



Research Article

## Nephroprotective Effect of Hydroalcoholic Extract *Allium jesdianum* Boiss against Carbon Tetrachloride Induced Nephrotoxicity via Stress Oxidative in Mice

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### Article Info

#### Article History:

Received: 22 August 2017

Revised: 27 October 2017

Accepted: 13 December 2017

ePublished: 20 June 2018

#### Keywords:

-*Allium jesdianum* Boiss  
-Carbon Tetrachloride  
-Nephrotoxicity  
-Oxidative Stress  
-Mice

### ABSTRACT

**Background:** Nephrotoxicity is one of the most common renal problems that especially occur when the body is exposed to drugs or chemical reagents. *Allium jesdianum* Boiss is the largest and most important plants genus of onion family that possesses many pharmacological effects. The present study was undertaken to investigate the effect *Allium jesdianum* Boiss in the treatment of nephrotoxicity induced by carbon tetrachloride (CCl<sub>4</sub>).

**Methods:** Forty two male mice were randomly divided into six groups; control, sham, CCl<sub>4</sub> (1 ml/Kg, i.p) single dose, *Allium* (500, 1000 and 2000 mg/kg) + CCl<sub>4</sub>. Blood urea nitrogen (BUN) and serum creatinine (Cr) evaluated in serum. Glutathione (GSH), catalase (CAT), malondialdehyde (MDA) and reactive oxygen species (ROS) were analyzed in kidney tissue homogenate and done the microscopic studies of renal tissue.

**Results:** The results indicated a significant increase of serum BUN and Cr as well as MDA and ROS levels and decrease of GSH and CAT in CCl<sub>4</sub> treated mice when compared with the control group ( $p < 0.001$ ), whereas all studied endpoints were significantly altered in pretreatment with *Allium* extract when compared with CCl<sub>4</sub> treated mice ( $p < 0.001$ ). Renal histopathology indicated normal appearances reduced in CCl<sub>4</sub> treated mice and *Allium* extract administration improved changes in renal tissue.

**Conclusion:** Administration of the hydroalcoholic extract of *Allium jesdianum* Boiss could prevent nephrotoxicity induced by CCl<sub>4</sub>. The protective potential may involve the powerful antioxidant of this plant by eliminating free radicals induced by CCl<sub>4</sub>.

### Introduction

Nephrotoxicity is one of the most common renal problems that especially occurs when the body is exposed to drugs or chemical reagents.<sup>1</sup> The kidney eliminates the metabolic waste, xenobiotic and regulates the water and ion content in the blood. The kidneys possess the common enzymes for metabolizing xenobiotic that mostly localize in proximal tubular cells.<sup>2</sup> CCl<sub>4</sub> is an industrial solvent that widely use to induce oxidative stress in liver laboratory animals. It has been demonstrated that liver is not only the target organ of CCl<sub>4</sub> toxicity, but also its toxicity affects in other organs such as the kidney, lung, testis, brain and blood. It has also been reported that CCl<sub>4</sub> causes renal damage through generation of free radicals. Kidney tissue has great affinity for CCl<sub>4</sub> due to the predominant presence of the cytochrome P450 in the renal cortex. CCl<sub>4</sub> toxicity is caused by its bioactivation to trichloromethyl free radical by cytochrome P<sub>450</sub>. The trichloromethyl radical reacts with oxygen to form trichloromethyl peroxy radical that is one reactive oxygen species (ROS). In order to cope with the excess of free

radicals produced organisms have developed enzymatic and non-enzymatic antioxidant systems to scavenge or detoxify ROS. Change of antioxidant status with CCl<sub>4</sub> may potentially cause nephropathies.<sup>3-11</sup>

Antioxidants have the ability to protect the body from damage caused by free radicals. This antioxidant capacity can be explored for the development of safe medicines against the progressive diseases. Based on growing interest in free radical biology and the efficacy of antioxidants in protection against oxidative stress has posed interest in the use of antioxidants as a tool to prevent or decelerate the progression of oxidative stress.<sup>12-16</sup> *Allium jesdianum* Boiss (*Allium*) is the largest and most important plant genus of alliaceae family (Figure 1) that its extract and isolated compounds possess pharmacological effects. Previous studies have indicated several biological activities for *Allium* species including antioxidant, antibacterial, antiviral, antiparasitic, anticarcinogenic, antifungal, anti-protozoa and anti-diabetic and also effects on the cardiovascular system and treatment of common cold.<sup>17-21</sup>

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Despite the favorable ethnopharmacological properties of *Allium*, the protective effect against nephrotoxicity of CCl<sub>4</sub> has not previously been explored. The aim of the present study is to investigate the protective effect of *Allium* against CCl<sub>4</sub> induced- nephrotoxicity in mice.



Figure 1. *Allium jesdianum* Boiss.<sup>22</sup>

## Materials and Methods

### Chemicals

Ammonium molybdate, thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2',7'-dichlorofluorescein diacetate (DCFH-DA), CCl<sub>4</sub> and glutathione (GSH) were purchased from Sigma-Aldrich (St Louis, Missouri, USA) and 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) was obtained from Merck (Darmstadt, Germany). Blood urea nitrogen (BUN) and Creatinine (Cr) kits were obtained from biochemical assay kits (Pars Azmoon, Iran). *Allium Jesdianum* Boiss purchased from the local herbal market in Ahvaz, Khuzestan province, Iran. And other chemicals were of the highest grade commercially available.

### Animals

In this experimental study, adult male Naval Medical Research Institute (NMRI) mice in the weight range of 25-30 g were obtained from the animal facility of Ahvaz Jundishapur University of Medical Sciences (AJUMS) laboratory which is fully accredited by AJUMS animal care guidelines with an ethics committee grantee (IR.AJUMS.REC.1395.02). The mice were used in the experiment at the age of 8–9 weeks. Animals housed in an air-conditioned room with controlled temperature of 20±4°C, humidity of 10% and maintained on a 12h light/12h dark cycle with free access to food and water. All experiments were carried out after one week.

### Extraction Preparation

At first, the leaves of the plant were identified by botanist from the Division of Pharmacognosy, Ahvaz Jundishapur University of Medical Sciences (number voucher: A-0138). Soxhlet method was used for extraction. Briefly, plants were washed with water, dried and powdered in a grinding mill and plant powder was soaked overnight of ethanol 70% in a large balloon for 3 days at room temperature. The pooled solvents were combined and

filtered through a paper and then filtrates were concentrated under reduced pressure in a rotary evaporator. The yield of the extract then was distilled and placed in the oven 37 °C and finally its dry extract is obtained.

### Experimental Design

In this study, forty two male mice were randomly divided into six groups (n=7). The mice of 1<sup>st</sup> group as control received normal saline orally for 5 days, 2<sup>nd</sup> group as sham received olive oil orally for 5 days, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> group received 500 mg/kg, 1000 mg/kg and 2000 mg/kg of *Allium* extract orally for 5 days and on the fifth day an hour after oral *Allium* extract administration, received the single dose of CCl<sub>4</sub> (1 ml/kg, i.p), CCl<sub>4</sub> dissolved in olive oil. 6<sup>th</sup> group received CCl<sub>4</sub> (1 ml/kg, i.p) a single dose. The doses of *Allium* and 5 days of treatment with regards to dose-response and toxicity studies at Ahvaz Jundishapur University of medical Sciences and previous studies were selected.<sup>23</sup>

### Sample Collection

24 hours after administration of the last doses of CCl<sub>4</sub> and *Allium*, animals were anesthetized by ketamine-xylazine and serum samples were obtained by heart puncture. Blood collection and centrifuging at 3000 rpm for 15 minutes and stored at -20 °C until analysis. Kidney was isolated and quickly washed with normal saline. One kidney was homogenate for kidney tissue biochemical and another portion was fixed in 10% formalin for histological studies.

### Serum Biochemical Assessments

Serum samples were transferred to the microtubes and BUN and Cr of serum samples were measured by using an Autoanalyzer device (BT 3000, Italy) and biochemical assay kits (Pars Azmoon, Iran).<sup>24-26</sup>

### Preparation of Homogenized Kidney Tissue

100 mg of kidney tissue in phosphate buffer (1 ml, 1 mM, and pH 7.4) (1/10 W/V) was homogenized using glass homogenizer, and then tissue homogenate was centrifuged at 12,000×g for 30 min at 4 °C. The supernatant was separated from sediment and used for GSH, MDA, ROS and CAT parameters.

### Tissue Biochemical Parameters

#### Determination of GSH Amount in Kidney Tissue

The GSH level in kidney tissue homogenate was measured using the Ellman's method and with Ellman's Reagent (DTNB) (27, 28). Briefly, TCA (20%) along with EDTA (1 mM) was added to the tissue homogenate (500 µl). Then, were mixed and centrifuged (10 min, 3000 rpm) and tissue homogenate (200 µl) was added to DTNB (1.8 ml, 0.1 mM) and then was incubated for 20 min at room temperature. The absorbance was read at 412 nm by spectrophotometer (UV-1650 PC, Shimadzu, Japan). Results were reported as mol/g tissue.<sup>27,28</sup>

### Determination of Catalase Activity in kidney tissue

CAT activity in kidney tissue homogenate was determined by a modified procedure of L. Goth; Accordingly, in a tube tris-HCl (500 µl, 0.05 mmol) was added per H<sub>2</sub>O<sub>2</sub> (1 ml) and tissue homogenate (50 µl) then were mixed and incubated for 10 min in room temperature, after then ammonium molybdate (500 µl, 4%) was added and absorbance was read at 410 nm. The result was expressed as U/g tissue.<sup>29</sup>

### Determination of MDA in Kidney Tissue

Lipid peroxidation amount in kidney tissue homogenate was measured based on the malondialdehyde. The reaction of MDA with TBA produces a purple color with maximum absorbance at 532 nm.<sup>30</sup> Briefly, in one test tube tissue homogenate (1 ml) was added to 2 ml TBA solution (0.67% W/V) then was mixed and kept in boiling water (100 °C) for 15 min. Then the mixture was cooled and centrifuged (3000 rpm, 10 min) and the supernatant separated. Ultimately, the MDA level was measured as nmol/g tissue.<sup>31</sup>

### Determination of Reactive Oxygen Species in Kidney Tissue

ROS intensity in the kidney tissue homogenate was measured using DCFDA method. The peroxides enzyme in cell, converted DCFDA into highly fluorescent DCF. Briefly, kidney tissue homogenate (10% W/V) were prepared in ice-cold Tris-HCl buffer 40 mM (pH 7.4). Then 2 ml kidney tissue homogenate was mixed with 40 µl of 1.25 mM DCFDA after then was incubated in a water bath 37 °C for 15 min. Measurement based on the intensity of fluorescence was determined at 488 nm excitation and 525 nm emission wavelength using a fluorimeter (Perkin-Elmer, LS-50 B, united Kingdom).<sup>32</sup>

### Histopathological Studies

The all mice kidneys were immediately fixed at 10%

formalin for 24 hours, afterward embedded in paraffin. Then, they were dehydrated through soaking in alcohol and xylol, respectively. Finally, after preparation of 5µ tissue sections using rotary microtome, the hematoxylin and eosin (H&E) staining technique was performed. The histopathological changes were examined using light microscope.

### Statistical Analysis

All results were analyzed using GraphPad Prism software (Version 5; GraphPad Software, Inc., La Jolla, CA) and expressed as mean values ± SE (n = 7). Comparisons between groups were evaluated by one-way ANOVA followed by a post hoc test. A value of p < 0.05 was considered to be statistically significant.

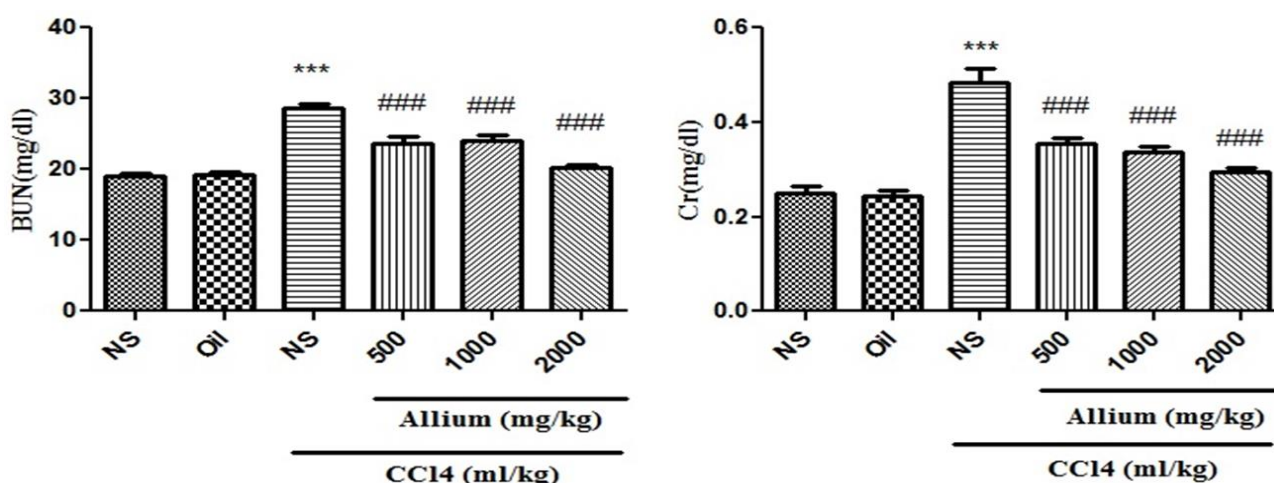
### Results

#### Effect of the Extract of *Allium* on Serum Analysis

Serum concentration of Cr and BUN are the marker tests for the injuries and dysfunction of kidney, therefore, the effect of CCl<sub>4</sub> administration on the changes of above chemical makers are presented in (figure 2). Treatment with CCl<sub>4</sub> significantly (P < 0.001) increased the serum level of Cr and BUN when compared to the control group. Pretreatment with the doses 500, 1000 and 2000 mg/kg extract of *Allium* caused a significant decrease in the serum BUN, Cr levels when compared with CCl<sub>4</sub>-treated mice (p < 0.001).

#### Effect of the Extract of *Allium* on Catalase Activity and GSH Kidney Tissue

As shown in Figure 3, CCl<sub>4</sub> caused a significant decrease in CAT activity and GSH amount in compared with control groups (p < 0.05). Pretreatment with *Allium* extract (500, 1000 and 2000 mg/kg) led to a significant increase in GSH level and CAT activity when compared with CCl<sub>4</sub>-treated mice (p < 0.001).



**Figure 2.** Effect of *Allium* extract on the serum BUN and Cr levels. (Mean ± SE; n = 7).

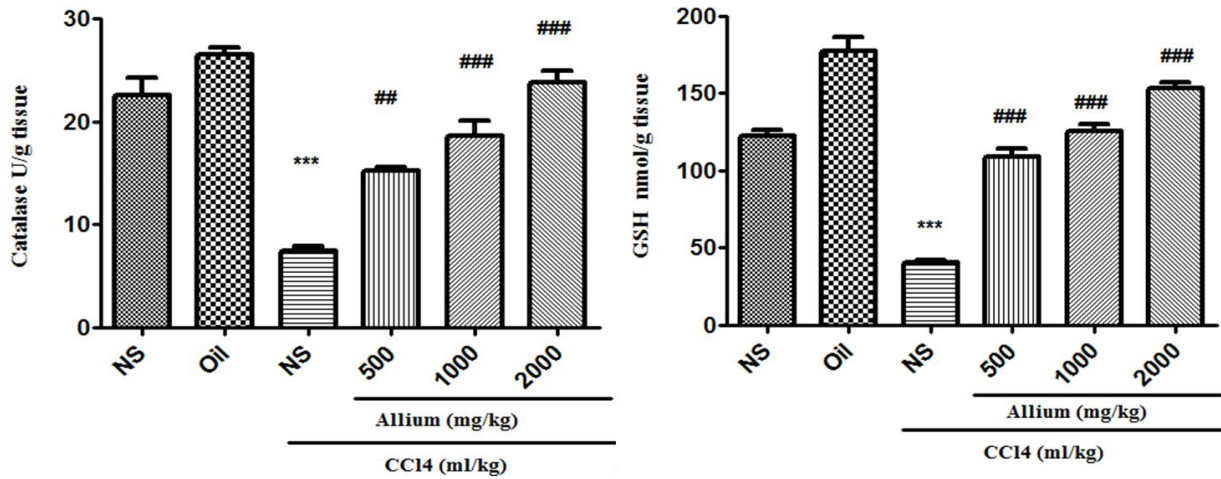
\*: designate significant difference from control groups (normal saline and sham), \*\*\* P < 0.001.

#: designate significant difference from CCl<sub>4</sub>-treated group, ### p < 0.001.

NS: Normal saline

BUN: Blood urea nitrogen

Cr: Creatinine



**Figure 3.** Effect of *Allium* extract on the GSH level and CAT activity. (Mean ± SE; n = 7). \*: designate significant difference from control groups (normal saline and sham), \*\*\* P<0.001. #: designate significant difference from CCl<sub>4</sub>-treated group. ##P<0.01 and ###P<0.001. NS: Normal saline GSH: Glutathione CAT: Catalase

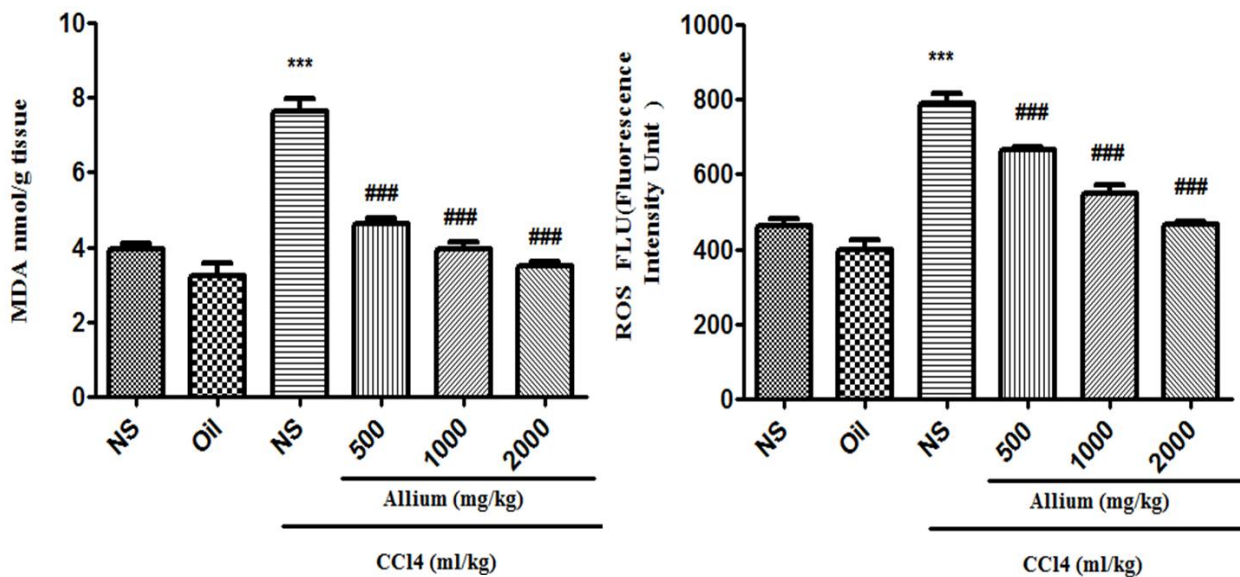
**Effect of the Extract of Allium on MDA and Reactive Oxygen Species Levels Kidney Tissue**

As shown in Figure 4, treatment to CCl<sub>4</sub> caused a significant increase in the levels of MDA and ROS in kidney tissue in mice when compared with control group (p<0.001). While, administration of *Allium* extract at the doses of 500, 1000 and 2000 mg/kg demonstrated a significant decrease in MDA and ROS levels in the kidney tissue when compared with CCl<sub>4</sub> group (p<0.001).

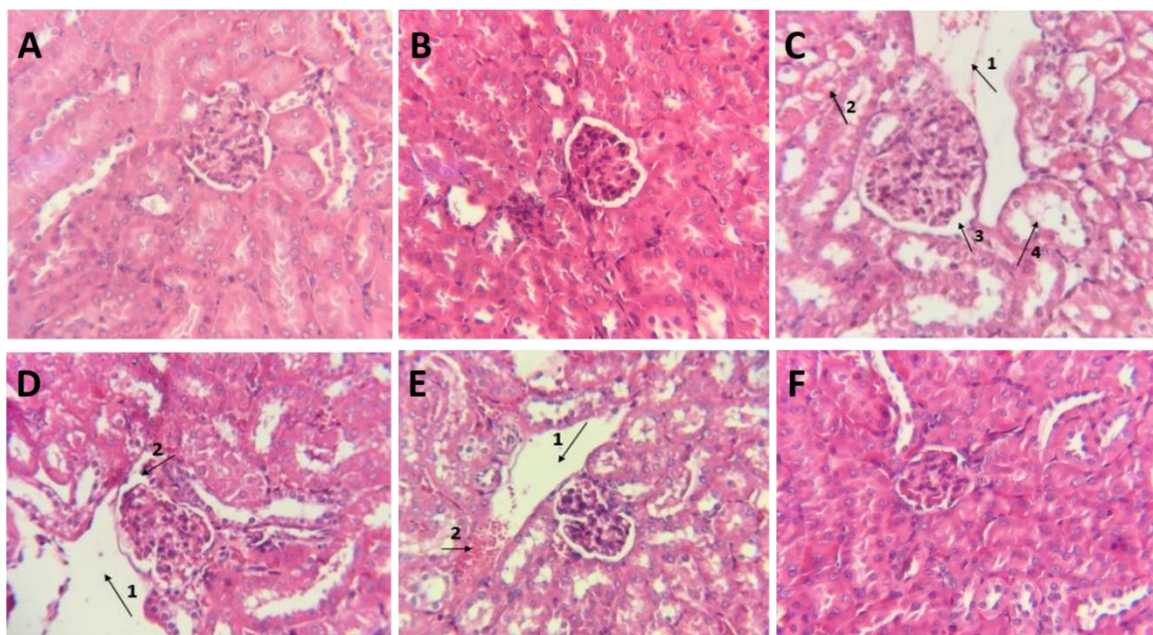
**Effect of the Extract of Allium on Renal Histopathology**

The results of histopathology showed that the degree of damage in the kidney tissues among the treated groups

was different before and after the use of *Allium* extract. Treated mice with normal saline and olive oil shown a normal tubular and glomerular structures (Figure 5A and 5B). Treated mice with CCl<sub>4</sub> shown widening of glomerular renal space, vascular congestion, degeneration of epithelial cells of tubules and widening of the tubular lumen (Figure 5C). Mice that pretreated with dose 500 , 1000 and 2000 mg/kg of *Allium*, showing less widening of glomerular space and tubular lumen, less degeneration of tubular epithelial cells and vascular congestion which these changes in group 2000 mg/kg shown more complete improvement when compared to other extract receiving groups (Figures 5D, 5E and 5F).



**Figure 4.** Effect of *Allium* extract on the MDA and ROS levels. (Mean ± SE; n = 7). \*: designate significant difference from control groups (normal saline and sham), \*\*\* P<0.001. #: designate significant difference from CCl<sub>4</sub>-treated group. ### P< 0.001. NS: Normal saline MDA: Malondialdehyde ROS: Reactive oxygen species



**Figure 5.** Photomicrograph shown histopathological section of kidney tissue from each group (original magnification: X 40). (A & B) Control and sham group; normal tubular and glomerular structures. (C) CCl<sub>4</sub> group; widening of the tubular lumen (arrow 1), vascular congestion (arrow 2), winding of glomerular (arrow 3), degeneration of epithelial cells of tubules (arrow 4). (D) *Allium* extract (500 mg/kg) + CCl<sub>4</sub>; widening of the tubular lumen (arrow 1), winding of glomerular (arrow 2). (E) *Allium* extract (1000 mg/kg) + CCl<sub>4</sub>; widening of the tubular lumen (arrow 1), vascular congestion (arrow 2). (F) *Allium* extract (2000 mg/kg) + CCl<sub>4</sub>; normal tubular and glomerular structures.

## Discussion

The finding of this study indicate that CCl<sub>4</sub> can induce nephrotoxicity through the increase BUN, Cr, MDA and ROS levels and decrease GSH and CAT activity and also can cause histopathological alterations in kidney tissue. In agreement with this study a number of reports clearly have demonstrated that CCl<sub>4</sub> leads to disorders in kidney tissue as well as increases serum BUN, Cr levels and also alters antioxidant and oxidative stress parameters. Also *Ruprah et al* showed that exposure to this solvent cause acute and chronic renal injury in animals.<sup>11,33</sup> Further, administration of the *Allium* extract could improve nephrotoxicity induce by CCl<sub>4</sub> via change serum and tissue biochemical parameters and also improved histopathological alterations in kidney tissue when compared with CCl<sub>4</sub> group. In this study all used doses of *Allium* extract decreased BUN, Cr, MDA and ROS and increased GSH and CAT levels. Thus, it can be suggested that *Allium* extract may prevent nephrotoxicity induced by CCl<sub>4</sub> in administration doses 500, 1000 and 2000 mg/kg. It is well-known that serum BUN and Cr are two main indicators for evaluation renal function.<sup>34</sup> Increases in BUN and Cr amounts occur through the kidney dysfunction or damage.<sup>35</sup> Some studies showed an increase of serum urea and Cr levels in kidney injury by CCl<sub>4</sub>.<sup>3</sup> The results of *Moneim et al* study indicate that serum BUN and Cr levels significantly increased in CCl<sub>4</sub> – induced nephrotoxicity.<sup>36</sup> *Sakr et al* also showed that serum urea and Cr levels increase in renal damage by CCl<sub>4</sub>.<sup>37</sup> Hence, it can be suggested that, present finding is in concomitant with previous studies.

There are various chemicals and drugs which cause

damage to renal tissue by ROS production. It well- known that CCl<sub>4</sub> induce ROS, deplete antioxidant defenses and cause oxidative stress in renal tissues. CCl<sub>4</sub> metabolized by cytochrome P<sub>450</sub> and generates a highly reactive free radical that can cause damage to cell membrane, lipids, proteins and DNA.<sup>38</sup> Various studies have demonstrated that CCl<sub>4</sub> causes free radical generation in many tissues including kidney.<sup>33</sup> So suggested that ROS generation as one of the postulated mechanisms in the pathogenesis of CCl<sub>4</sub>.

Endogenous GSH use to estimate the non-enzymatic antioxidant capacity of a tissue for inhibition the damage associated to free radicals<sup>39</sup> and a good indicator of the capacity of antioxidant defenses is the enzymatic antioxidant that it can be presumed, in this study in mice renal tissues non-enzymatic and enzymatic antioxidant defenses were conceded by CCl<sub>4</sub>. Considering that the decreasing of GSH levels, it is imaginable that this decrease may reflect increased ROS generation by CCl<sub>4</sub> that is eliminated GSH. Metabolism of CCl<sub>4</sub> by the P<sub>450</sub> enzymes and generation of (<sup>0</sup>CCl<sub>3</sub>) radicals initiates the process of lipid peroxidation that is the most important mechanism in the pathogenesis of renal damage induced by CCl<sub>4</sub>.<sup>40</sup>

We cannot emphasize exclusively that CCl<sub>4</sub> metabolites may directly reacted with GSH, and reduce its concentration and cause an increase in MDA level in renal tissue. But, previous studies have shown that trichloromethyl radicals (<sup>0</sup>CCl<sub>3</sub>) react with sulfhydryl groups (SH) of GSH and other proteins thiol and alter the redox status of cells.<sup>41</sup> In addition, decrease in the activity of primary antioxidant enzyme; CAT may be due

accumulation of ROS in renal tissue that represents increased level of H<sub>2</sub>O<sub>2</sub> in renal tissues of this study. The inhibition of antioxidant system may cause the accumulation of H<sub>2</sub>O<sub>2</sub> or other reactive oxygen species/reactive nitrogen species that all of them have aided to decrease CAT activity.<sup>41</sup> The activity of CAT is an suitable indirect way to measure redox status in tissues<sup>42</sup> and MDA is an indicator of oxidative stress and is a final product of lipid peroxidation that is the result of the attack ROS to lipid membranes.<sup>43</sup> Therefore, ROS indirectly can be estimate with the amount of MDA.<sup>44</sup>

The levels of GSH and CAT decreased in CCl<sub>4</sub>-treated group and MDA and ROS levels increased that was returned by treatment of *Allium* extract. Increased GSH level and/ or its rate of synthesis represent enhanced protection against oxidative stress induced by CCl<sub>4</sub>. Results obtained in this study suggest the preventive effects of *Allium* extract against the CCl<sub>4</sub> could be attributed to its high level of phenol compounds and other antioxidants.<sup>45,46</sup>

*Stajner et al* showed that leaves of different of wild *Allium* species possess well-defined antioxidant activity which their antioxidant abilities are related to content of their flavonoids and carotenoids and could be used as a source of natural non-toxic antioxidants in food, cosmetic and pharmaceutical industries.<sup>47</sup>

Histopathology alterations in nephrotoxicity induced by CCl<sub>4</sub> is characterized by several alterations such as tubular epithelial cells alterations including vacuolization, atrophy, detachment of epithelial cells, tubular necrosis and widening of tubular lumen with these histopathological changes, the capacity of tubular absorption and functional of nephrons may have been altered as a result of renal dysfunction and injury.<sup>48</sup> *Alm Eldeen et al* study showed appearance of the necrotic areas, cellular infiltration, atrophied renal glomerular and degeneration renal tubule lining with widening of the renal lumens.<sup>49</sup> The present results revealed widening of glomerular renal space ,vascular congestion , degeneration of epithelial cells of tubules and widening of the tubular lumen after induction nephrotoxicity by CCl<sub>4</sub>, then the present data is consistent with the previous studies. Our results indicated that the *Allium* leaves utilization is successful in ameliorating the renal histopathological changes induced by CCl<sub>4</sub>. The observed effects of the *Allium* leaves can be related to its phenolic content. Flavonoids have shown their antioxidant properties through scavenging or chelating free radicals. One of the proposed influence mechanisms of the *Allium* extract in these changes could be suggested that renal protective effect of it has been occurred through its antioxidant properties, but future studies are required to clarify the exact.

### Conclusion

In present study the protective role the extract of *Allium* leaves at different levels was evaluated. This protective potential may involve the powerful antioxidant properties of this plant by eliminating free radicals and oxidative

damages induced by CCl<sub>4</sub>. This study confirmed the scientific evidence for pharmacological use this plant in renal injuries.

### Acknowledgement

This paper is issued from the thesis of Maryam Danesh Pajou and was financially supported by Medicinal Plants Research Center (Grant number: MPRC-9502) provided by the Vice Chancellor of Research, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

### Conflict of interests

The authors claim that there is no conflict of interest.

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