. and Dubery, I.A. (2003) Fluorescence Microplate Assay for the Detection of Oxidative Burst Products in Tobacco Cell Suspensions Using 2',7'-Dichlorofluorescein. *Methods in Cell Science*, **25**, 115-122. https://doi.org/10.1007/s11022-004-3851-6

[33] Myhre, O., Andersen, J.M., Aarnes, H. and Fonnum, F. (2003) Evaluation of the Probes 2',7'-Dichlorofluorescin Diacetate, Luminol, and Lucigenin as Indicators of Reactive Species Formation. *Biochemical Pharmacology*, **65**, 1575-1582. https://doi.org/10.1016/S0006-2952(03)00083-2

[34] Yim, M.B., Chock, P.B. and Stadtman, E.R. (1990) Copper, Zinc Superoxide Dismutase Catalyzes Hydroxyl Radical Production from Hydrogen Peroxide. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 5006-5010. https://doi.org/10.1073/pnas.87.13.5006

[35] Harris, E.D. (1992) Regulation of Antioxidant Enzymes. *FASEB Journal*, **6**, 2675-2683. https://doi.org/10.1096/fasebj.6.9.1612291

[36] Dierickx, P.J., Nuffel, G.V. and Alvarez, I. (1999) Glutathione Protection against Hydro Gen Peroxide, Tert-Butyl Hydroperoxide and Diamide Cytotoxicity in Rat Hepatoma-Derived Fa32 Cells. *Human & Experimental Toxicology*, **18**, 627-633. https://doi.org/10.1191/096032799678839482

[37] Kadiiska, M.B., Gladen, B.C., Baird, D.D., Dikalova, A.E., Sohal, R.S., Hatch, G.E., Jones, D.P., Mason, R.P. and Barrett, J.C. (2000) Biomarkers of Oxidative Stress Study: Are Plasma Antioxidants Markers of CCl_4 Poisoning? *Free Radical Biology and Medicine*, **28**, 838-845. https://doi.org/10.1016/S0891-5849(00)00198-2

[38] Acworth, I.N., McCabe, D.R. and Maher, T.J. (2017) The Analysis of Free Radicals, Their Reaction Products, and Antioxidants. In: Baskin, S. and Salem, H., Eds., *Oxidants, Antioxidants and Free Radicals*, Routledge, Boca Raton, 23-77. https://doi.org/10.1201/9780203744673-2

[39] Guayerbas, N., Puerto, M., Ferrandez, M.D. and De La Fuente, M. (2002) A Diet Supplemented with Thiolic Anti-Oxidants Improves Leucocyte Function in Two Strains of Prematurely Ageing Mice. *Clinical and Experimental Pharmacology and Physiology*, **29**, 1009-1014. https://doi.org/10.1046/j.1440-1681.2002.03758.x

[40] McCarty, M.F. and DiNicolantonio, J.J. (2015) An Increased Need for Dietary Cysteine in Support of Glutathione Synthesis May Underlie the Increased Risk for Mortality Associated with Low Protein Intake in the Elderly. *Age*, **37**, Article No. 96. https://doi.org/10.1007/s11357-015-9823-8

[41] Grudzien, M. and Rapak, A. (2018) Effect of Natural Compounds on NK Cell Activation. *Journal of Immunology Research*, **2018**, Article ID: 4868417 https://doi.org/10.1155/2018/4868417

[42] Kho, A., Choi, B., Lee, S., Hong, D., Lee, S., Jeong, J., Park, K.H., Song, H., Choi, H. and Suh, S. (2018) Effects of Protocatechuic Acid (PCA) on Global Cerebral Ischemia-Induced Hippocampal Neuronal Death. *International Journal of Molecular Sciences*, **19**, 1420. https://doi.org/10.3390/ijms19051420

[43] Fujiwara, N., Som, A.T., Pham, L.-D., Lee, B.J., Mandeville, E.T., Lo, E.H. and Arai, K. (2016) A Free Radical Scavenger Edaravone Suppresses Systemic Inflammatory Responses in a Rat Transient Focal Ischemia Model. *Neuroscience Letters*, **633**, 7-13. https://doi.org/10.1016/j.neulet.2016.08.048

[44] Suzuki, H., Matsumori, A., Matoba, Y., Kyu, B.S., Tanaka, A., Fujita, J. and Sasayama, S. (1993) Enhanced Expression of Superoxide Dismutase Messenger RNA in viral Myocarditis. An SH-Dependent Reduction of Its Expression and Myocardial Injury. *Journal of Clinical Investigation*, **91**, 2727-2733. https://doi.org/10.1172/JCI116513

[45] Liu, J.Y., Chen, C.C., Wang, W.H., Hsu, J.D., Yang, M.Y. and Wang, C.J. (2006) The Protective Effects of *Hibiscus sabdariffa* Extract on CCl_4-Induced Liver Fibrosis in Rats. *Food and Chemical Toxicology*, **44**, 336-343. https://doi.org/10.1016/j.fct.2005.08.003

[46] Paradis, V., Kollinger, M., Fabre, M., Holstege, A., Poynard, T. and Bedossa, P. (1997) *In Situ* Detection of Lipid Peroxidation By-Products in Chronic Liver Diseases. *Hepatology*, **26**, 135-142. https://doi.org/10.1002/hep.510260118

[47] Davenport, D.M. and Wargovich, M.J. (2005) Modulation of Cytochrome P450 Enzymes by Organosulfur Compounds from Garlic. *Food and Chemical Toxicology*, **43**, 1753-1762. https://doi.org/10.1016/j.fct.2005.05.018