

Hepatoprotective activity of *Allium paradoxum*

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Abstract. – **OBJECTIVE,** Recent studies show that free radicals are important mediators of hepatic injury induced by carbon tetrachloride. *Allium (A.) paradoxum* showed antioxidant and antihemolytic activities. This work was conducted to determine the possible protective effect of this plant against hepatotoxicity.

MATERIALS AND METHODS, Subcutaneous injection of 3 ml/kg carbon tetrachloride diluted in olive oil (1:1 dilution) was employed for inducing acute liver toxicity. The protective effect of aerial parts and bulbs of *A. paradoxum* at flowering stage were determined.

RESULTS, Both aerial parts and bulbs extracts at the doses 500 and 750 mg/kg, i.p. offered significant hepatoprotective effect by reducing the serum marker enzymes, serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Histopathological studies further confirmed the hepatoprotective activity of aerial parts and bulbs extracts when compared with the CCl₄ treated groups.

CONCLUSION, Extracts of *A. paradoxum* showed significant hepatoprotective activity compared with control group.

Key Words:

Allium paradoxum, CCl₄ induced hepatotoxicity, Alkaline phosphatase, Hepatoprotective activity

Introduction

The polyhalogenated compound such as CCl₄ is a well-known hepatotoxic compound, and exposure to this chemical is known to result in hepatocellular necrosis in rodents¹. Depending on the dose of exposure to CCl₄ and prior exposure to other chemicals, extensive liver dysfunction results in total hepatic failure and animal lethality²⁻⁴. There are many natural products such as plant and traditional herbal formulation available for the protec-

tive of liver against injury induced by hepatotoxin. More than 600 commercial herbal products with claimed hepatoprotective role are being sold in all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to show hepatoprotective role. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy⁵. Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds which are being evaluated⁶. The genus *Allium* [*Alliaceae* family) has more than 600 species all over the world, among them just a few species have been consumed so far as vegetables, spices or ornamental plants⁷. *A. paradoxum* (M.B.) G. Don., which is a cultivated vegetable and spice used in home gardens. This species, which is locally called Alezi, was found as a cultivated vegetable and spice in home gardens of northern area of Iran especially in Mazandaran⁸. The plant is used to prepare a variety of local and special foods. Cysteine sulfoxides, alliinase activity and antioxidant activity of *A. paradoxum* have been reported recently^{7,9}. Considering its good antioxidant activity, its hepatoprotective activity needs to be investigated using a toxicant that directly causes hepatic toxication. One such toxicant is CCl₄, which induces hepatotoxicity in mice. The goal of this study was to determine the hepatoprotective effect of aerial parts and bulbs extract of *A. paradoxum* in mice.

Material and Methods

Plant Materials and Preparation of Extract

Allium paradoxum aerial parts and bulbs were collected from Sari Mountains, Mazandaran,

Iran and identified by Dr. B. Eslami, Assistance Professor of Plant Systematic and Ecology. The materials were oven dried at 38°C, for 5 days. Dried materials were coarsely ground (2-3 mm) before extraction. Materials were extracted by percolation method using methanol (80/20 w/w) for 24 h at room temperature. Extracts were filtered and concentrated under reduced pressure at 40°C using a rotary evaporator until a crude solid extracts were obtained which were then freeze-dried for complete solvent removal. The yields were 15.5 and 27% for bulbs and aerial parts, respectively.

Animal

The study was performed on male NMRI mice of approximately the same age-group and body weight (2-3 weeks; 20-25 g), housed in ventilated animal rooms at a temperature of $24 \pm 2^\circ\text{C}$ with a 12 h light/dark cycle and $60 \pm 5\%$ humidity. They were fed with standard laboratory animal feed, manufactured by Pasture Institute, Tehran, Iran. Water was provided *ad libitum*. Experiments were performed between 10:00 h and 14:00 h. All experiments were performed according to the norms of the Ethical Committee of University of Mazandaran, Babolsar which is in accordance with the National Guidelines for animal care and use.

Experimental Protocol

Animals were divided into four groups, each group containing ten animals. Group I (normal control) received normal saline for 5 days. Group II (induction control) received CCl_4 3 ml/kg, SC., 1:1 dilution with olive oil on 3rd day. Groups III, IV received extract (500 and 750 mg/kg, i.p.) respectively for 5 days and CCl_4 induction on 3rd day. On the 6th day, the animals were sacrificed under ether anesthesia, and blood and liver samples were collected¹⁰. The blood was allowed to

clot for 30 min; serum was separated by centrifuging at 37°C and was used for biochemical estimations. For histopathological study, liver tissue was quickly removed after autopsy and fixed in 10% formosaline. Serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) were determined spectrophotometrically (UV – Visible EZ201, Perkin Elmer, Waltham, MA, USA) from serum samples using commercially available kits (Ziestchem. Diagnostics, CAT No. 10-513, Tehran, Iran).

Histopathological Studies

The liver was collected and immediately fixed in 10% Formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 μ) were prepared and then stained with hematoxylin and eosin (H & E) dye for photomicroscopic observations.

Statistical Analysis

The values are presented as means \pm SD. Differences between group means were estimated using a one-way ANOVA followed by Duncan's multiple range test. Results were considered statistically significant when $p < 0.05$.

Results and Discussion

Hepatoprotective effect of *A. paradoxum* bulbs and aerial parts extracts against CCl_4 -induced hepatotoxicity in mice have been showed in Table I. CCl_4 (3 ml/kg SC.) when injected as single dose on 3rd day, caused marked hepatotoxicity showing significant ($p < 0.001$) increase in serum aspartate aminotransferase (AST) (555.3%) and alkaline phosphatase (ALP) (190.0%) as compared to normal control animals. The methanol extract of *A.*

Table I. Effect of *Allium paradoxum* on AST and ALP levels in carbon tetrachloride-induced hepatotoxic mice.

Groups	AST U/L	ALP U/L
Normal	98.333 \pm 16.74	143.33 \pm 25.65
CCl_4 control (3 ml/kg, SC.)	644.33 \pm 16.92 (555.3)***	415.67 \pm 23.58 (190.0)***
Bulbs extract-treated (500 mg/kg, i.p.)	280.33 \pm 19.50 (185.9)***	219.00 \pm 11.0 (52.8)*
Bulbs extract-treated (750 mg/kg, i.p.)	199.67 \pm 8.73 (103.1)**	166.0 \pm 18.52 (15.8)*
Aerial parts extract-treated (500 mg/kg, i.p.)	135.00 \pm 10.8 (37.3)*	211.67 \pm 15.36 (47.7)*
Aerial parts extract-treated (750 mg/kg, i.p.)	106.0 \pm 6.0 (7.8)*	165.0 \pm 5.0 (15.1)*

Values are mean \pm SD (n = 10). Data for normal animals are considered as base-line data; there was no significant base-line difference between the groups. Percentage increase (in parentheses) is calculated with reference to normal control. * $p < 0.05$ versus control group, ** $p < 0.01$ versus control group, *** $p < 0.001$ versus control group.

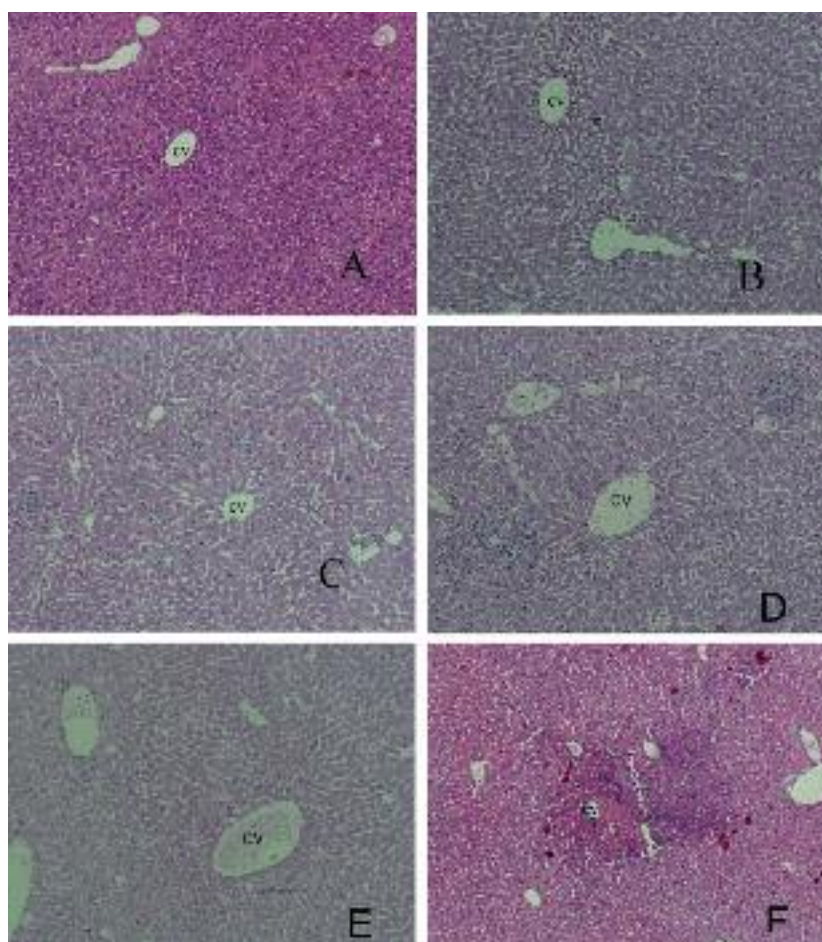


Figure 1. The photomicrography of liver sections from mice treated with CCl_4 , the post-doses of extracts at 500 and 750 mg/kg/day for 5 consecutive days vehicle. **A**, Liver section of normal group. **B**, Liver section of the CCl_4 -treated mice and treated with aerial parts extract at 500 mg/kg. **C**, Liver section of the CCl_4 -treated mice and treated with aerial parts extract at 750 mg/kg. **D**, liver section of the CCl_4 -treated mice and treated with bulbs extract at 500 mg/kg. **E**, Liver section of the CCl_4 -treated mice and treated with bulbs extract at 750 mg/kg. **F**, Liver section of the control group treated with CCl_4 .

paradoxum aerial parts-treated mice differed from normal control mice by an elevated concentration of serum AST (37.3 and 7.8%) and ALP (47.7 and 15.1%) at doses 500 and 750 mg/kg/day, i.p. respectively. These parameters were found to be statistically significantly different as compared to normal mice (Table I). The methanol extract of bulbs-treated mice differed from normal control mice by an elevated concentration of serum AST (185.9 and 103.0%) and ALP (52.8 and 15.8%) at doses 500 and 750 mg/kg/day, i.p. respectively. These parameters were found to be statistically significantly different as compared to normal mice, too (Table I). The effect was dose-dependent which indicated activation of the regeneration process.

A single dose of carbon tetrachloride caused toxicity in the liver. Both extracts at dose of 750 mg/kg/day exhibited maximum protection in the

liver (Figure 1). The histological changes associated with the hepatoprotective activity in two dosages of extracts basically supported the measuring of the serum enzyme activities. There was no abnormal appearance or histological changes in the liver of normal group, which received olive oil only (Figure 1A). However, CCl_4 administration caused classical damage in the mice liver at 72 h, as demonstrated by severe apoptosis, hepatocyte necrosis, inflammatory cells infiltration and sinusoidal dilation (Figure 1F). The administration of bulbs and aerial parts extracts at dose of 500 and 750 could largely rescue the severity of CCl_4 -induced liver intoxication. The higher doses (750 mg/kg) were most effective (Figure 1B-C-D-E). Bulbs treatment at doses 750 mg/kg produced extensive piece mild necrosis and mild apoptosis and high inflammatory cell

filtration (Figure 1E). Bulb extract at lower dose (500 mg/kg) showed moderate necrosis, moderate apoptosis, high inflammatory cell filtration and sinusoidal dilation (Figure 1D). In aerial parts, treatment at doses 750 mg/kg showed moderate to high inflammatory cell filtration and mild apoptosis (Figure 1C) that were better than other doses in preventing the toxic changes induced by CCl₄ administration. Aerial parts extract at lower doses (500 mg/kg), showed moderate necrosis, moderate apoptosis, high inflammatory cell filtration and sinusoidal dilation and confluent necrosis (Figure 1B).

The need to identify alternative natural and safe sources of antioxidants arose and the search for natural antioxidants, especially of plant origin, has notably increased in recent years¹¹. One of the most sensitive and dramatic indicators of hepatic failure is the release of intracellular enzymes, such as serum AST and alkaline phosphatase in the circulation after CCl₄ administration. The elevated activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of the cell membranes in liver¹². The stabilization of these enzyme levels by the extract is a clear indication of the improvement of the functional status of the liver. Also, recovery in serum AST and alkaline phosphatase activities with the administration of other *Allium* species are reported e.g. *A. sativum*^{13,14}, *A. fistulosum* L¹⁵ and *A. cepa*¹⁴. Also In the present investigation administration of single dose of CCl₄ caused toxicity in the liver, because CCl₄ is capable of initiating cell injury and the cellular sites for free radical generation include mitochondria, endoplasmic reticulum and plasma membrane. Recoument in these enzymes activity after administration of extracts may be ascribed to the antioxidant property of the extracts. The mitochondrial damage may be prevented when oxidative stress is suppressed.

Conclusions

Present study improved the hepatoprotective effect of aerial parts and bulbs extract of *A. paradoxum* on hepatic injuries induced by CCl₄, which might be considered to be therapeutic effect in clinical situations. These results can be useful as a starting point of view for further applications of this plant or its constituents in pharmaceutical preparations after performing clinical researches.

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