



Histopathological and Biomedical Parameters Determination in the Protective Effect of Hydroalcoholic Extract of *Allium Jesdianum* on Hepatotoxicity Induced by Bromobenzene

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Abstract

Allium Jesdianum (AJ) is the native plant mostly grown in Middle East region that has the excellent pharmacological properties. In this study, we evaluated the hepatoprotective effect of hydroalcoholic extract of AJ on liver injury induced by Bromobenzene (BB) in male mice. Animals were randomly divided into five groups, control group received normal saline plus olive oil, groups 2-4 received (500, 1000 and 2000 mg/kg) AJ extract plus BB for 5 days and 5th group received BB (460 mg/kg). On the fifth day all groups received hexobarbital sodium (25 mg/kg, i.p). It should be noted that sleeping time of the all mice were recorded. After 24 hours the mice were sacrificed. By serum and tissue biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), reduced glutathione (GSH), malondialdehyde (MDA), and histopathological studies were measured. The results showed that BB significantly increased sleeping time, ALT, AST, and MDA levels besides and decreased GSH level compared with the control group. AJ extract at doses 1000 and 2000 mg/kg showed a significant alter in all studied endpoints and dose 2000 mg/kg showed a marked improvement in histopathological examination. The present finding indicated that administration of the hydroalcoholic extract of AJ could prevent hepatotoxicity induced by BB via improvement serum and tissue parameters and histopathological alterations in liver tissue.

Keywords: Bromobenzene; *Allium Jesdianum*; Hepatoprotective; Antioxidants, Glutathione, Malondialdehyde.

1. Introduction

Liver is the vital organ in the body and has the major role in excretion and metabolism of drugs and xenobiotics. Liver diseases are one of the main causes of mortality and morbidity

in the world [1]. Acute liver failure (ALF) causes hepatic damage by drugs, poisons, viral hepatitis, ischemia, and other causes [2, 3]. Organic solvents utilize in diverse industrial processes and may be related to

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hepatotoxicity[4].BB is one organic solvent and the toxic chemical that uses for organic agents synthesis especially for production of phenyl magnesium bromide during the process of water chlorination and also uses as an additive in motor oil[5, 6].BB can enter the body via oral or dermal exposure. The metabolism of BB in the liver leads to hepatotoxicity by primary cytochrome P450-dependent [7]. BB-induced hepatotoxicity via formation of reactive epoxide intermediate [8].Cytochrome P450 monooxygenase hydrolysis BB to BB-3, 4-epoxide (less stable) and 2, 3 epoxide (more stable) that are highly electrophilic compounds. These metabolites bind to GSH and deplete hepatic GSH levels. As a result decrease in the first line of defenses against intracellular reactive oxygen species (ROS) and lead to next events including mitochondrial dysfunction, increased lipid peroxidation, oxidative stress, inflammation and subsequently liver injury and hepatocyte necrosis [9-12].

In recent years, herbal medicines due to their wide biological and medicinal activities have been used for the treatment of a large number of diseases [13, 14]. Because of failure in the treatment of many diseases, unwanted side effects of chemical drugs and increased

resistance to drugs particularly the antibiotics have led to use medicinal plants [15, 16]. The genus *Allium* is important sources of dietary flavonoids and phenolic compounds which have antioxidant properties [17]. AJ species belong to Liliaceae family, the bulbar and durable plant which grows up in altitudes of Middle East region [18]. This plant is used in diet and of course in traditionally treatment for digestive pains, like rheumatic [19].The purpose of this study is to evaluate the hepatoprotective effect of hydroalcoholic extract of AJ on hepatotoxicity induced by BB in male mice.

2. Materials and Methods

2.1. Chemicals and Animals

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). BB was purchased from Roche chemical company (Germany). Male Albino mice (25-30g) were prepared from Animal house of medical science of Jundishapur University, Ahvaz, Iran. The animals were housed in polycarbonate cages under temperature $25\pm 2^{\circ}\text{C}$ and 12 hours dark-light cycle and were fed with standard diet and watered ad libitum.

2.2. Preparation of Hydroalcoholic Extract of AJ

AJ plant was taxonomically identified and confirmed at Medicinal Plant Research Center medical science of Jundishapur University. The plant was dried and milled, and then 30 gram of powered material was put into the Soxhlet extractor with 100 ml of 80%

ethanol/water for extraction. The dried extraction by oven was used in this experiment.

2.3. Experimental Design

Thirty five male mice were randomly divided into 5 groups (n=7).

Group 1: control group received olive oil (0.2 ml) for 5 days.

Group 2: Mice received BB (460 mg/kg) for 5 day.

Group 3: Mice received AJ extract (500 mg/kg) plus BB (460 mg/kg) for 5 day

Group 4: Mice received AJ extract (1000 mg/kg) plus BB (460 mg/kg) for 5 day.

Group5: Mice received AJ extract (2000 mg/kg) and BB (460 mg/kg) for 5 day.

All mice treated by gavage and BB solved in olive oil.

On the fifth day, all groups received hexobarbital sodium (25 mg/kg, i.p) and sleeping time of all mice was recorded.

After 24 hours of the last dosage, the animals were sacrificed. The blood samples were collected and centrifuged at 3,000 rpm for 10 min at 4°C. The serums were separated and stored at -80°C for evaluation of the activities of AST and ALT by the method of Reitman's and Frankel [20]. A piece of liver was removed and fixed in 10% formalin solution for histological examination. The other piece was isolated and homogenized in phosphate buffer solution (PBS) (0.1 M, PH: 7.4) and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was used for the estimation of GSH, MDA, and protein levels.

2.4. Protein Estimation

The protein content of various samples was determined according to the method of Bradford by using bovine serum albumin [21].

2.5. Determination of GSH

GSH level was measured by the method Ellman [22]. Briefly liver homogenate was added with equal volume of trichloroacetic acid (TCA) (20%) and EDTA (1mM) for precipitation of the tissue protein. The mixture was shaken for 5 min and centrifuged for 10 min at 2,000 rpm. Supernatant (200µl) was transferred to new tube and added DTNB (0.1 mM, 1.8ml) and mixed with PBS. The absorbance was recorded at 412 nm. The GSH level in liver was expressed as µg/mg protein.

2.6. Lipid Peroxidation Assay

Liver homogenate was used for measurement lipid peroxidation level by reaction MDA with thiobarbituric acid (TBA) according to Draper and Hadley [23]. Briefly liver homogenate (0.5 ml) and TCA (1 ml) were mixed and centrifuged at 2,500g for 10 min. Then TBA (1 ml, 0.67%) and supernatant (0.5 ml) were incubated for 30 min at 90 °C and then cooled. Absorbance of complex was determined at 532 nm. Lipid peroxidation was expressed as nmol/mg protein of TBA reactive substances.

2.7. Pathological Preparation

The liver slices of all groups were fixed in a 10% buffered formalin and embedded in paraffin. Sections of 5µm stained with

hematoxylin and eosin (H&E) for study of histopathological changes.

2.8. Statistical Analysis

All data were expressed as mean \pm SD and statistical analysis was carried out using SPSS software. The Comparisons between groups were evaluated by one-way ANOVA followed by a post hoc test to determine the significant differences between the groups. P values < 0.05 were considered statistically significant.

3. Results and Discussion

Liver is one of the vital organs and main site for metabolism of foreign compounds entering the body. It has broad functions including detoxification, protein synthesis, and production of enzyme for digestion [24, 25]. A number of industrial chemical agents and drugs can cause serious cellular injuries in various organs of the body. Often these injuries are mediated by free radicals or removal of endogenous antioxidants such as GSH [26, 27]. BB is a toxic industrial material

which is known for causing centrilobular hepatic necrosis via formation of reactive epoxides intermediate. This compound lead to liver injury which can be disruption in hepatic enzymes [28, 29]. In the present study, the protective effect of hydroalcoholic extract of AJ against BB induced hepatotoxicity was investigated by assessing the levels of hepatic enzymes (ALT and AST), sleeping time, MDA and GSH in the studied groups. Treatment with BB showed a significant increase in sleeping time (47.8 ± 2.6) compared to the control group (28.7 ± 1.6) which can be related to liver enzymes abnormality while treated groups with AJ extract at doses 1000 and 2000 mg/kg can improve sleeping time, respectively (38.1 ± 1.06 , 34.1 ± 1.06) compared to BB group (47.8 ± 2.6) (Fig 1) which represents improved in liver function [30].

Also our results showed a significant increase in the serum levels of ALT and AST in the BB-treated mice. Pretreatment with hydroalcoholic extract of AJ could reverse the liver damages in a dose-dependent manner and

Table 1. Effect of hydroalcoholic extract of AJ and BB on ALT and AST levels.

Groups	ALT (U/I)	AST(U /I)
1) Normal saline + olive oil	75.42 \pm 25.39	153.85 \pm 20.87
2) BB group (460 mg/kg)	160.71 \pm 22.58 *	430.42 \pm 92.55 *
3) AJ extract (500 mg/kg) +BB	136.57 \pm 13.91	350.14 \pm 72.28
4) AJ extract (1000 mg/kg) +BB	123.14 \pm 13.59 #	248.42 \pm 39.18#
5) AJ extract (2000 mg/kg) +BB	108.28 \pm 27.04 #	233.42 \pm 136#

(Mean \pm SE; n = 7). *: significantly different from the control group, p < 0.05. #: significantly different from the BB group, p < 0.05.

the best results were observed in doses of 1000 and 2000 mg/kg (Table 1). This effect may be due to the prevention of release these intracellular enzymes into the circulation due to its membrane stabilizing activity [24]. Antioxidant activity may protect the biological

systems from injurious of free radicals induced by xenobiotic. Liver has variety of redox systems, which among them GSH acts as an intracellular non-enzymatic biological antioxidant and protect cells against ROS [10, 31].

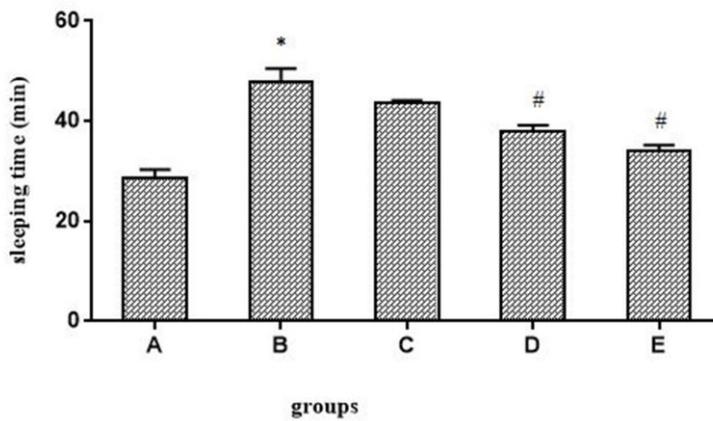


Figure 1. Effect of extract of AJ and BB on the mean of sleeping time in the studied groups. **A:** normal saline + olive oil (0.2 ml), **B:** normal saline + BB, **C:** AJ extract (500 mg/ kg) + BB, **D:** AJ extract (1000mg/kg) + BB, **E:** AJ extract (2000 mg/ kg) + BB. Values are mean \pm SD for seven rats each group. *: significantly different from the control group at $p < 0.05$. #: significantly different from the positive control group at $p < 0.05$.

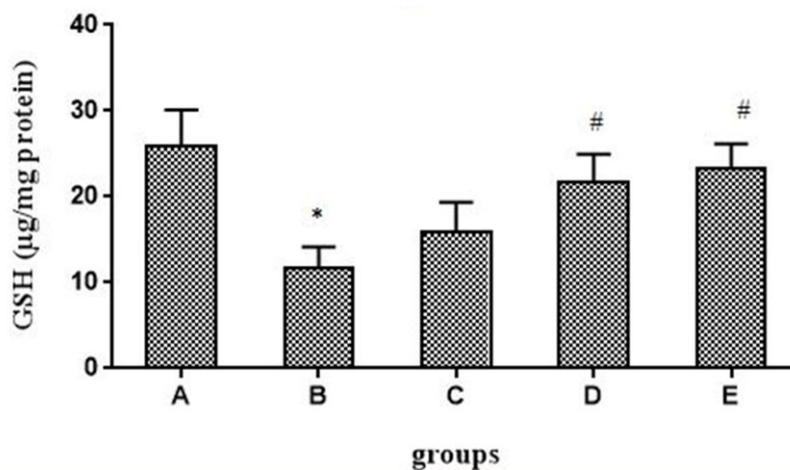


Figure 2. Effect of hydroalcoholic extract of AJ and BB on GSH level in the studied groups. **A:** normal saline + olive oil, **B:** BB group, **C:** AJ extract (500 mg/kg) + BB, **D:** AJ extract (1000 mg/kg) + BB, **E:** AJ extract (2000 mg/kg) + BB. (Mean \pm SE; $n = 7$). *: significantly different from the control group, $p < 0.05$. #: significantly different from the BB group, $p < 0.05$.

Oxidative stress can stimulate the formation of variety oxidant mediator that can disrupt liver function. Lipid peroxidation know as one of index of membrane damage induced by oxidative stress and occurs naturally in the

tissues rich of polyunsaturated fatty acids and leads to alterations in the structure and function of the membranes [31, 32].These observations indicate that the mechanisms hepatotoxicity are associated with the

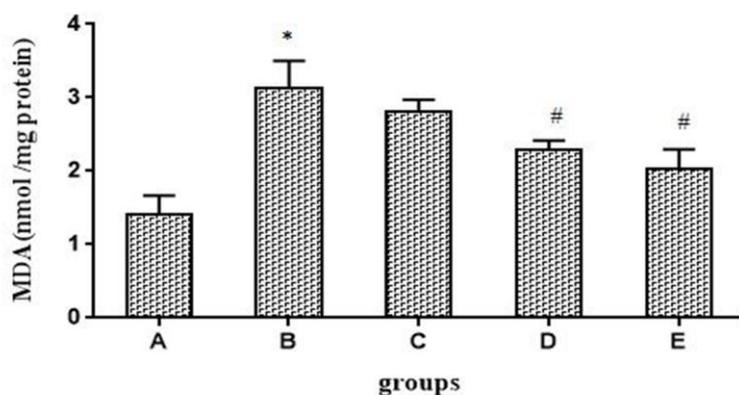


Figure 3. Effect of hydroalcoholic extract of AJ and BB on MDA level in the studied groups. **A:** normal saline + olive oil, **B:** BB group, **C:** AJ extract (500 mg/kg) + BB, **D:** AJ extract (1000 mg/kg) + BB, **E:** AJ extract (2000 mg/kg) + BB. (Mean \pm SE; n = 7). *: significantly different from the control group, $p < 0.05$. #: significantly different from the BB group, $p < 0.05$.

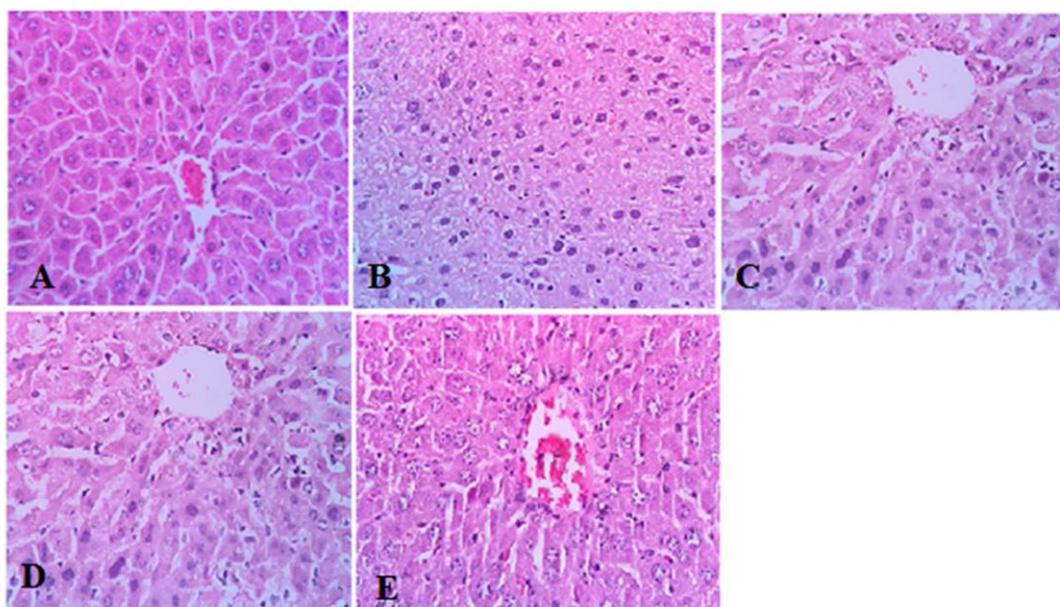


Figure 4. Histopathological observations of liver tissues were performed with Hematoxylin and Eosin, magnification x 100. **A:** Normal group, **B:** BB treated group **C, D and E:** 500, 1000 and 2000 mg/kg of AJ extract + BB, respectively.

depletion of GSH and free radical generation.

Our investigation demonstrated a significant decrease in GSH level of group treated with BB while in the pretreated groups with hydroalcoholic extract of AJ (1000 and 2000 mg/kg) a significant increase in GSH level (Fig 2) was showed. Also, MDA level was significantly elevated as a marker of lipid peroxidation in the BB-treated group while pre-treatment of hydroalcoholic extract of AJ(1000 and 2000 mg/kg) could restore the MDA level to normal (Fig 3). In the study conducted by Mansour et al, the effect of the antioxidant alpha lipoic acid (ALA) assess on liver necrosis induced by BB in rat and biochemical parameters such as ALT, AST, isocitrate dehydrogenase (ICDH), GSH, MDA, nitric oxide (NO) and total protein (TP) were measured. Their findings showed that pretreatment with ALA increased GSH, while the normalized MDA, ICDH, ALT, AST, and NO levels together with preservation of damage to the hepatocyte construction indicated effect of hepatoprotective ALA against BB -induced liver damage [27]. In another study Vahdani et al showed the effects of hydrophilic extract of AJ on ethylene glycol-induced renal stone in rat. Their finding demonstrated that hydrophilic extract of AJ can prevent calcium oxalate stones in rat, but in urinary and serum parameters renal wasn't significant [18].

Histopathological examination of liver tissue of the control group showed normal hepatic building with distinct hepatic cells (Fig 4A). In the treated group with BB congestion, severe fatty changes, pyknotic nuclei, necrosis,

disorderly sinusoidal spaces, and ballooning degeneration were showed (Fig4B). Administration of 500 mg/kg of AJ extract showed mild improvement, also inflammation and lobular order disorganization were limited (Fig 4C). With increasing dose of AJ extract further improvement were achieved and at a dose of 2000 mg/kg only inflammation and swelling of the cells was observed (Fig 4D and 4E). Our observations showed that histopathological findings confirmed the above results and indicated that hydroalcoholic extract of AJ reduced BB-induced hepatotoxicity in the dose dependent manner and led to protect liver from hepatotoxicity induced by BB.

4. Conclusion

Our findings indicated the hepatoprotective effect of hydroalcoholic extract of AJ against BB induced liver damage in mice. This hepatoprotective effect may be mediated antioxidant properties of AJ extract. *Allium Jesdianum* can use a substitute therapeutic agent against oxidative damage induced by toxic chemicals.

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Reference

- [1] Girish C, Pradhan SC. Drug development for liver diseases: focus on picroliv, ellagic acid and curcumin. *Fundam Clin Pharmacol.* 2008;22(6):623-32.
- [2] Bantel H, Schulze-Osthoff K. Mechanisms of cell death in acute liver failure. *Front Physiol.* 2012;3(79):1-8.
- [3] Kalantari H, Kooshapur H, Rezaii F, Ranjbari N, Moosavi M. Study of the protective effect of *Raphanus sativus* (radish) seed in liver toxicity induced by carbon tetrachloride in mice. *Jundishapur J Nat Pharm Prod.* 2009;4(1):24-31.
- [4] Brautbar N, Williams J. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. *Int J Hyg Environ Health.* 2002;205(6):479-91.
- [5] El-Sharaky A, Newairy A, Kamel M, Eweda S. Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. *Food Chem Toxicol.* 2009;47(7):1584-90.
- [6] Vedi M, Sabina EP. Assessment of hepatoprotective and nephroprotective potential of withaferin A on bromobenzene-induced injury in Swiss albino mice: possible involvement of mitochondrial dysfunction and inflammation. *Cell Biol. Toxicol.* 2016;32(5):373-90.
- [7] Sabina EP, Martin SJ, Vedi M, Lavinya BU, Benny B, ST AS, et al. Protective role of food supplement *Spirulina fusiformis* in chemical induced hepatotoxicity: a bromobenzene model in rats. *Int. j. pharm. Res.* 2014;4(1):04-9.
- [8] Cheol Park J, Chul Kim S, Moon Hur J, Choi SH, Yeon Lee K, Won Choi J. Anti-hepatotoxic effects of *Rosa rugosa* root and its compound, rosamultin, in rats intoxicated with bromobenzene. *J Med Food.* 2004;7(4):436-41.
- [9] Vedi M, Rasool M, Sabina EP. Amelioration of bromobenzene hepatotoxicity by *Withania somnifera* pretreatment: role of mitochondrial oxidative stress. *Toxicol Rep.* 2014;1:629-38.
- [10] Hamed M, Ali S. Effects of black seed oil on resolution of hepato-renal toxicity induced by bromobenzene in rats. *Eur Rev Med Pharmacol Sci.* 2013;17(5):569-81.
- [11] Yoshioka H, Fukaya S, Fukuishi N, Nagatsu A, Nonogaki T, Onosaka S. Bromobenzene-induced lethal toxicity in mouse is prevented by pretreatment with zinc sulfate. *Chem. Biol. Interact.* 2016;254:117-23.
- [12] Mahima V, Martin S, Lavinya B, Yashodhara B, Monisha S, Shreni T, et al. Ameliorative effects of *Spirulina fusiformis* against bromobenzene induced nephrotoxicity in rats: A Biochemical and Histoarchitectural study. *Asian J. Biochem.* 2014;9(1):57-64.
- [13] Lorigooini Z, Ayatollahi SA, Amidi S, Kobarfard F. Evaluation of anti-platelet aggregation effect of some *Allium* species. *iranJ Pharm Res: IJPR.* 2015;14(4):1225.
- [14] Kshirsagar A, Mohite R, Aggrawal A, Suralkar U. Hepato-protective medicinal plants of Ayurveda: a review. *Asian J Pharm Clin Res.* 2011;4(3):1-8.
- [15] Rios J, Recio M. Medicinal plants and antimicrobial activity. *J Ethnopharmacol.* 2005;100(1):80-4.
- [16] Arif T, Bhosale J, Kumar N, Mandal T, Bendre R, Lavekar G, et al. Natural products—antifungal agents derived from plants. *J Asian Nat Prod Res.* 2009;11(7):621-38.
- [17] Tepe B, Sokmen M, Akpulat HA, Sokmen A. In vitro antioxidant activities of the methanol extracts of five *Allium* species from Turkey. *Food Chem.* 2005;92(1):89-92.
- [18] Vahdani R, Mehrabi S, Malekzadeh J, Jannesar R, Sadeghi H, Shafaeifar A. Effect of hydrophilic extract of *Allium jesdianum* on ethylene glycol-induced renal stone in male wistar rats. *Armaghane danesh.* 2012;16(6):566-77.
- [19] Gholami A, Arabestani MR, Ahmadi M. Evaluation of antibacterial activity of aqueous and methanol extracts of *Allium Jesdianum* plant on a number of pathogenic bacteria resistant to antibiotics. *PSJ.* 2016;14(4):18-26.

- [20] Pradeep K, Mohan C, Anand K, Karthikeyan S. Effect of pretreatment of *Cassia fistula* Linn. leaf extract against subacute CCl₄ induced hepatotoxicity in rats. *Indian J Exp Biol.* 2005;43(6):526.
- [21] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976;72(1-2):248-54.
- [22] Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam R, Sengupta P, et al. Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R. Br. on paracetamol-induced hepatotoxicity in rats. *Trop J Pharm Res.* 2007;6(3):755-65.
- [23] Soudani N, Amara IB, Sefi M, Boudawara T, Zeghal N. Effects of selenium on chromium (VI)-induced hepatotoxicity in adult rats. *Exp Toxicol Pathol.* 2011;63(6):541-8.
- [24] Forouzandeh H, Azemi ME, Rashidi I, Goudarzi M, Kalantari H. Study of the protective effect of *Teucrium polium* L. extract on acetaminophen-induced hepatotoxicity in mice. *Iran J Pharm Res.* 2013;12(1):123-9.
- [25] Rajesh M, Latha M. Protective activity of *Glycyrrhiza glabra* Linn. on carbon tetrachloride-induced peroxidative damage *Indian J. Pharmacol.* 2004;36(5):284.
- [26] Noguchi T, Fong K-L, Lai EK, Alexander SS, King MM, Olson L, et al. Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of carbon tetrachloride to the trichloromethyl radical. *Biochem Pharmacol.* 1982;31(5):615-24.
- [27] Mansour DF, EL-Denshary ES, Bahgat AK, Nada SA. Hep atop roective Effect of Alpha Lipoic Acid Against Bromobenzene-Induced Liver Damage in Rats. *Med J Cairo Univ.* 2009;77(4).
- [28] Park J-C, Hur J-M, Hwang Y-H, Choi M-R, Kim S-N, Choi J-W. Hepatoprotective activities of *Rosa davurica* root extract in rats intoxicated with bromobenzene. *J. Life Sci.* 2003;13(2):230-5.
- [29] Kalantari H, Nazari Z, Keliddar A, Foruozandeh H, Kalantar M. Study of the Protective Effect of Livregol against Liver Toxicity Caused by Bromobenzene in Mice. *IJPS.* 2015;10(2):11-20
- [30] Gilani A-UH, Janbaz KH. Preventive and curative effects of *Artemisia absinthium* on acetaminophen and CCl₄-induced hepatotoxicity. *Gen Pharmacol-Vasc S.* 1995;26(2):309-15.
- [31] Messarah M, Klibet F, Boumendjel A, Abdennour C, Bouzerna N, Boulakoud MS, et al. Hepatoprotective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. *Exp Toxicol Pathol.* 2012;64(3):167-74.
- [32] Gopi S, Setty OH. Protective effect of *Phyllanthus fraternus* against bromobenzene induced mitochondrial dysfunction in rat liver mitochondria. *Food Chem Toxicol.* 2010;48(8):2170-5.

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