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Effect of Garlic, Vitamin C, Vitamin E–Selenium against Bioaccumulated Organolead-Induced Cellular Injury in Liver and Spleen of Albino Rats: Pilot Study

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Abstract

Exposure to Lead (Pb^{2+}) in its organic and inorganic forms is known to have deleterious effects on the health of individuals. Several studies showed that these effects are due to accumulation in vital organs and induction of oxidative stress.

This study examines the possibility of three different antioxidant substances (Aqueous garlic extract, Vitamin C and Vitamin E – Selenium) to act as chelating agents against organolead toxicity (lead acetate) by decreasing its accumulation in liver and spleen.

To achieve this purpose, forty-eight adult male albino rats were divided into eight equal groups; Group I: Control received normal water, Group II: received lead acetate (100 mg/kg/day; i.p), Group III: received garlic (100 mg/kg; orally), Group IV: received Lead (100 mg/kg/day; i.p) + Garlic (100 mg/kg; orally), Group V: received Vitamin C (100 mg/kg; orally), Group VI: received Lead (100 mg/kg/day; i.p) + Vitamin C (100 mg/kg; orally). Group VII: received Vitamin E (100 mg/kg; orally) + Selenium (0.5 mg/kg; orally) and Group VIII: received Lead (100 mg/kg/day; i.p) + Vitamin E (100 mg/kg; orally) + Selenium (0.5 mg/kg; orally). The experiments were performed over four consecutive weeks, after which blood was withdrawn from rats by heart puncture and animals were sacrificed by cervical dislocation; the liver and spleen were removed to quantify their lead content by Flame Atomic Absorption Spectroscopy (FAAS).

Analysis of the serum showed that lead acetate has significantly elevated the activity of the ALT, AST, and LDH compared to the control group, and the three selected antioxidant substances were able to minimize the activity of these enzymes significantly in comparison with lead acetate group. In addition, the results from the FAAS showed that lead concentration has significantly increased in the liver and spleen of the lead acetate group compared to the control group, and that treatment with antioxidants was not effective in reducing that effect.

It is concluded that the selected antioxidants had an ameliorative effect against the hepatotoxicity induced by lead acetate but could not be considered the suitable heavy metal chelator to overcome the bioaccumulated lead in the vital organs.

Keywords: Antioxidants, Bioaccumulation, Biochemical Markers, Lead Acetate, Metal Chelators

1. Introduction

The body of organisms is constructed with blend of different elements. Some chemical elements like carbon, hydrogen, oxygen and nitrogen are able to produce biological macromolecules which are vital for biochemical reactions and physiological functions in the organisms, and these organic biomolecules are the fundamental components of carbohydrates, protein, lipid and nucleic acid (Jonsson *et al*. 2017). Other groups of elements are minerals, or essential trace elements are important with certain daily magnitudes, any deficiency in these required elements could develop disorders that could lead to death. A group of chemical elements like gold, silver, lead, mercury, arsenic and cadmium are not essential at all, and the presence of these toxic metals in the body induce oxidative stress status (Johnston *et al*, 2010; Maraqa *et al*. 2015; Matović *et al*. 2015).

Lead is considered a heavy metal that causes toxicity by bioaccumulation in organs and interfering with cellular signals and metabolic reactions (Brochin *et al*. 2014; Gillis *et al*. 2012; Xia *et al*. 2018). Environmental and occupational exposures are considered the significant sources of lead toxicity to humans, as the human body could absorb inorganic lead following inhalation, oral, and dermal administration (Abadin *et al*. 2007).

The prolonged exposures to lead, especially to the occupationally exposed workers resulted in decreasing the activity of delta-aminolevulinic acid dehydratase (δ-

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ALAD; a key enzyme in heme biosynthesis (Shraideh *et al*. 2019) and influence the expression of calmodulin-related genes (Hussain, 2018). Also, these exposures had adverse effects on erythrocyte morphology by surface deformability and invaginations on plasma membrane (Shraideh *et al*. 2019). In addition, lead exposure induces oxidative stress by elevation of the level of superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), lipid peroxidation, and decline the parameters of reduced glutathione (GSH) and total antioxidant capacity (TAOC) (Shraideh *et al*. 2018).

Antioxidants are chemical molecules mostly extracted from medicinal plants. These molecules are considered safe and effective treatment strategy to attenuate the oxidative stress status induced by hazardous environmental factors (Forni *et al*. 2019). Garlic contains chemicals with antioxidant activities; its extract contains four main classes of antioxidant compounds (alliin, allicin, allyl cysteine, and allyl disulfide), and these chemicals exhibit different patterns of free radicals scavenging capacity to protect cellular components from radical damage (Chung, 2006). In vitro studies using a cell-free system, it was reported that aged garlic extract has more inhibition capacity toward advanced glycation end products, higher total phenol content, more potent antioxidant properties compared to fresh garlic extract (Elosta *et al*. 2017).

L-Ascorbic acid; commonly known as vitamin C, is a water-soluble molecule with molecular formula $C_6H_8O_6$, and a molecular weight 176.12 g/mol. Vitamin C has a useful role as a potent antioxidant by scavenging oxygen and nitrogen oxide species such as superoxide radical ion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen (Paciolla *et al*. 2019). Besides, it could conjugate with reactive lipid peroxide products such as malondialdehyde and 4-hydroxynonenal to neutralize the cellular stress condition. Thus, vitamin C has vital processes in the protection of cellular components from free radical-induced damage (Barja *et al*. 2014).

α-Tocopherol, commonly called vitamin E, has a chemical formula $C_{29}H_{50}O_2$, and a molecular weight of 430.7 g/mol. This molecule has low solubility in water due to its hydrophobic repulsion; it has been reported that vitamin E is a fat-soluble compound. Vitamin E has antioxidant activity by reducing the oxidative stress biomarkers like malondialdehyde content, protein carbonyl content, nitric oxide, xanthine oxidase, and increasing the activity of superoxide dismutase and catalase enzymes in rats with oxidative damage caused by formaldehyde (Gulec *et al*. 2006). The same study has reported that vitamin E has anti-inflammatory and anti-hepatotoxic properties (Gulec *et al*. 2006). Vitamin E has a protective effect against genotoxicity by decreasing sister chromatin exchanges and chromosomal aberration in cultured human lymphocytes with chromosomal damage induced by a platinum-based anticancer drug called oxaliplatin (Alqudah *et al*. 2018).

Selenium (Se) is a necessary trace element in the human body. Human body contains multiple families of selenium-dependent proteins such as glutathione peroxidases, thioredoxin reductases, thioredoxin-glutathione reductase, iodothyronine deiodinases, selenophosphate synthetase selenoprotein H, I, K, M, N, O, P, T, V, and W (Zoidis *et al*. 2018). These proteins are antioxidant enzymes with anti-inflammatory, anti-viral, and chemopreventive properties (Zoidis *et al*. 2018).

The main points of this study are to evaluate the gastric administration of garlic extract, vitamin C and vitamin E-Se against the hepatotoxicity induced by organolead (lead acetate) throughout measuring of some liver enzymes, and to illustrate the ability of these selected antioxidants for chelating the bioaccumulated organolead in liver and spleen using albino rats as animal model.

2. Materials and Methods

2.1. Chemicals

Lead acetate trihydrate, vitamin C, vitamin E, and selenium, were purchased from (Sigma, USA). Nitric acid (HNO_3), hydrogen peroxide (H_2O_2), and lead standard solution were purchased from (Scharlau, Spain). All analytical grade solutions were prepared using deionized water.

2.2. Animals

This study was ethically approved by the scientific committee in the School of Science at the University of Jordan. Forty-eight adult male albino rats (6-8 weeks old, weight 140 ± 25 g) were purchased from (Animal household / The University of Jordan). These rats were randomly divided into 8 groups, each containing 6 rats. The volume of chemicals administrated to rats was 0.5 ml intraperitoneal injection via insulin syringes for lead acetate, and 0.5 ml oral administration via plastic gastric tube for antioxidant solutions. Aqueous garlic extract was prepared according to (Belguith *et al*. 2010) considering the concentration of administration dose needed for this study.

Group I (Ctrl): Control receives normal water.

Group II (Pb): Lead (Pb) as lead acetate (100 mg/kg/day; i.p).

Group III (Gar): Garlic (100 mg/kg; orally).

Group IV (Pb + Gar): Lead (100 mg/kg/day; i.p) + Garlic (100 mg/kg; orally).

Group V (Vit C): Vitamin C (100 mg/kg; orally).

Group VI (Pb + Vit C): Lead (100 mg/kg/day; i.p) + Vitamin C (100 mg/kg; orally).

Group VII (Vit E-Se): Vitamin E (100 mg/kg; orally) + Selenium (0.5 mg/kg; orally).

Group VIII (Pb + Vit E-Se): Lead (100 mg/kg/day; i.p) + Vitamin E (100 mg/kg; orally) + Selenium (0.5 mg/kg; orally).

This experiment was operated and repeated daily on all animal groups for four consecutive weeks. Rats were kept in well-ventilated room at room temperature, 12/12-hours day/night period with open access to standard animal chew as feeding material with *ad libitum* for drinking water. Adult male rats were preferred to be chosen in this study because it is generally known that the adult female of mammals, including rats, could periodically enter in menstruation cycle, which could result in rhythmic fluctuations during measurement for the activity of liver enzymes.

2.3. Blood and organs collection

After four weeks, blood was withdrawn from rats by heart puncture using 3 ml disposable syringes under general ether anesthesia. Blood was left to clot in room temperature under dark condition for 1 hour and then centrifuged at 3800 rpm for 10 minutes to obtain serum for enzymatic liver tests. The liver and spleen were isolated from rats after cervical dislocation. These samples were washed with phosphate buffered saline (PBS; 0.1 M, pH 7.2) and stored in freezer at -20 ^{o}C until lead analysis.

2.4. Biochemical tests

The activity of three liver enzymes were estimated include; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). The chemicals kits were purchased from (BioSystem, Spain), and the process of measuring the enzymatic activity was operated according to manufacturer instructions using the serum. These colorimetric assays were performed using 1 cm light path UV-VIS single beam spectrophotometer (LI-295, Lasany, India) at wavelength (λ) of 340 nm.

2.5. Quantification of lead concentration in selected organs

All analytical grade solutions were prepared using deionized water. Glassware and tools were washed and rinsed with deionized water, dried in the oven, soaked in 10% HNO_3 for 24 hours prior to use. This method was operated according to (Massadeh[a] *et al*. 2007) with some modifications. Five grams of liver or spleen were weighed and dried in the oven at 105 ^{O}C for 18 hours. A weight of 0.2 grams from each dried sample was transferred into Teflon digestion vessel and allowed for acid digestion using a mixture of 5 ml of HNO_3 with few drops of 30% v/v H_2O_2 at 80 ^{O}C for 18 hours with continuous shaking. After cooling, 5 ml of 2% HNO_3 were added to the digested extract, filtered with Whatman filter paper, and then the residue filtrate was completed to 25 ml using 2% HCO_3. This residue was directly used to determine the concentration of lead in the sample. Determination of lead in liver and spleen samples was performed using flame atomic absorption spectroscopy (SpectrAA 250 Plus, Varian, Australia). The hollow-cathode lamp of lead (Pb^{+2}) was operated at λ = 283.3 nm with a spectral bandpass of 0.7 nm.

2.6. Accuracy and precision of flame atomic absorption spectroscopy

Every running of 12 samples was repeated with control blank and testing several quality control (QC) solutions. The results were within 5% of the QC values. For every sample, two replicates were taken, and each replicate was read three times and the mean reading was used for calculation purposes. In addition, concentrations of (0.1, 0.5, 1, 2, 5, 10, 15, 20 ppm) of lead standard solution prepared from stock solution were used for calibration check along with blank solution for calculating the concentration of the sample by linear calibration curve. The method was optimized and partially validated in the Department of Chemistry at The University of Jordan, Amman, Jordan.

2.7. Statistical analysis

The results from data were inserted, analyzed, and designed in figures and table using statistical software (GraphPad Prism 7.0.0, IBM, USA). The concentration differences among the groups for each individual test was tested using one-way ANOVA followed by Tukey *post hoc* considering p values <0.05, <0.01, <0.001 as significant, highly significant, extreme significant, respectively. Graph bars in figures were represent mean ± SEM. The standard error of the mean (SEM) seems to be more accurate to be used during the interpretation of our data since each treatment group contains 6 rats only. Linear calibration curve was designed for calculating the unknown concentrations in samples and the linear regression data for each organ in this study was illustrated in the table to observe the accuracy and precision of the obtained results from flame atomic absorption spectroscopy.

3. Results

3.1. Enzymatic activity of alanine aminotransferase (ALT)

The results of this enzyme activity are illustrated in Figure (1A). The mean values of Gar, Vit C, and Vit E-Se groups for ALT did not show any significant differences against the control group at P<0.05. Pb group showed increasing in ALT activity with extremely significant result against Ctrl group at P<0.001. In addition, it had high significant result against Gar, Vit C, and Vit E-Se at P<0.001, and high significant result against Vit E-Se group at P<0.01. The groups of Pb+Gar, Pb+Vit C, and Pb+Vit E-Se did not show any significant differences against the Ctrl group at P<0.05. Also, Pb+Gar and Pb+Vit E-Se groups showed no significant differences against Pb group. Besides, Pb+Vit C group showed high significant result against Pb group at P<0.01.The inter-group comparison Gar vs. Pb+Gar; Vit C vs. Pb+Vit C; Vit E-Se vs. Pb+Vit E-Se) did not show any significant differences at P<0.05.

3.2. Enzymatic activity of alanine aminotransferase (AST)

The results of this enzyme are illustrated in Figure (1B). The mean values for Gar, Vit C, and Vit E-Se groups did not show any significant differences against the control group at P<0.05. Pb group showed about two folds increase in AST activity compared to control with extreme significant different at P<0.001. Also, Pb group showed different significant results against Vit C, Vit E-Se and Gar at P<0.05, P<0.01, and P<0.001, respectively. The result for Pb+Gar group showed no significant difference against control. Furthermore, Pb+Vit C and Pb+Vit E-Se group showed extreme significant differences against control group at P<0.001. The results of AST enzyme for Pb+Gar group showed a significant difference against Pb group at P<0.05. Besides, Pb+Vit C and Pb+Vit E-Se groups did not show any significant differences against Pb group at P<0.05. The inter-group comparison (Gar vs. Pb+Gar; Vit C vs. Pb+Vit C; Vit E-Se vs. Pb+Vit E-Se) showed no significant difference between Gar and Pb+Gar, extreme significant difference between Vit C and Pb+Vit C at P<0.001, and high significant between Vit E-Se and Pb+Vit E-Se at P<0.01.

3.3. Enzymatic activity of lactate dehydrogenase (LDH)

The results of this enzyme are illustrated in Figure (1C). The mean values of LDH enzyme for Gar, Vit C, and Vit E-Se groups did not show any significant differences against the control group at P<0.05. Pb group showed elevated level of LDH activity up to six folds compared to control group with extreme significant result at P<0.001. In addition, Gar, Vit C, and Vit E-Se groups had extreme differences against Pb group at P<0.001. Pb+Gar group showed about two folds increasing in the enzymatic level of LDH compared to control and Gar groups with extreme significant results at P<0.001, and three folds decreasing compared to Pb group with extreme significant difference at P<0.001. Also, Pb+Vit C group showed extreme significant differences against control, Pb, and Vit C groups at P<0.001. The enzymatic activity of LDH in Pb+Vit E-Se group illustrated extreme significant changes against control, Pb, and Vit E-Se at P<0.001.

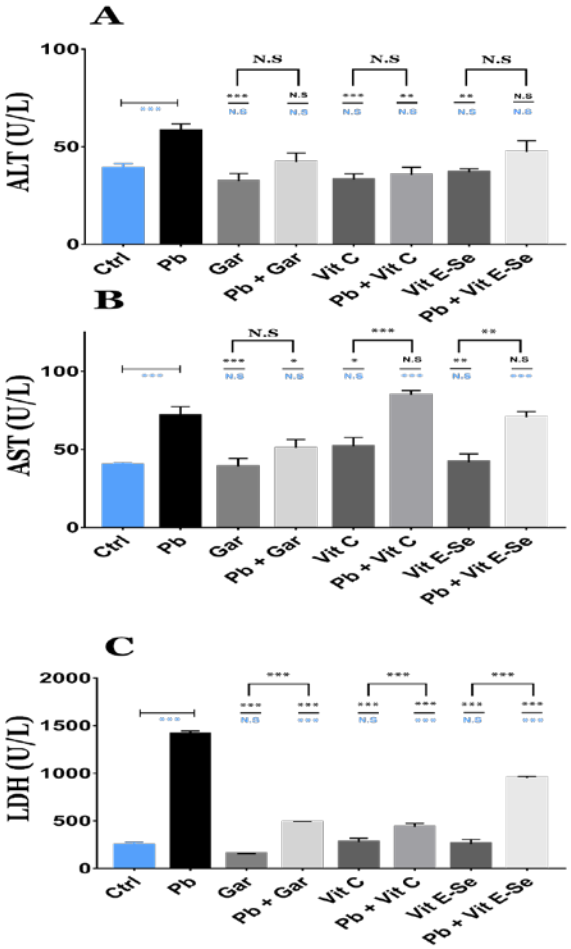

Figure 1. Graph bars represent the effect of aqueous garlic extract (Gar), Vitamin C (Vit C), and Vitamin E-Selenium (Vit E-Se) on some hepatic enzymes in parallel with the toxicity induced by the organolead (Pb). Figure (1-A) for Alanine aminotransferase (ALT); Figure (1-B) for Aspartate aminotransferase (AST); Figure (1-C) for Lactate dehydrogenase (LDH). Each bar represents the mean value of that treatment group to the biochemical test, and the error bar represents the standard error of mean value. Significant values against the control group written in blue color, whereas the significant values against the organolead group written in black color. The black-capped lines represent the significance between the inter-groups. Signs of (*, **, ***) represent P<0.05, P<0.01, P<0.001, respectively. N.S represents no significance when P≥0.05.

3.4. Concentrations of bioaccumulated organolead in liver

The data of linear regression for quantification of lead in liver samples is illustrated in (Table 1). Pb group showed about 17 folds increasing compared to Ctrl with statistically extreme significant difference at P<0.001 (Figure 2A & C). Groups of Pb+Gar, Pb+Vit C, and Pb+Vit E-Se did not show any significant differences against Pb group but showed extreme significant differences against Ctrl group at P<0.001, except Pb+Vit C at P<0.01.

3.5. Concentrations of bioaccumulated organolead in spleen

The data of linear regression for quantification of lead in spleen samples is illustrated in (Table 1). Pb group showed about 16 folds increasing compared to control with statistically highly significant difference at P<0.01 (Figure 2B & D). In addition, the groups of Pb+Gar, Pb+Vit C, and Pb+Vit E-Se did not show any significant differences against Pb group at P<0.05. Besides, these three treatment groups showed highly significant differences against control group at P<0.01.

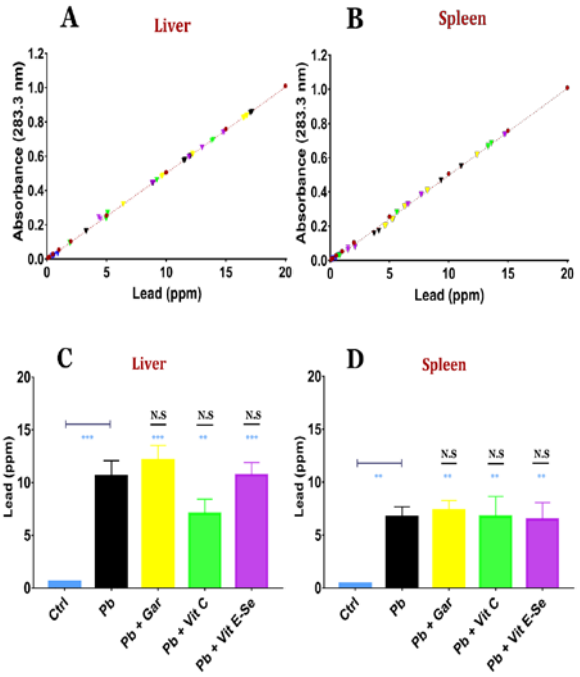

Figure 2. Results of flame atomic absorption spectroscopy (FAAS) for the concentrations of lead (Pb 2+) in the liver and spleen organs of rats after four weeks of organolead (lead acetate) exposures. Figure 2 (A-B) illustrates the absorbance generated from the liver (A) and spleen (B) of different experimental groups. Each colored inverted triangle dot represents the result of lead concentration of a single sample. The blue triangles for the control group; black for organolead; yellow for organolead + aqueous garlic extract; green for organolead + vitamin C; violet for organolead + vitamin E-Selenium. The red circular dots represent the lead standards, and the red line represents the slope of the linear calibration curve for calculating the results (absorbance versus concentration). Figure 2 (C-D) represents the bar graphs that indicates the mean ± SEM of lead concentration per group in liver (C) and spleen (D). Significant values against the control group written in blue color, whereas the significant values against the organolead group written in black color. Signs of (*, **, ***) represent P<0.05, P<0.01, P<0.001, respectively. N.S represents no significance when P≥0.05.

Table 1. Linear regressing data from the statistical software for the results of lead concentration in liver and spleen obtained by flame atomic absorption spectroscopy (FAAS).

	Information of linear regression data	*Liver*	*Spleen*
Best-fit values ± SE	Slope	0.05029 ± 0.0001514	0.05083 ± 0.0002836
	Y-intercept	-0.001902 ± 0.001485	-0.01091 ± 0.002138
	X-intercept	0.03782	0.2147
	1/slope	19.88	19.67
95% Confidence Intervals	Slope	0.04999 to 0.05059	0.05026 to 0.0514
	Y-intercept	-0.004876 to 0.001072	-0.01519 to -0.006629
	X-intercept	-0.02141 to 0.09649	0.1315 to 0.2965
Goodness of Fit and Equation	R square	0.9995	0.9982
	Sy.x	0.006792	0.01095
	Equation	Y = 0.05029*X - 0.001902	Y = 0.05083*X - 0.01091
Is slope significantly non-zero?	F	110295	32113
	DFn, DFd	1, 57	1, 57
	P value	<0.0001	<0.0001
	Deviation from zero?	Significant	Significant

Note: This table was obtained from the statistical software after inserting the absorbance of standard concentrations and the absorbance from the samples of different experimental groups in this study by using flame atomic absorption spectroscopy. This linear regression data could deliver perception about precision and accuracy for this technique to quantify the concentrations of lead in the selected vital organs.

4. Discussion

Prolonged exposure to lead in its organic and inorganic forms induces cellular toxicity on different organisms, and this fact is consistent with the results of this study. This study showed that organolead (lead acetate) elevates liver enzymes activity of ALT, AST, and LDH. Also, quantification of lead concentration in the liver and spleen showed significant increases in the bioaccumulated metallic lead more in the liver than spleen. A previous study showed that sub-lethal exposure to lead acetate increases the enzymatic activity of ALT, AST, LDH, alkaline phosphatase, inhibition of cholinesterase activity and fluctuates the hematological and hormonal parameters in male wistar rats (Ibrahim *et al*. 2012). Also, lead acetate could induce histopathological alterations in the liver of rats which involve blood congestion with dilation of central veins and portal triads, in addition to hepatic vacuolization and degeneration (Albishtue *et al.* 2020).

This study showed that aqueous garlic extract, vitamin C and vitamin E-Se have ameliorative effect against the hepatotoxicity induced by lead acetate exposure by decreasing the activity of ALT, AST and LDH. A previous study showed that both the aqueous garlic extract and vitamin E elevate epididymal sperm count, increase the percentage of sperm motility and viability, enhance some oxidative stress biomarkers and increase the concentration of testosterone and luteinizing hormones in rats exposed to lead acetate (Asadpour *et al*. 2013). It was reported that high levels of ascorbic acid supplementation will reduce lead-induced toxic effects by slightly increasing RBCs count, hematocrit, hemoglobin, and decrease the elevation of liver enzymes in juvenile rockfish *Sebastes schlegelii* (Kim *et al*. 2017).

The second part of this study was performed to evaluate the ability of the three selected antioxidants to chelate the bioaccumulation lead in liver and spleen of the experimental animals. The results of our findings showed that these antioxidants were inactive to achieve this purpose and could not decrease the high lead accumulation in the selected organs in this study, with an exception for vitamin C that showed partial chelating ability in the liver, but not in the spleen.

Ascorbic acid showed an ameliorative effect against the toxicity of heavy metals by reducing the accumulated level of cadmium and mercury and lowering the levels of creatinine, urea, uric acid, and cystatin C protein in kidneys of heavy metals exposed rabbits (Ali *et al.* 2019). Another study stated that aqueous garlic extract could decrease the accumulated lead in different organs such as liver, kidney, heart, spleen, red blood cells in Balb/c mice exposed to 1 ppm of lead (II) nitrate $Pb(NO_3)_2$ (Massadeh[b] *et al*. 2007), this finding is not consistent with our findings due to differences in chemical structure and dosage concentration of lead compound. A recent study illustrated that vitamin C and E significantly decreases the bioaccumulation heavy metals like lead acetate, cadmium chloride, and mercuric chloride in liver, gills, muscle, and plasma of carp fish (Sahiti *et al*. 2020).

The use of lead chelation therapies such as dimercaprol, edetate calcium disodium, and succimer is considered a controversial topic with the recommendation of their uptake in case of critical purpose, taking into consideration the concern from their potential risk of adverse drug events and lead remobilization inside human body (Gracia and Snodgrass, 2007). A recent study has suggested new therapeutic strategies against heavy metal poisoning by mixing metal chelators with antioxidants to improve excretion of heavy metal bioaccumulation and

reduce oxidative stress, which leads to restoring cell viability and inhibiting apoptosis (Kim *et al*. 2019).

5. Conclusion

The findings of this study suggest that aqueous garlic extract, vitamin C, vitamin E-Se could be considered active compounds to reduce the hepatotoxicity induced by lead acetate, but fail to chelate the bioaccumulated organolead in the liver and the spleen in albino rats.

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Conflict of interest

Authors declare that no conflict of interest was associated with this work.

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