

# *Allium Jesdianum* Extract Improve Acetaminophen-Induced Hepatic Failure through Inhibition of Oxidative/Nitrosative Stress

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## Key Words

*Allium jesdianum*, Oxidative stress, Acetaminophen, Hepatotoxicity

## Abstract

**Objectives:** *Allium jesdianum* (Aj) is a medicinal plant that has highlighted pharmacological features. In this study, the effects of Aj extract were examined on acetaminophen (APAP)-induced hepatic failure in rats.

**Methods:** Methanolic fraction of hydro-alcoholic extract of Aj was obtained by silica gel column chromatography method. Animals were randomly divided into four groups each containing six rats and treated by gavage as follows: the first and second groups received normal saline, the third and fourth groups were received with 50 and 100 mg/kg of Aj extract, respectively. After two consecutive weeks, the groups 2-4 were given a single dose of APAP (2 g/kg). After 48 hours, blood and liver samples were collected for biochemical and histological examinations.

**Results:** The findings of the study demonstrated that APAP caused a significant increase in ALT ( $P < 0.001$ ), AST ( $P < 0.001$ ), LDH ( $P < 0.001$ ), ALP ( $P < 0.001$ ) serum

levels, hepatic lipid peroxidation (LPO;  $P < 0.001$ ) and nitric oxide (NO;  $P < 0.001$ ). In this regard, APAP led to the depletion of the total antioxidant capacity (TAC;  $P < 0.001$ ), glutathione and total thiol groups (TTGs;  $P < 0.001$ ), and structural change in the liver. In the Aj extract groups, a considerable improvement was found in the hepatic function alongside the histopathologic changes.

**Conclusion:** This investigation indicated that the influential effects of Aj extract in APAP-induced hepatic failure might depend on its effect on improving oxidant/antioxidant balance in hepatic tissue.

## 1. Introduction

Acetaminophen (acetyl-para-aminophenol; APAP), has been used extensively but it can cause hepatotoxicity when more than therapeutic doses are used [1]. About 80% of APAP is conjugated directly to sulfated and glucuronidated metabolites and secreted in bile or urine. A slight portion of APAP (about 5%-10%) is metabolized to N-acetyl-para-benzo-quinone imine (NAPQI) by hepatic cytochrome CYP 2E1 which is an extremely reactive toxic metabolite [1, 2]. This electrophilic metabolite can binds covalently to some of the intracellular molecules such as DNA, deplete glutathione (GSH) which may cause oxidative stress, and alter calcium and/or thiol status in hepatocellular, all of which cause hepatic failure [2, 3].

Received: Mar 01, 2019 Reviewed: Jul 18, 2019 Accepted: Nov 25, 2019

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Medicinal herbs and/or herbal supplements have been mostly applied to sustain or upgrade health, and the community believes that natural herbal compounds have no side effects and they are safe. The World Health Organization (WHO) recited that 80% of people in developing countries use herbal medicine to treat their health problems [4-6]. Among these, many studies have encouraged the use of *Allium* species as food and/or medicine so that people all over the world consume *Allium* vegetables especially onions and garlic, as a part of their daily diet. Various studies have been conducted to investigate the potential health-promoting effects of *Allium* species [7-9]. Beneficial properties of these species on hypertension, cardiovascular risk factors and lipid profile seemed to be based on various recent meta-analyses [10-13]. Furthermore, several studies have shown that some of the *Allium* species contain antioxidant properties and can inhibit oxidative damage induced by oxidants agents [14, 15].

*Allium jesdianum* (Aj) is a type of the *Allium* that can be found in the Zagros Mountains of Iran. Recently, some useful effects of Aj like antibacterial and antifungal activity have been confirmed [16, 17]. However, there has not been a lot of information about the therapeutic effects of Aj on hepatic function. Hence, the aim of the present study was to examine the effects of Aj extract against APAP-induced liver dysfunction in male rats.

## 2. Materials and methods

### 2.1. Chemicals

Acetaminophen (98% purity, CAS#: 103-90-2), 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB), N-(1-naphthyl) ethylenediamine dihydrochloride (NED), bovine serum albumin (BSA), 2-thiobarbituric acid (TBA), and 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

### 2.2. Plant materials: collection and extraction procedure

The Aj was gathered from the Zagros Mountains, Kurdistan province, Iran. The collected plant was identified by herbarium unit, faculty of pharmacy, Hamadan University of Medical Sciences (HUMS), Hamadan, Iran with the code number of (NO: 403). The plant samples were dried and powdered. The extraction process was conducted using methanol and water (1:1) for 72 hours. Then, the resulting extract was filtered; and condensed with a rotary evaporator. The total extraction process was repeated three times. In the next stage, n-hexane, chloroform, ethyl acetate and methanol fractions of hydroalcoholic extract were prepared, respectively by column chromatography. For this purpose, a column with dimensions of 8 × 17 cm was used with a silica gel (70-30 mesh) as a solid phase. Finally, the Ferric reducing/antioxidant power assay (FRAP) was

performed on the fractions and the methanolic fraction as an effective fraction was selected and maintained at the temperature of 4°C.

### 2.3. Animals

Male Wistar rats (250±20 g) were taken from the animal house of HUMS, Hamadan, Iran, and quarantined for one week before use. The animals were monitored in the suitable laboratory condition that had the cycle of the 12 h light/dark at the temperature of 22-25°C, and were provided with the standard diet and water ad libitum. The investigation procedure was confirmed by the Ethics Committee of HUMS (ethical ID: IR.UMSHA.REC.1396.336).

### 2.4. Safety dose determination

According to pilot studies, the safety dose of Aj extract was evaluated to be in the range of 50-200 mg/kg through determining hepatic enzymes activity.

### 2.5. Experimental protocol and groups

The animals were divided into four groups of six, as follows: the first and second groups received normal saline, the third and fourth groups were treated with 50 and 100 mg/kg of Aj extract, respectively. After two weeks, the groups 2-4 were given a single dose of APAP (2 g/kg). After 48 hours, the rats were anaesthetized with ketamine and xylazine (5:1 ratio) [18]. Following laparotomy surgery, blood specimens were obtained from the animals' heart, allowed to clot, and for 10 min, they were centrifuged at 3000 g to acquire clear serum. The samples of the serum were kept at -20°C for biochemical examinations. The liver was isolated from rats, and part of the hepatic tissue was homogenized in phosphate-buffered saline (PBS, pH 7.4). After centrifuging samples at 3000 g/4°C for 10 min, its supernatant was removed for biochemical examinations. Another part of the hepatic tissue was fixed in formalin (10%) for histopathological examination.

### 2.6. Serum enzymes analysis

Enzymatic colorimetric kits were used to assay serum alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity (Pars Azmun kit, Iran).

### 2.7. Nitric oxide assay

Griess reagent (1% sulfanilamide, 0.1% NED, and 2.5% phosphoric acid) was used to identify liver nitric oxide (NO) as described by Nili-Ahmadabadi et al. [19].

## 2.8. Lipid peroxidation assay

Thiobarbituric acid-reactive substances (TBARS) method was used for lipid peroxidation (LPO) assay, as described by Nili-Ahmadabadi et al. [19]. Briefly, homogenized liver tissue were mixed with TBA (0.2%) in H<sub>2</sub>SO<sub>4</sub> (0.05 M) and heated for 30 min in boiling water bath. By-products of LPO were extracted by n-butanol and absorbance was determined at 532 nm. Malondialdehyde (MDA) was used as a standard and data were expressed as nmol/mg protein.

## 2.9. Total antioxidant capacity assay

Total antioxidant capacity (TAC) was determined in hepatic tissue by measuring their ability to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup>. The reaction between TPTZ reagent and Fe<sup>2+</sup> gives a blue color with a maximum absorbance at 593 nm [20]. Data were expressed as nmol/mg protein.

## 2.10. Total thiol groups assay

Total thiol groups (TTGs) were determined spectrophotometrically by DTNB reagent. This reagent reacts with the thiol molecules to yield a yellow colored compound, which has a peak absorbance at 412 nm [21]. Data were expressed as μmol/mg protein.

## 2.11. Glutathione assay

The GSH hepatic levels were measured according to the kit brochure from ZellBio GmbH Company, Germany. Data were expressed as nmol/mg protein.

## 2.12. Protein assay

At the end of each experiment, protein content was as-

sayed using the Bradford method in the crude homogenate of hepatic tissue. The BSA was used as standard to calculate protein content [20].

## 2.13. Histopathological examination

A part of the hepatic tissue was removed from the each rat and held in 10% formalin for at least 24 h. The paraffin-embedded block was obtained by automatic tissue processor, Autotechnique. In the next stage, the tissue was cut into 4 μm thick parts by a rotating microtome with at least three cross-sections of tissue on each slide. The samples were stained by hematoxylin and eosin (H&E) dye for histopathological analysis [22].

## 2.13. Statistical analysis

The data were described using the mean and standard deviation (SD). The differences were determined, at 95% confidence, using the analysis of variance (ANOVA) and Turkey's test to account for multiple comparisons. All analyses were executed by Graph Pad Prism software (version 6.0).

## 3. Results

### 3.1. The efficiency of *Aj* fractions

The yield of hydroalcoholic extract was 32.67%, and the yield of n-hexane, chloroform, ethyl acetate and the methanolic fraction of hydroalcoholic extracts were 0.94, 0.39, 0.79 and 74.78%, respectively.

### 3.2. The effects of *Aj* extract on liver function biomarkers

The results indicated no sign of toxicity up to 100 mg/kg of *Aj* extract. There was an increase in ALP ( $P < 0.001$ ) and LDH ( $P < 0.001$ ) serum activity in the dose of 200 mg/kg *Aj*

**Table 1** The effects of *Allium jesdianum* (*Aj*) extract on liver function tests.

Groups	ALT activity (U/L)	AST activity (U/L)	ALP activity (U/L)	LDH activity (U/L)
Control	43.38±7.04	74.70±11.50	120.66±12.48	157.00±10.30
<i>Aj</i> extract (50 mg/kg)	37.89±5.78	70.08±10.7	128.57±5.68	155.99±10.45
<i>Aj</i> extract (100 mg/kg)	40.53±3.79	74.98±7.78	124.04±8.13	163.98±10.47
<i>Aj</i> extract (200 mg/kg)	43.05±6.83	79.20±10.71	138.33±7.07***	259.04±18.37***

extract in comparison with control (Table 1). The administration of APAP significantly increased ALT ( $P < 0.001$ ), AST ( $P < 0.001$ ), ALP ( $P < 0.001$ ) and LDH ( $P < 0.001$ ) serum activity in comparison with the control group (Fig. 1A-1D, respectively). The serum levels of liver enzymes (ALT, AST, LDH and ALP) in animals exposed to APAP could be decreased by the Aj extract.

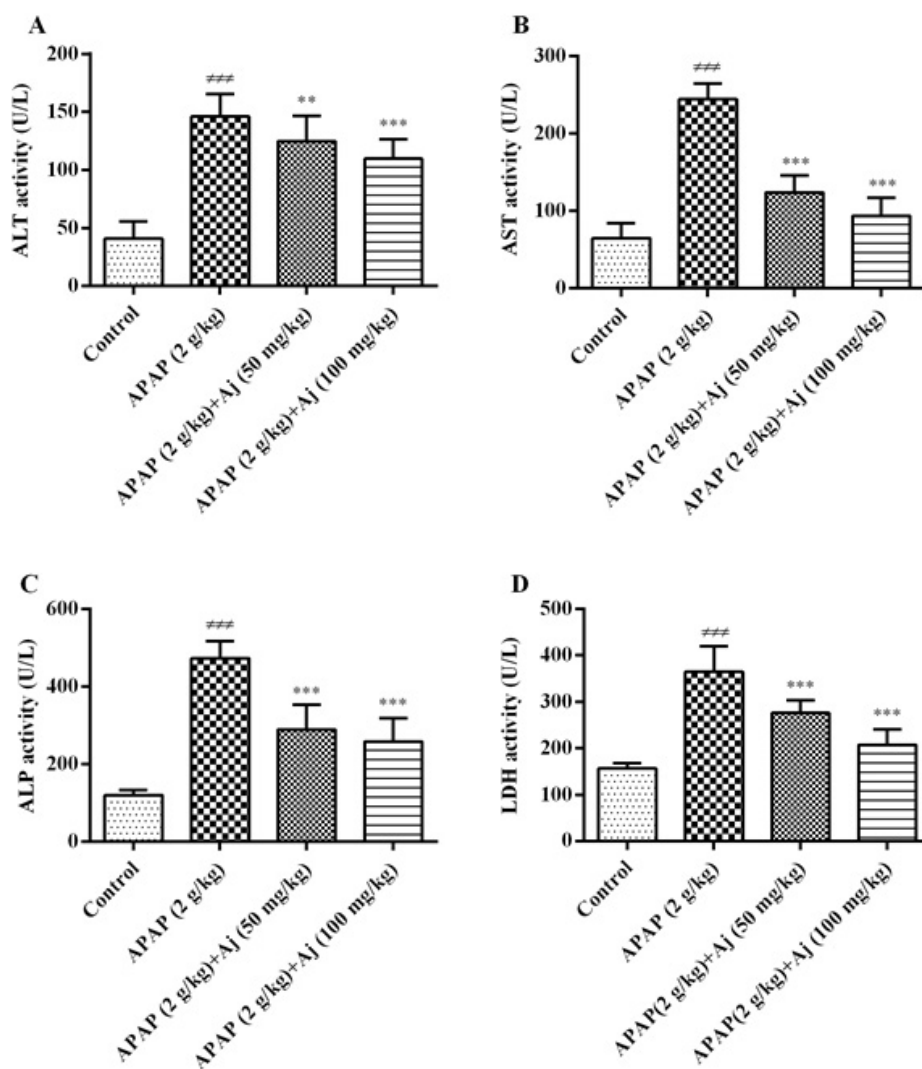
### 3.3. The effects of *Aj* extract on oxidative stress biomarkers

Following APAP administration, the levels of LPO (Fig. 2A) and NO (Fig. 2B) were increased ( $P < 0.001$ ) and TAC (Fig. 3A), as well as TTGs and GSH (Fig. 3B and 3C, respec-

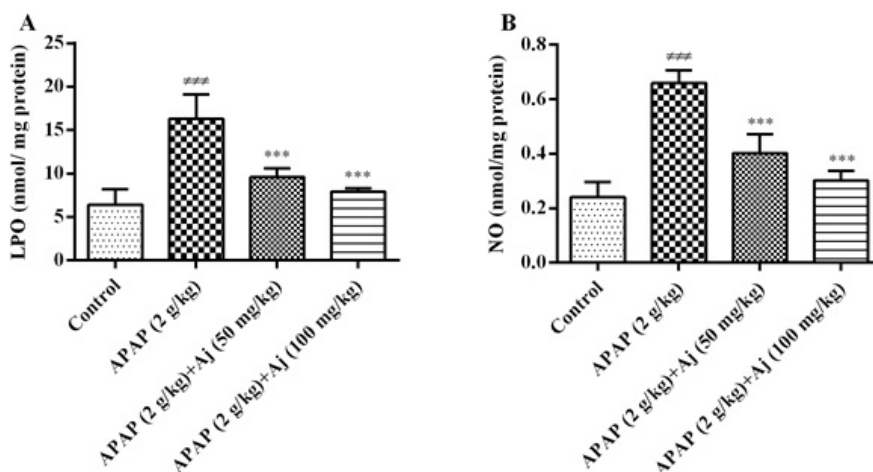
tively) levels, were decreased in liver tissue compared to control group ( $P < 0.001$ ). In addition, a significant improvement was observed in TTGs ( $P < 0.01$ ), and GSH ( $P < 0.05$ ) levels following pre-treatment with the Aj extract (Fig. 3B and 3C, respectively). In this regard, the levels of NO and LPO were decreased in treatment groups ( $P < 0.001$ ).

### 3.3. The effects of *Aj* extract on oxidative stress biomarkers

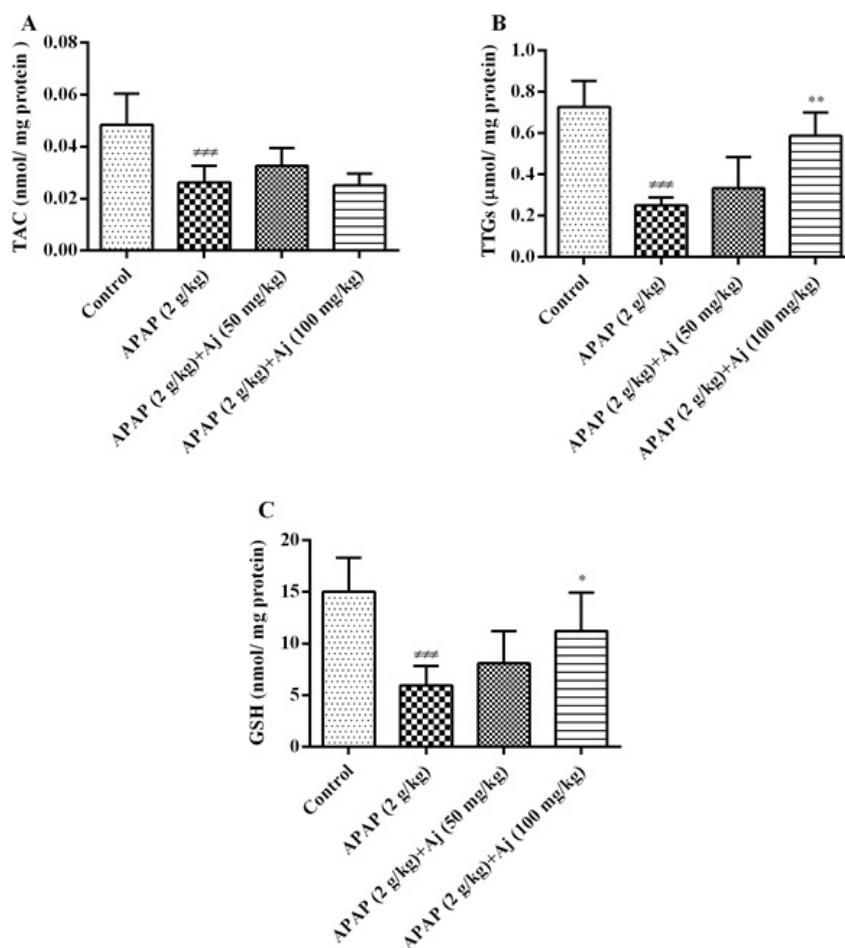
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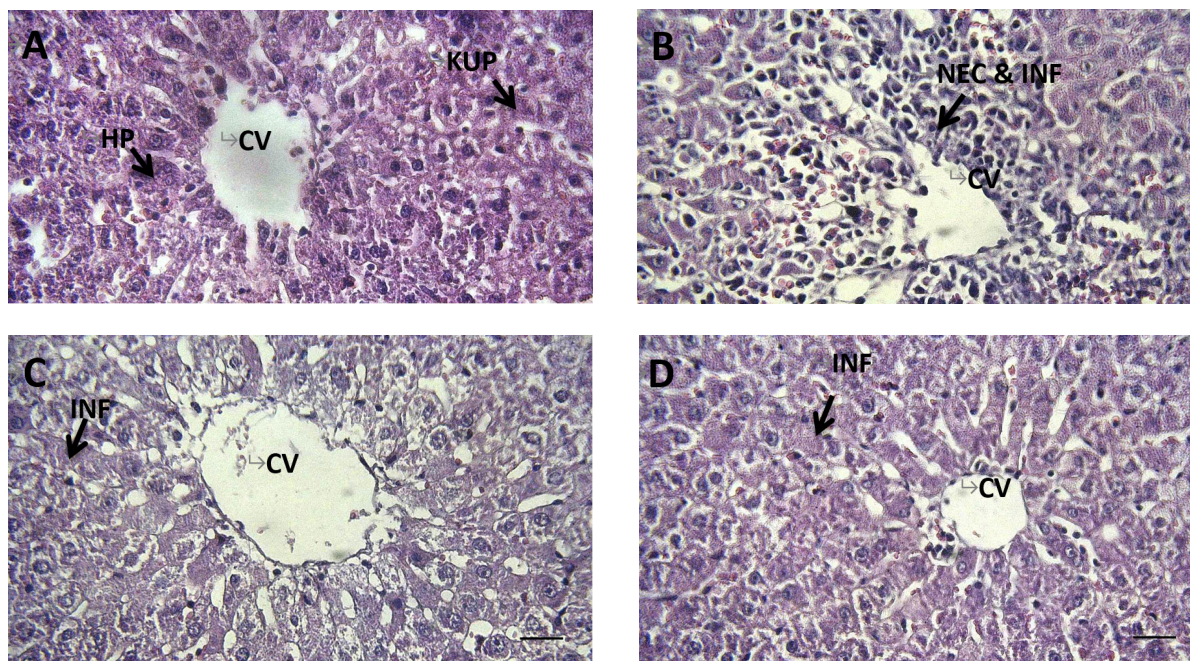
**Figure 1** Effects of *Allium Jesdianum* (*Aj*) on serum enzymes of acetaminophen (APAP)-exposed wistar rat. Statistical analysis used one-way ANOVA with Tukey's test. Values are expressed as means $\pm$ SD, n=6 for each group. ### $P < 0.001$  vs control group; \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs APAP group. ALT: alanine aminotransferase (A); AST: aspartate aminotransferase (B); ALP: alkaline phosphatase (C); LDH: lactate dehydrogenase (D).



**Figure 2** Effects of *Allium jesdianum* (Aj) on oxidant biomarkers of acetaminophen (APAP)-exposed wistar rat. Statistical analysis used one-way ANOVA with Tukey's test. Values are expressed as means±SD, n=6 for each group. <sup>###</sup>  $P < 0.001$  vs control group; <sup>\*\*\*</sup>  $P < 0.01$  vs APAP group. LPO: lipid peroxidation (A); NO: nitric oxide (B).



**Figure 3** Effects of *Allium jesdianum* (Aj) on antioxidative biomarkers of acetaminophen (APAP)-exposed wistar rat. Statistical analysis used one-way ANOVA with Tukey's test. Values are expressed as means±SD, n=6 for each group. <sup>###</sup>  $P < 0.001$  vs control group; <sup>\*</sup>  $P < 0.05$ , <sup>\*\*</sup>  $P < 0.001$  vs APAP group. TAC: total antioxidant capacity (A); TTGs: total thiol groups (B); GSH: glutathione (C).



**Figure 4** Acetaminophen (APAP) (2 g/kg); C, Aj extract (50 mg/kg) + APAP (2 g/kg); D, Aj extract (100 mg/kg) + APAP (2 g/kg). Original magnification of all images is  $\times 40$ . CV, central vein; HP, hepatocyte; KUP: Kupfer cells; NEC: necrosis; INF: infiltration.

tively) levels, were decreased in liver tissue compared to control group ( $P < 0.001$ ). In addition, a significant improvement was observed in TTGs ( $P < 0.01$ ), and GSH ( $P < 0.05$ ) levels following pre-treatment with the Aj extract (Fig. 3B and 3C, respectively). In this regard, the levels of NO and LPO were decreased in treatment groups ( $P < 0.001$ ).

### 3.4. Histopathological experiments

As shown in Fig. 4, mononuclear cell infiltration, dilation of sinusoids, vascular congestion, necrosis as well as the increased number of Kupffer cells were found in hepatic tissue of APAP-treated animals. Following the pre-treatment with various amount of Aj extract, a significant improvement was observed in some of the pathological symptoms such as necrosis and vascular congestion.

## 4. Discussion

In the current study, the safe dose of the Aj extract was found to be 50-100 mg/kg. In the high dose of Aj extract (200 mg/kg), a significant increase was seen in ALP and LDH serum levels alongside the histopathologic changes. It seems that Allium species contained substances that are metabolized to reactive and hepatotoxic substances when consumed high levels [23]. For instance, sodium n-propylthiosulfate and N-propyl disulfide are very toxic organosulfur compounds present in some of the Allium

species. These phytochemical constituents may cause a remarkable decrease in the activity of glucose-6-phosphate dehydrogenase (G6PD), whereas the latter can reduce glutathione level in the erythrocyte [24]. This changes leads to the vulnerability of erythrocyte to oxidative damage and subsequent hemolytic anemia, which can be associated with an increase in some serum biomarkers, especially LDH [25].

Contrary to our findings, Kalantari et al. showed that Aj extract had therapeutic effects at doses of 1000 and 2000 mg/kg in hepatotoxicity induced by bromobenzene, in mice model [26]. This discrepancy could be explained due to the difference in the type of extract and animal models.

As our data shown, APAP could induce liver toxicity and increase the levels of ALT, AST, ALP, and LDH which was consistent with the other literature [27-30]. When the integrity of the hepatocellular membrane disappeared, ALT and AST leaked into the bloodstream, and their level increased in blood [26]. The increased levels of ALP could be because of the biliary tract injury and/or increased biliary pressure [31, 32]. In the groups receiving the Aj extract, a remarkable decrease was observed in liver indices which may be associated with improving the integrity of the hepatocellular membrane in animals.

Since the toxicity of APAP has been associated with oxidative pathways, LPO, and NO levels (as oxidant markers), TAC, TTGs and GSH levels (as antioxidant indices), were assessed in the hepatic tissue. Significant increase in NO and LPO levels and reduction of antioxidant indices, such as TTGs, GSH and TAC, indicate the occurrences of oxidative stress which is in line with the other literature [33-37].

Overall, NAPQI has been known as the major metabo-

lite of APAP, which in high levels is caused reduction in the hepatocyte GSH levels. Following APAP poisoning, the excessive production of NAPQI breaks the SH-group in macromolecules, such as proteins, nucleic acids, and membranes, leading to hepatocellular dysfunction. An increased in LPO level may lead to the membrane detriment and alter the homeostasis of calcium after decreasing GSH levels and thiol storages [38].

Additionally, increased NO may be associated with APAP-inducing effects on the nitric oxide synthase (NOS) and occurrence of nitrosative stress [39]. The experimental studies showed that the excess NO reacted with superoxide anion to make peroxynitrite radicals [1, 40-42]. The role of the peroxynitrite radicals in the development of hepatic failure is confirmed through the NOS inhibitor and subsequently inhibition of endogenous NO generation as described by Gardner et al. [39]. These radicals deplete the intracellular reduced GSH leading to the increase in the vulnerability to the oxidative stress [43]. In treatment groups, a remarkable reduction was found in liver oxidant indexes (LPO and NO). Furthermore, this extract improved liver antioxidant state that may be associated with the antioxidant-related properties of *Allium* species [44, 45]. It seemed that the high values of sulfur-containing constituents in the *Allium* species like diallyl sulfide, S-methyl cysteine 2,5-dimethylthiophene and 1-propylmercaptan could recover TTGs and GSH levels in treated rats [46]. Previously, Chiu et al. showed that 2,5-dimethylthiophene and 1-propylmercaptan may contribute to the antioxidative and antimutagenic properties of *Allium* species [47]. Diallyl sulfide, a garlic constituent, protects the liver from the warm hepatic ischemia-reperfusion injury through decreasing oxidative stress and inhibition of CYP2E1 [48]. S-methyl cysteine is effective in improving inflammation, and oxidative stress in rats fed with fructose rich diet [49].

Despite an increase in TTM and GSH contents there was no increase in TAC. This might be due to the fact that the FRAP cannot be useful in precisely determining the thiol-containing mixtures because this experiment does not totally react with thiol-type antioxidants like glutathione [50]. However, the phytochemical analysis of the some of the *Allium* species showed the presence of several phytoconstituents such as, phenols and flavonoids which may have therapeutic effects against liver oxidative damages [51, 52].

## 5. Conclusion

This study showed that, although the Aj extract was toxic at the amount of 200 mg/kg, its lower doses had protective effects on APAP hepatotoxicity. The Aj extract may moderate liver damage through decreasing the oxidant markers (LPO and NO) and restoring the antioxidant thiol groups that it could improve the oxidative/antioxidant balance in hepatic tissue. Our findings may expand the applications of Aj plant and offer an herbal supplement with antioxidant and hepatoprotective properties in the human diet.

## Acknowledgment

The financial support for conducting the present study was provided by the Vice-chancellor of Research and Technology, Hamadan University of Medical Sciences, Hamadan, I.R. Iran (Grant No. 9605032795).

## Conflict of interest

No conflicts of interest were declared by the authors.

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