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S-allyl cysteine, a bioactive ingredient of Allium sativum, alleviates carbon tetrachloride-induced acute renal dysfunction in the mouse

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Abstract

Background and Objective: Kidney diseases are endangering conditions to public health. Carbon tetrachloride (CCL4)-induced model of acute kidney injury (AKI) is a reliable model for studying renal damage under different conditions. S-allyl cysteine (SAC) is a natural organosulfur compound in aged garlic extract with multiple protective effects. In this study, possible preventive effect of SAC in CCl4 model of AKI was investigated.

Materials and Methods: For induction of AKI, CCl4 (10 ml/kg body weight; 0.175% in olive oil) was intraperitoneally injected and SAC was given orally at doses of 25 or 100 mg/kg. Functional markers of kidney were determined besides renal analysis of oxidative stress and inflammatory indices.

Results: SAC pretreatment at a dose of 100 mg/kg for 1 week before CCL4 challenge significantly and markedly reduced level of blood urea nitrogen (BUN), malondialdehyde (MDA), reactive oxygen species (ROS), tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and significantly enhanced superoxide dismutase (SOD) activity and with no significant effect on creatinine and catalase activity. In addition, such valuable effects were not observed for SAC at a dose of 25 mg/kg in CCL4-exposed group.

Conclusion: Findings of this study indicated beneficial effect of SAC subsequent to CCL4-induced kidney injury that is partly mediated through its regulation of oxidative and inflammatory events and upregulating some of the antioxidants.

Keywords: Acute kidney injury, Carbon tetrachloride, S-allyl cysteine, Inflammation, Oxidative stress

1. Introduction

Kidneys are the most pivotal and highly metabolic organ that excrete excess water and electrolytes in addition to toxicants in the mammalian species. Kidney diseases are prominent and endangering conditions for public health. Incidence rate of kidney injuries is rising with a sudden slope in recent decades (1,2). General rate of morbidity and mortality for kidney diseases is associated with great economic burden for the healthcare systems (3).

Air pollution and sulfur oxides, environmental toxicants such as CCl4, drug overdose, and alcohol damage the kidney tissue (4). Multiple mechanisms including oxidative stress, apoptosis, activation and release of inflammatory cytokines, and even endothelial dysfunction are responsible for renal damage (4). Carbon tetrachloride (CCL4)-induced model of renal injury is a reliable model to investigate further kidney damage (4).

CCL4 in the liver is metabolized into trichloromethyl radicals by cytochrome P450 with final formation of reactive and damaging trichloromethyl peroxy radicals. Such agents alter lipid metabolism and reduce membrane permeability which finally leads to degeneration and necrosis of tissue cells (5).

Involved mechanism for CCl4-induced injury is due to

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oxidative damage caused by lipid peroxidation which initiates after the conversion of CCl4 to free radicals of highly toxic trichloromethyl radicals (•CCl3) and trichloromethyl peroxyl radical (•CCl3O2).

Many herbal compounds can prevent and even protect against kidney damage which may be used in clinical practice. S-allyl cysteine (SAC) is a natural organosulfur compound in aged garlic extract. SAC is easily absorbed through the intestine and its bioavailability is rather high (6).

SAC has shown multiple beneficial effects in different models of humankind diseases and there is still no scientific report against its adverse effects and it has even been shown that its consumption has advantageous pharmacological effects (7, 8). SAC has shown many beneficial effects such as attenuation of oxidative stress, suppression of inflammation and apoptosis, and also its protection of tissues against toxic chemicals (6, 9). In this study, valuable effect of SAC in CCL4-induced model of acute kidney injury (AKI) was investigated.

2. Materials and Methods

2.1. Animals

Male mice (NMRI, 20-25 g) were obtained from Razi Institute (Karaj, Iran). All mice had adaption for 1 week with controlled conditions for temperature at 21-23°C, humidity at about 45% and with 12/12 photoperiods). They had also free access to diet and water. Mentioned procedures were approved by Ethics Committee of Iran University of Medical Sceinces (no. IR.IUMS.FMD.REC.1398.007).

2.2. Experimental design and treatments

Mice were randomly divided into 5 groups consisting of control, SAC 100-treated control, CCL4, SAC 25-treated CCL4, and SAC 100-treated CCL4. Mice in CCl4 group were i.p. injected with CCl4 (10 ml/kg, 0.175% in olive oil) (10) 1 h after the last treatment of SAC. Treatment groups received oral SAC on a daily basis, started 1 week up to one h before CCl4 injection. Administered dose of SAC was according to its ability to attenuate acute Renal injury due to lipopolysaccharide/ d-galactosamine in the mouse (11). After 1 day, mice were killed following deep anesthesia with ketamine (120 mg/kg) and blood samples through the heart and kidney tissues were obtained for biochemical assessments. Blood samples were kept at room temperature for 20 min and were then centrifuged at 3000 rpm for 10 min to isolate serum. Serum level of BUN and creatinine was measured using its proper kits from Pars Azmun Co. (Tehran, Iran).

2.3. Renal evaluation of oxidative stress

After preparing Renal homogenate in 150 mM Tris-HCl lysis buffer with pH at 7.4 and centrifuging them, its supernatant was used for determination of oxidative stress factors. Level of MDA as a known index of lipid peroxidation was measured using MDA assay reagent (SigmaAldrich, USA) (8,9). ROS level was measured using DCF-DA with its conversion into dichlorofluorescein in the presence of ROS radicals (12). Activity of the enzyme SOD was obtained using its related kit from Cayman Chemical (USA). Catalase activity was determined using its specific kit (KiaZist, Iran). Bradford method was used for determination of total protein level (13).

Renal levels of inflammatory factors were determined by means of sandwich Elisa protocol (antibodies for TNF-α were obtained from SigmaAldrich (USA) and antibodies for IL-6 were obtained from Santa Cruz Biotechnology, Inc. (USA)).

2.4. Statistical analysis

Findings are brought as means SEM. After verification of normal distribution of data using Shapiro-Wilk test, data analysis was performed by one-way ANOVA and Tukey post-test. In all tests, p value less than 0.05 was taken as significant.

3. Results

3.1. The effect of SAC on serum levels of BUN and creatinine

Measurement of serum level of creatinine (Fig. 1A) and BUN (Fig. 1B) in different groups indicated that administration of SAC at a dose of 100 mg/kg to the control animals is not associated with significant and marked changes of BUN and creatinine ($p>0.05$). In addition, CCL4 group had higher levels of creatinine ($p<0.01$) and BUN ($p<0.001$) at a significant level versus the control group. Such significant increase was also observed at a lower level in CCL4 group treated with SACA at a dose of 25 mg/kg for creatinine ($p<0.05$) and BUN ($p<0.001$) as compared to the CCL4 group. In contrast, CCL4 group receiving SAC at a dose of 100 mg/kg had lower level of creatinine ($p>0.05$) and BUN ($p<0.05$) versus the CCL4 group.

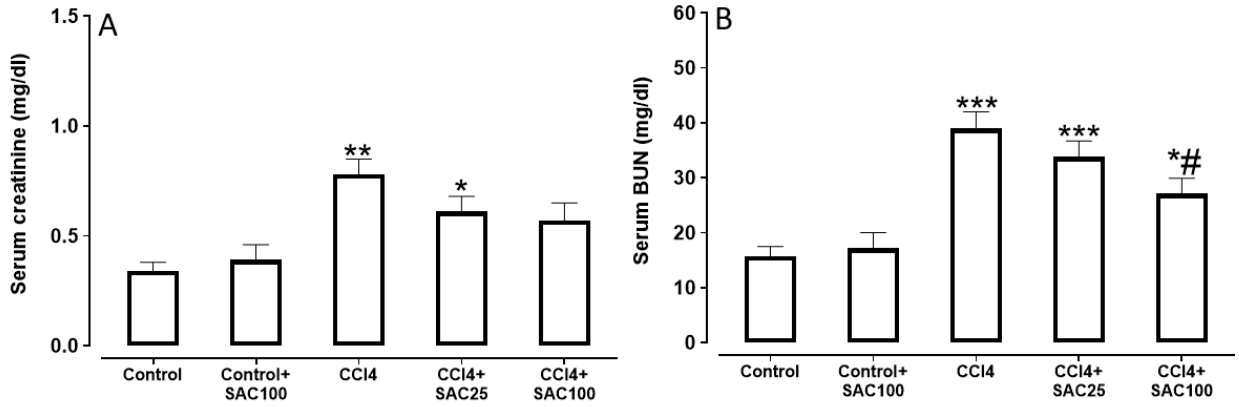

Fig. 1. The effect of SAC on serum levels of creatinine (A) and BUN (B). * p<0.05, ** p<0.01, *** p<0.001 versus the control group, # p<0.05 versus the CCL4 group. Results are shown in means ± SEM (n = 7 in each group).

3.2. The effect of SAC on oxidant factors

Measurement of kidney tissue levels of MDA as a marker of lipid peroxidation (Fig. 2A) and ROS (Fig. 2B) in different groups showed that SAC administration at a dose of 100 mg/kg to the control animals is not associated with significant and noticeable changes of MDA and ROS (p>0.05). In addition, CCL4 group had higher kidney levels of MDA (p<0.01) and ROS (p<0.01) at a significant level versus the control group. Such significant increase was only noted in CCL4 group receiving SAC at a dose of 25 mg/kg for MDA (p<0.01) and not for ROS (p>0.05) versus the CCL4 group. In contrast, CCL4 group given SAC at a dose of 100 mg/kg had significantly lower levels of MDA (p<0.05) and ROS (p<0.05) as compared to the CCL4 group.

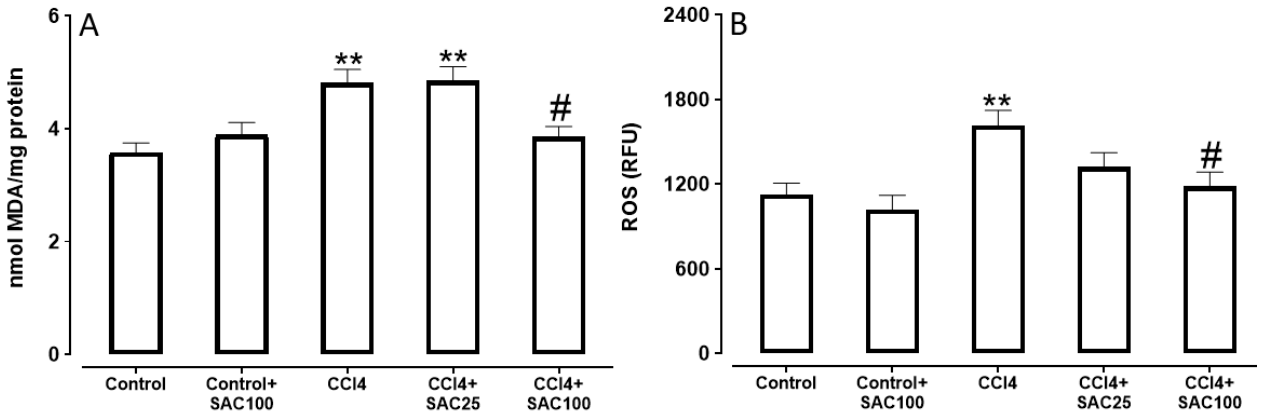

Fig. 2: The effect of SAC on renal tissue level of MDA (A) and ROS (B). ** p<0.01versus the control group, # p<0.05 versus the CCL4 group. Results are presented as mean ± SEM (n = 7 in each group).

3.3. The effect of SAC on renal antioxidant system

Measurement of renal levels of antioxidants consisting of SOD activity (Fig. 3A) and catalase activity (Fig. 3B) showed that SAC given at a dose of 100 mg/kg to the control group did not produce significant changes of activity of catalase and SOD (p>0.05). In addition, CCL4 group had lower renal level of catalase activity (p<0.01) and SOD activity (p<0.001) at a significant level versus the control group. Such significant decrease was also noted in CCL4 group given SAC at a dose of 25 mg/kg for catalase (p<0.05) and SOD (p<0.01) versus the CCL4-injured group. Conversely, CCL4 group treated with SAC at a dose of 100 mg/kg had significantly higher levels of SOD activity (p<0.01) and with no significant elevation of catalase activity (p>0.05) relative to the CCL4 group.

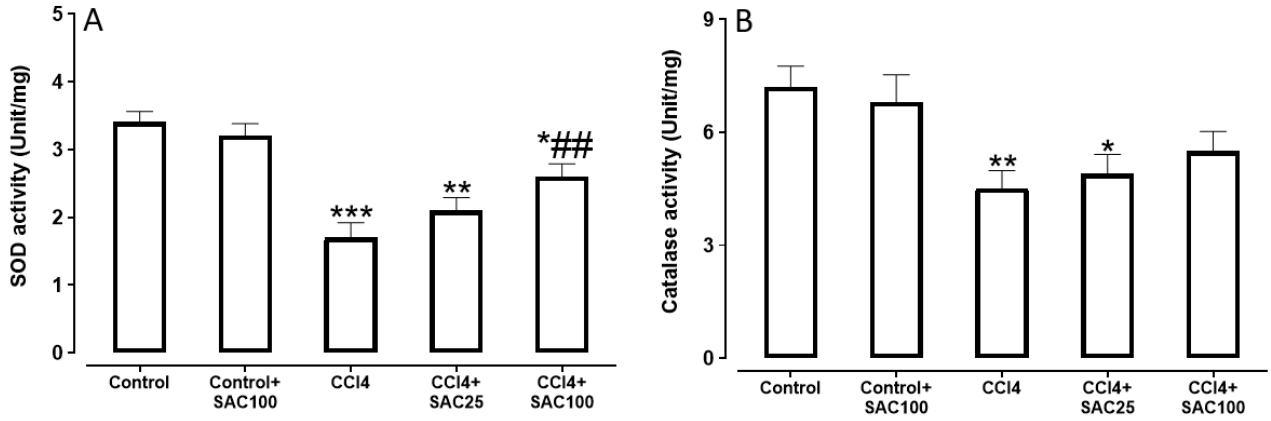

Fig. 3. The effect of SAC on renal levels of SOD activity (A) and catalase activity (B). * p<0.05, ** p<0.01, *** p<0.001 versus the control group, ## p<0.01 versus the CCL4 group. Results are shown in means ± SEM (n = 7/group).

3.4. The effect of SAC on inflammatory factors

Measurement of kidney tissue levels of inflammatory indices including TNFα (Fig. 4A) and IL-6 (Fig. 4B) showed that SAC given at a dose of 100 mg/kg to the control animals is not associated with significant changes of TNFα and IL-6 (p>0.05). In addition, CCL4 group had higher renal levels of TNF (p<0.001) and IL-6 (p<0.001) at a significant level as compared to the control group. Such significant increase was also obtained for CCL4 group given SAC at a dose of 25 mg/kg for TNFα (p<0.01) and IL-6 (p<0.01) relative to the CCL4 group. In contrast, CCL4 group given SAC at a dose of 100 mg/kg had significantly lower levels of TNFα (p<0.01) and IL-6 (p<0.01) when compared to the CCL4 group.

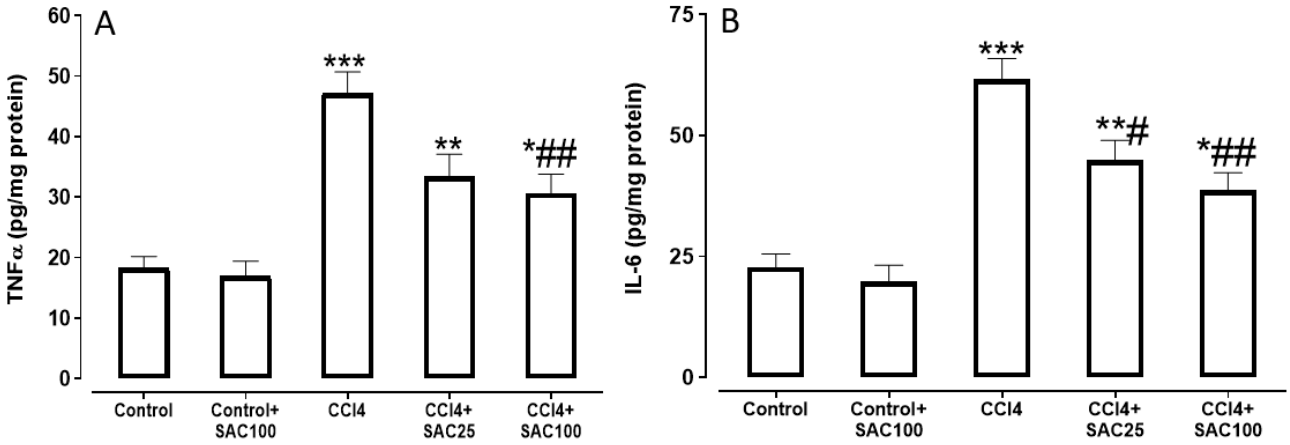

Fig. 4. The effect of SAC on renal level of TNFα (A) and IL-6 (B). * p<0.05, ** p<0.01, *** p<0.001 versus the control group, # p<0.05, ## p<0.01 versus the CCL4 group. Results are shown in mean ± SEM (n = 7 in each group).

4. Discussion

Obtained results showed that SAC pretreatment dose-dependently can reduce CCL4-induced renal dysfunction. In this regard, pretreatment with this medicinal plant product lowered renal oxidative stress and inflammation.

It has been shown that CCl4 toxicant is converted to dangerous trichloromethyl free radical (CCl3) and trichloromethyl peroxy radical (OOCCl3) by the action of cytochrome P450 complex. Such free radicals generated during CCl4 exposure can damage cell membrane unsaturated fatty acids which is associated with lipid peroxidation and production of oxygen lipid radicals (5, 14). A marked and significant increase of serum BUN and creatinine is an indicator of renal damage due to CCL4. Our results showed that

SAC given at 100 mg/kg could attenuate CCl4-induced liver dysfunction and injury. Such beneficial effect for SAC as an active ingredient of garlic has been reported in acute renal dysfunction induced by a combination of lipopolysaccharide and d-galactosamine in the mouse (11).

Overproduction of various free radicals following CCl4 exposure elevates ROS level and MDA amount as an indicator of lipid peroxidation. A marked increase in tissue ROS and MDA following CCL4 shows oxidative damage in the tissue and failure of the antioxidant defensive system to scavenge related free radicals (5, 14). Significant reduction of these factors was noted after the administration of SAC in our study which shows that this bioactive compound can protect the kidneys against CCL4-induced oxidative stress burden. Of related relevance, it has been demonstrated that astaxanthin-SAC diester can protect pancreatic beta cells against high glucose-provoked toxicity through alteration of oxidative stress and apoptosis (15) and SAC alone can protect bovine mammary epithelial cells against heat stress-induced damage through regulation of Nrf2/HO-1 cascade and oxidative stress (8).

Renal injury is developed following an inflammatory event. ROS produced by CCl4 damages tissue cells and provokes release of different pro-inflammatory factors. TNF-α which is an inflammatory factor provokes immune-related cells to produce multiple cytokines such as IL-1b and IL-6 (5, 14). In this research, SAC administration as a bioactive compound in garlic at a dose of 100 mg/kg to CCL4 group was able to significantly ameliorate kidney tissue inflammation, as demonstrated by lower levels of TNF-a and IL-6. Anti-inflammatory activity of SAC has been reported in different studies. In this regard, protective effect of SAC against asthmatic neonatal rats has been attributed to its anti-inflammatory effect, as was evident by lower levels of inflammatory cytokines such as TNF-α, IL-6, and IL-1β (16). In addition, SAC administration can attenuate acute liver dysfunction due to a combination of lipopolysaccharide and d-galactosamine in the mouse through attenuation of oxidative stress, neutrophil infiltration, inflammation, and apoptosis (11). Likewise, anti-inflammatory effect of SAC was also noted in our CCL4 model of acute kidney injury.

Conclusion

To conclude, results of this study demonstrated that beneficial property of SAC subsequent to CCL4-provoked kidney injury that is mediated through its regulation of oxidative and inflammatory events and enhancement of some antioxidants.

Acknowledgment

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Compliance with ethical standards

All experimental procedures of this research study were approved by Ethics Committee of Iran University of Medical Sciences (no. IR.IUMS.FMD.REC.1398.007).

Conflict of interest

The authors declare that they have no competing interest.

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