Study of the Interaction Effect Between Parsley *Petroselinum crispum* and Cadmium on Lipid Profile, Lipid Peroxidation and Catalase Activity of Albino Mice Males' Liver and Kidney

Huda A. Yousuf [1*], Fawzi S. Al-Zubaidi [1] , Waleed H. Yousif [2]
[1] Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
[2] College of Applied Biotechnology, Al-Nahrain University, Baghdad, Iraq.

Abstract

This study was designed to investigate the effect of different cadmium concentrations on albino mice males' oxidative balance through lipid profile, malondialdehyde (MDA) concentration and catalase (CAT) activity estimation in liver and kidney. Parsley *Petroselinum crispum* was chosen to detect its effect as a natural antioxidant.

Five groups of albino mice males (10 mice each) were treated with (0.0, 0.25, 0.5, 0.75 and 7.5) mg Cd/kg b.w. orally by using gavages needle for 60 days according to 5 days/week protocol, another five mice groups were treated with the same cadmium concentrations mentioned above and 0.1 ml of parsley *Petroselinum crispum* juice.

The results showed an adverse effect of cadmium on mice oxidative balance, while parsley showed an effective antioxidant effect which was revealed through lipid profile protection, MDA concentrations decrease and CAT activity increase.

Keyword: cadmium, parsley *Petroselinum crispum*, lipid profile, malondialdehyde (MDA), catalase (CAT).

دراسة التأثير التداخلي بين نبات المعدنوس *Petroselinum crispum* والكادميوم على صورة الدهون وبيروكسدة الدهون وفعالية إنزيم الكاتاليز في كبد وكلى ذكور الفئران البيض.

هدى عبد المسيح يوسف [1*] ، فوزي شناوه الزبيدي [1] ، وليد حميد يوسف [2]
[1] قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.
[2] كلية التقنيات الحيوية التطبيقية ، جامعة النهرين ، بغداد ، العراق.

الخلاصة:

صُمّمت هذه الدراسة للتحري عن تأثير التراكيز المختلفة من الكادميوم على التوازن التأكسدي لذكور الفئران البيض من خلال تقدير صورة الدهون وتركيز المالوندايالديهالد MDA وفعالية إنزيم الكاتاليز CAT في الكبد والكلى. تمّ اختيار المعدنوس *Petroselinum crispum* للكشف عن تأثيره كمضاد تأكسدي طبيعي. عومِلت خمس مجموعات من ذكور الفئران البيض مقسمة إلى عشرة فئران لكل مجموعة بتراكيز مختلفة من الكادميوم (0.0 و 0.25 و 0.5 و 0.75 و 7.5) ملغم كادميوم/ كغم وزن جسم بطريقة التجريع الفموي الإجباري لمدة 60 يوما بواقع 5 أيام في الاسبوع وخمس مجموعات أخرى بنفس التقسيم عومِلت بنفس تراكيز الكادميوم المذكورة أعلاه و 0.1 مل من عصير المعدنوس. أظهرت النتائج تأثيراً ضاراً للكادميوم على

*Email: huda_maloyan@yahoo.com

التوازن التأكسدي للفئران، بينما أظهر المعدنوس تأثيراً مضاداً للتأكسد والذي أتضح من خلال حماية صورة الدهون وانخفاض تركيز المالونداياالديهايد MDA وزيادة تأثير إنزيم الكاتاليز.

Introduction:

Cadmium is not essential for plants, animals nor humans; its presence in live organisms is unwanted and harmful [1]. It is a potent human carcinogen which has been classified as a category number 1 human carcinogen by the International Agency for Research on Cancers (IARC), and the National Toxicology Program of the USA. Occupational exposure to cadmium has been associated with cancers of the prostate, pancreas and kidney [2] and has been accepted as a pulmonary carcinogen by (IARC) in human [3]. Liver and kidney tissues are the two main targets of cadmium [4].For the general population, the main exposure source to cadmium is the food [5]. Cadmium is a redox stable metal, so it can't induce the production of free radicals by itself; instead it can generate, by indirect mechanisms, some reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as superoxide radical, hydroxyl radical and nitric oxide; as well as some toxic-non radical hydrogen peroxide [6,7]. The mechanisms of cadmium-induced oxidative stress is not fully clarified [8] but one of the most interesting mechanisms is the interference of cadmium with other vital ions in the body such as elements zinc (Zn), calcium (Ca), iron (Fe) and copper (Cu), which resulted in many adverse effects in the body [9].

Administration of cadmium via different routes causes increased lipid peroxidation in membranes of erythrocytes and tissues such as liver, kidney, brain and testes [10]. Cadmium shows a high affinity for thiol ($^{-}$SH) [11]. When Cd enters the body, it reaches the liver within the first 6 hours and binds to metallothionein, which is rich in cystien, to produce Cd-metallothionein [12].

Cholesterol is water-insoluble transported inside lipoproteins (LDL and HDL). It is needed to insulate nerves, build up cell membranes and produce certain hormones [13]. Triglycerides are the most common type of fat in the body. LDL, HDL and VLDL compose the total cholesterol count in the body [14]. About one forth to one third of blood cholesterol is carried by HDL. Medical experts think that HDL tends to carry cholesterol away from the arteries and back to liver to pass from the body (for this reason HDL is called the good cholesterol) [15], while LDL is called bad cholesterol because of its ability to build up fatty deposits in artery walls and increase the risk of heart attack and stroke [14].

Fats and oils are important nutrients and energy sources that are composed mostly of triglycerols, where the latter consists of fatty acids which may vary in their chain length, degree of saturation, isomeric orientation of the double bonds and position. According to their saturation, fatty acids are classified into three types (Saturated fatty acids (SFA), Mono- unsaturated fatty acids (MUFA) and Poly-unsaturated fatty acids (PUFA) [15].

Lipid peroxidation is the oxidative deterioration of lipids containing a number of carbon-carbon double bonds [16] to form a lipid hydroperoxide (ROOH) which then react further. It is a chain-like reaction which consists of three stages: initiation, propagation and termination. Initiation can be occurred by transition metals (iron or copper), oxidative intracellular enzymes or free radicals. The reaction goes into further reactions to enter the propagation phase which terminated when oxygen supply is extinguished [17] or reacting with the antioxidants or other mechanisms [16]. The free radical-mediated oxidation is less susceptible in saturated and monounsaturated fatty acids than the poly unsaturated fatty acids and the rate of oxidation increased by the increasing of the number of double bonds [18]. Hydroperoxides are formed as the primary products in lipid peroxidation, they have toxic effects on cells and they may react with transition metals like iron or copper to form stable aldehyde such as Malondialdehyde (MDA) [19] which is a good marker of free radical-mediated damage, oxidative stress and a suitable biomarker for lipid peroxidation [15]. Lipid peroxidation is known now as a responsible for a number of diseases and clinical conditions including premature birth disorders, diabetes, Parkinson's disease, Alzheimer's disease, atherosclerosis, fibrosis and cancer, inflammatory liver injury and many others [16]. Malondialdehyde is well known as mutagenic and carcinogenic [7], it can react with DNA bases and induce mutation [20].

Catalase is an enzymatic antioxidant. It is a heme-containing enzyme that consists of four identical tetrahedral subunits contains a single ferroprotoporphyrin group per subunit [21, 22]. Catalase exclusively detoxifies hydrogen peroxide to water and oxygen [23,7, 24]. It also has functions to lower

the risk of hydroxyl radical formation from H_2O_2 [25]. It plays an important role during the oxidative stress in the adaptive response of cells [23].

Parsley *Petroselinum crispum*, which is a leafy vegetable belongs to the family Umbelliferae (Apiaceae) is known as a rich source of vitamins and minerals especially vitamins C, A and E; iron, calcium, phosphorus and manganese as well as many active chemical compounds. Therefore, it has many medicinal uses, the leaves are diuretic and are giving during the urinary tract infection while the fruit has a diuretic effect too in low doses but higher doses increase the contractility of the intestinal smooth muscles, bladder and uterus. Therefore the fruit is used to remove intestinal spasms, uterus recovery after birth and menstruation complaints, but it could be abortive, so that it is advisable not to be used in medicinal doses during pregnancy [26,27].

Materials and Methods:

Preparation of cadmium chloride solutions:

Cadmium chloride monohydrate, formula weight 201.33 was used to prepare the different concentrations of the treatment solutions, (0.0, 11.19, 22.39, 33.58 and 335.85) mg of $CdCl_2$ were dissolved in 100 ml of D.W. apart to prepare (0.0, 0.25, 0.5, 0.75 and 7.5) mg Cd^{+2}/ Kg b.w. respectively in each 0.1 ml of the solution.

Preparation of parsley juice:

One hundred and fifty grams of fresh, clean and sliced parsley were mixed with 250 ml of D.W. in a blender and filtered by clean gauze. After filtration, the final volume of parsley juice was 300 ml; 0.1 ml of this juice was given to each animal. The juice was freshly prepared every week and kept at 4ºC.

Animals management:

Healthy mature albino male mice at the age of 8-10 weeks and average weight 25 ± 5 g were purchased from the National Center for Drug Control and Research (NCDCR). One hundred male mice were divided randomly into 10 groups of ten animals each (5 animals/cage) in polypropylene cages. The animals were reared and treated at the animal house of Biotechnology Research Center/ Al-Nahrain University at the temperature of 25 ± 5 ºC and 12 ± 2 hours light/day. Diet and drinking water were given *ad libitum.*

Experimental design:

Animals were treated for 60 days orally by using gavages needle according to the protocol of 5 days/week. They were divided into the following groups:

Group (1): control animals, given 0.1 ml D.W. considered as control.
Group (2): given 0.1 ml of 0.25 mg Cd^{+2}/kg b.w.
Group (3): given 0.1 ml of 0.5 mg Cd^{+2}/kg b.w.
Group (4): given 0.1 ml of 0.75 mg Cd^{+2}/kg b.w.
Group (5): given 0.1 ml of 7.5 mg Cd^{+2}/kg b.w.
Group (6): given 0.1 ml of D.W. + 0.1 ml of parsley juice. Considered as a positive control.
Group (7): given 0.1 ml of 0.25 mg Cd^{+2}/kg b.w. + 0.1 ml of parsley juice.
Group (8): given 0.1 ml of 0.5 mg Cd^{+2}/kg b.w. + 0.1 ml of parsley juice.
Group (9): given 0.1 ml of 0.75 mg Cd^{+2}/kg b.w. + 0.1 ml of parsley juice.
Group (10): given 0.1 ml of 7.5 mg Cd^{+2}/kg b.w. + 0.1 ml of parsley juice.

Blood collection:

Blood was collected from the animals by heart puncture and placed into eppendorf tube then allowed to clot. Serum was separated after centrifugation for 10 minutes at 3000 rpm and kept at -20 ºC for the biochemical tests.

Lipid profile:

Total cholesterol, triglycerides and HDL were estimated using the procedure of lipid profile kits provided by Spinreact- Spain. While LDL was calculated using Friedwald equation.

Malondialdehyde (MDA) and Catalase assay:

Malondialdehyde (MDA) and Catalase were determined in liver and kidney using the procedure ofBiovision-USA kits. Two experimental concentrations (0.75 and 7.5 mg Cd/kg b.w.) were selected to determine changes in MDA levels and catalase activity.

Statistical analysis:

Statistical analysis was performed using the statistical analysis system [28]. The least significant difference (LSD) test was used to examine the significant effects of treatments and concentrations on parameter means in this study. P value less than 0.05 was considered as statistically significant.

Results and Discussion:

Effect of parsley on lipid profile in mice treated with cadmium:

Figures-1, 2, 3 and 4 show the effect of parsley on lipid profile in mice treated with cadmium.

Administration of parsley significantly (P<0.05) decreased serum cholesterol in mice treated with parsley and cadmium compared with mice treated with cadmium alone in all of cadmium concentrations except the concentration of 0.25 mg Cd/kg b.w. figure-1.

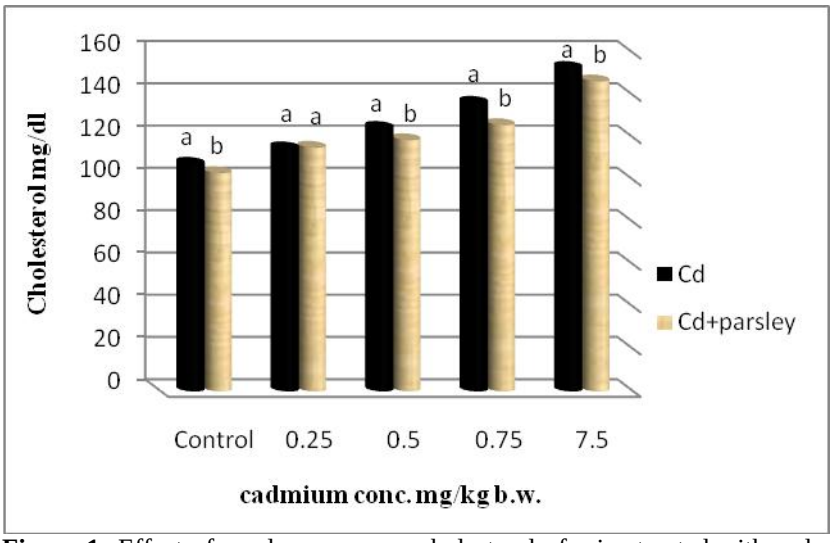

Figure 1- Effect of parsley on serum cholesterol of mice treated with cadmium Different letters over columns refer to significant (P<0.05) differences

Parsley caused non-significant (P>0.05) decrease in serum triglycerides of mice treated with parsley and cadmium compared to mice treated with cadmium alone figure-2.

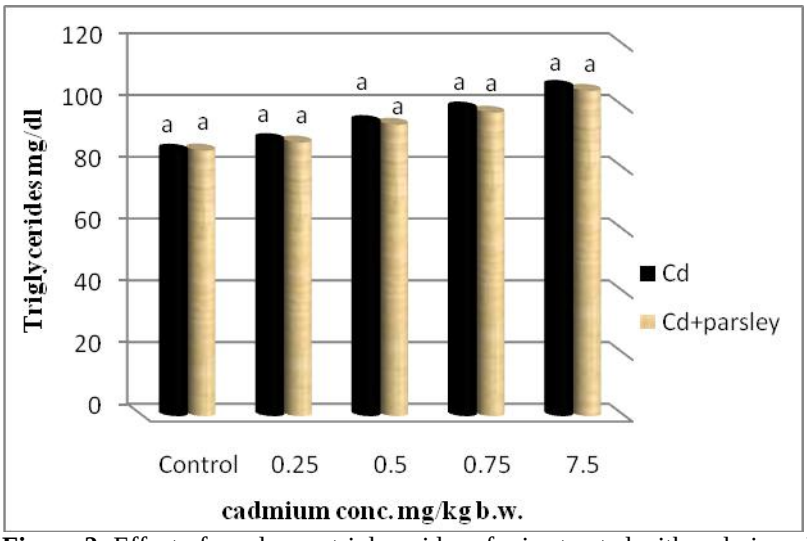

Figure 2- Effect of parsley on triglycerides of mice treated with cadmium .Different letters over columns refer to significant (P<0.05) differences

Figure-3 shows the effect of parsley on mice HDL compared with HDL of cadmium treated mice. Parsley showed non- significant (P>0.05) increase in HDL concentrations at 0.25 mg Cd/kg b.w., while significant (P<0.05) increase was shown at the concentration 0.5 mg Cd/kg b.w. due to parsley administration with cadmium compared with cadmium alone. Non- significant (P>0.05) decrease were

observed at control, 0.75 and 7.5 mg Cd/kg b.w. in HDL of parsley and cadmium treated mice compared with cadmium treated mice.

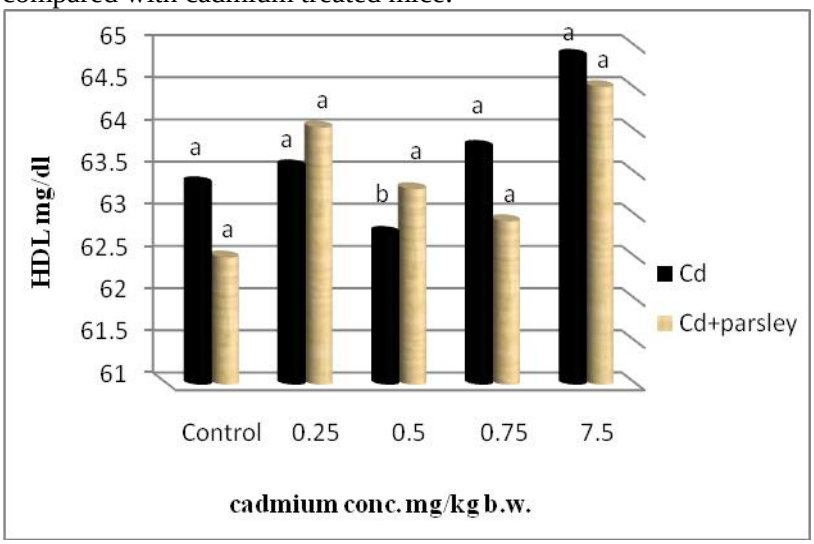

Figure 3- Effect of parsley on HDL of mice treated with cadmium..Different letters over columns refer to significant (P<0.05) differences

Parsley caused a significant (P<0.05) decrease in LDL of mice at cadmium concentrations of 0.5, 0.75 and 7.5 mg Cd/kg b.w. compared with cadmium alone treated mice. At the concentration of 0.25 mg Cd/kg b.w., LDL was 33.54 mg/dl and decreased to 33.50 mg/dl in cadmium + parsley group; but this decrement was non-significant (P>0.05). figure -4.

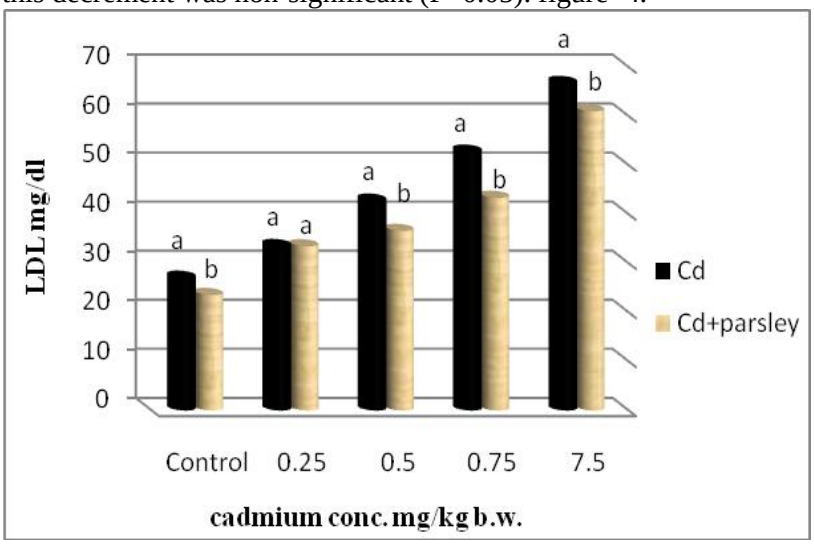

Figure 4- Effect of parsley on LDL of mice treated with cadmium **.**Different letters over columns refer to significant (P<0.05) differences

Cadmium treated mice had the highest mean values of cholesterol, triglycerides and LDL followed by cadmium + parsley groups; HDL values were fluctuated among the groups. Parsley had a protective role on lipid profile but the results were not totally significant (P>0.05) compared with cadmium alone. Increase the treatment duration and/or the doses administered; may show the significant effects on lipid profile more clearly.

The present study showed that parsley had protective effects on lipid profile which may attribute to the active compounds vitamins that parsley contains.

Parsley is rich with chemical active compounds, minerals and vitamins such as vitamin C which has the ability to activate the enzyme 7α-hydroxylase in the first step of bile acid synthesis; the activation of this enzyme will enhance the conversion of cholesterol into bile acid, hence resulting in decreasing serum levels of cholesterol [29]. Also, vit.C might have an inhibitory effect on HMG CoA reductase activity, thus inhibiting cholesterol biosynthesis [30].

Flavonoids, the most abundant chemical compounds in parsley were previously registered to contribute health promotion. They are suggested to lower blood cholesterol levels by diminishing the biosynthesis of cholesterol due to the ability of flavonoids to enhance the phosphorylation of HMG COA reductase indirectly [31].

The effects of cadmium exposure on rats' lipid levels were studied; after 7 weeks of exposure to 50 and 100 ppm of $CdCl_2$ via drinking water. A significant ($P<0.05$) increment was found in total cholesterol, triglycerides in both concentrations compared to control with non-significant changes in HDL [32]. The ameliorating effect of crude flavonoids extracted from parsley on rats exposed to 50 ppb of cadmium chloride in drinking water for 60 days were studied. A significant ($P<0.05$) increase was found in serum total cholesterol, triglycerides, LDL and VLDL concentrations with a significant ($P<0.05$) decrease in serum HDL concentration in cadmium treated rats. Parsley flavonoids extract at the concentration of 150 mg/kg b.w. caused a significant ($P<0.05$) decrease in total cholesterol, triglycerides, LDL and VLDL while HDL concentration significantly ($P<0.05$) increased [33].

Parsley was used to decrease the adverse effects upon lipid profile in a study designed to overload rabbits with methionine and monitor the probable effects against lipid profile and other physiological aspects. Apigenin; extracted from parsley was used to measure the interaction effects between methionine and apigenin on lipid profile. Total cholesterol was decreased from 141.0 mg/dl in methionine group to 102.0 mg/dl in methionine+apigenin group. Triglycerides decreased from 110.0-135.0 mg/dl in methionine and methionine+ apigenin group respectively. The same ameliorating effects of apigenin were observed with HDL and LDL mean values [34].

Effect of parsley on MDA levels in the liver and kidney of mice treated with cadmium:

Figure-5 shows that the administration of parsley with cadmium decreased MDA levels at the concentration of 0.75 mg Cd/kg b.w. significantly ($P<0.05$) from 0.497 nmol/mg in cadmium treated mice to 0.410 nmol/mg in cadmium and parsley treated mice. Also, parsley significantly ($P<0.05$) decreased MDA levels at the concentration 7.5 mg Cd/kg b.w. compared with cadmium alone.

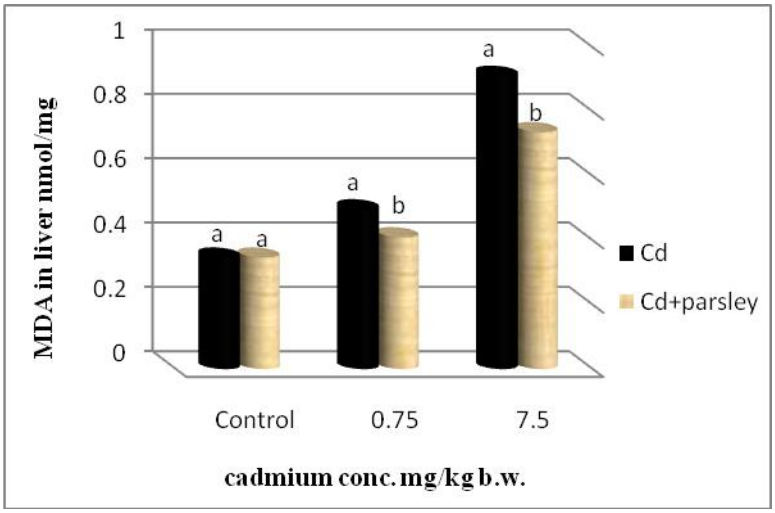

Figure 5- Effect of parsley on MDA levels in the liver of mice treated with cadmium .Different letters over columns refer to significant ($P<0.05$) differences

The effect of parsley on MDA levels in kidney are shown in figure-6. Parsley caused a significant ($P<0.05$) decrease in MDA levels in mice treated with 0.75 and 7.5 mg Cd/kg b.w. compared with cadmium alone.

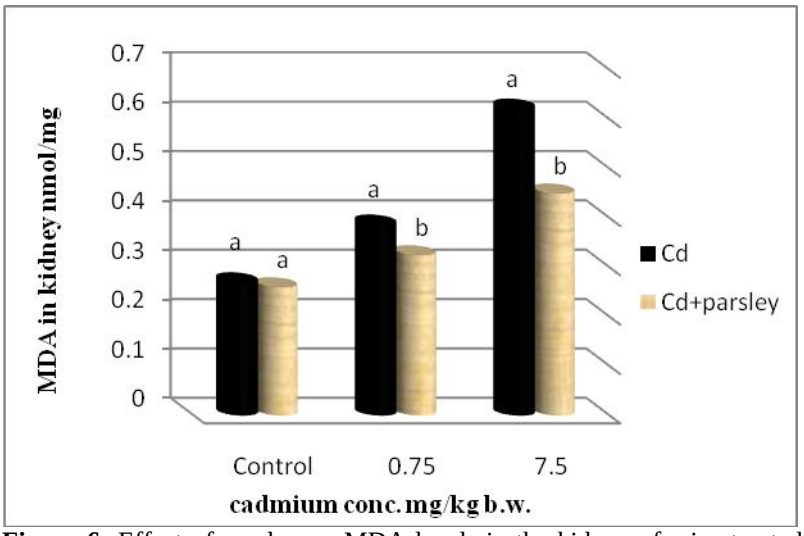

Figure 6- Effect of parsley on MDA levels in the kidney of mice treated with cadmium. Different letters over columns refer to significant (P<0.05) differences

Oxidative damage induced by long-term cadmium intoxication has been demonstrated by the increased lipid peroxidation and/or inhibition of antioxidant enzymes that are required to prevent such oxidative damage [35].

Cadmium induces oxidative damage due to its ability to indirect production of ROS such as hydrogen peroxide, superoxide radicals, hydroxyl radicals and lipid peroxides [36]. The exposure of male wistar rats to 50 and 100 ppm of cadmium chloride in their drinking water for 7 weeks revealed significant increases in MDA levels in the liver compared with the control [32]. Excessive production of free radicals or ROS is mainly responsible for peroxidation of cell membrane lipids and other unsaturated lipids (especially LDL) which are the chief mechanisms of cell damage leading to necrosis or apoptosis; the terminal product of lipid peroxidation is MDA. The determination of MDA levels is usually the most practical and reliable method for detecting and screening for oxidative stress [37].

In the present study, MDA levels in liver and kidney tissues increased proportionally with cadmium concentration. Such increment in lipid peroxidation has been attributed to alterations in the antioxidant defense system that normally protects the body against free radical toxicity. [24].

Parsley is known for its rich components of vit.C, β-carotene, essential oils and flavonoids which could bring its protective role against lipid peroxidation induced by cadmium. Flavonoids are suggested to scavenge free radicals or increase the production of glutathione S-transferase [38]; they also protect LDL and α-tocopherol (vit.E) from being oxidized [39]; While vitamin C which is considered as the most important water-soluble antioxidant in extracellular fluids due to its hydrophilic properties is capable of neutralizing ROS in the aqueous phase before the initiation of lipid peroxidation. At the same time vit.E is the major lipid soluble antioxidant and the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation and LDL from oxidative attack. Vitamin C has been cited for its capability in regenerating vit.E and thus restores its original function in the "antioxidant network" [36, 40].

Serum MDA concentrations were significantly (P<0.05) elevated from 0.37 µmol/dl at zero time to 0.99 µmol/dl after 60 days of cadmium administration, while administration of parsley flavonoids extract showed a significant (P<0.05) decrease in serum MDA concentrations (0.36 µmol/dl) compared with cadmium alone [33].

The antioxidants activities of parsley leave and stem phenolic extract were estimated *in vitro* by determining of free radical scavenging capacity of the extracts. Parsley leaves extract antioxidant capacity was 30.35 % while it reached more than 18 % in parsley stems extract [41]. The antioxidant capacities of ethanolic leaf extract of parsley at the dose of 0.5 mg/day for 20 days were studied by using brain stressed mice; the study revealed that leaves of parsley have many phytochemical constituents that may be responsible for many antioxidant activities especially the presence of flavonol glycoside [42].

Effect of parsley on catalase activity in the liver and kidney of mice treated with cadmium:

The effect of parsley on liver's catalase activity is shown in figure-7.

After intoxication with 0.75 mg Cd/kg b.w.; catalase activity reached 121.19 nmol/min/ml, while co-treatment with parsley significantly (P<0.05) raised the activity of catalase to 125.06 nmol/min/ml. Parsley significantly (P<0.05) increased catalase activity to 95.66 nmol/min/ml when co-administered with cadmium at the concentration of 7.5 mg Cd/kg b.w., while cadmium alone significantly (P<0.05) decreased catalase activity to 88.54.

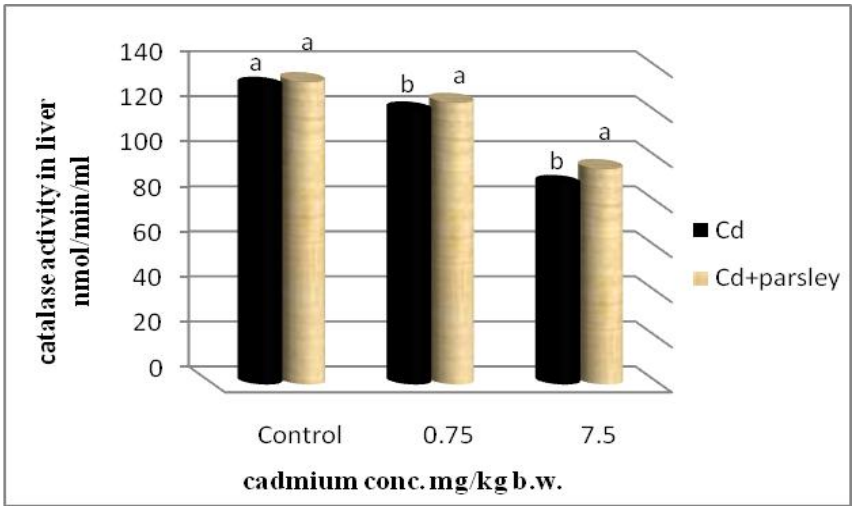

Figure 7- Effect of parsley on catalase activity in the liver of mice treated with cadmium. Different letters over columns refer to significant (P<0.05) differences

The effects of parsley on catalase activity in kidney of mice treated with cadmium are shown in figure-8. Significant (P<0.05) increase in kidney catalase activity observed due to the co-administration of parsley with 0.75 and 7.5 mg Cd/kg b.w. compared with the corresponding concentration of cadmium alone.

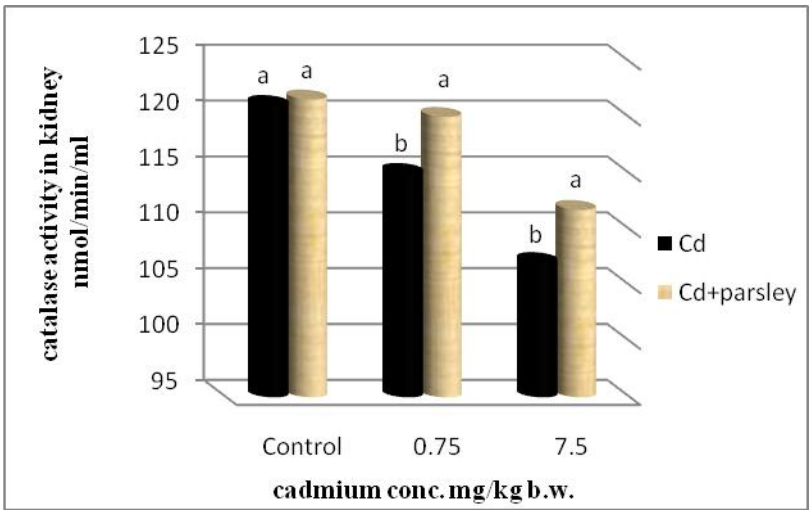

Figure 8- Effect of parsley on catalase activity in the kidney of mice treated with cadmium .Different letters over columns refer to significant (P<0.05) differences

According to the oxidative damage resulted from cadmium intoxication, catalase activity disturbs; sometimes it is increased while in others decreased.It was found that the increment of catalase activity in tissues is related to acute cadmium intoxication which may be attributed to the response to cadmium toxicity as a defense mechanism [43].In the present study, the results showed a decrease in catalase activity due to cadmium intoxication which was concentration-dependent.

There are many suggested mechanisms behind the inhibition of catalase activity; one of them is attributed to the possibility of high production of ROS and their increased intracellular accumulation which exceed the detoxification capacity of antioxidant enzymes with subsequent development of liver and kidney injury [44]. An interaction between cadmium and catalase subunits which contain iron as a composing element was suggested [45]. The presence of cadmium in the organism is well known to decrease the levels of iron in the blood, so the decreased activity of catalase could be resulted from iron deficiency [46].

The increase in catalase activity is due to the concomitant treatment of parsley with cadmium could be a result of vit.C, that parsley contains, properties as a potent scavenger of free oxygen radicals especially hydroxyl radical, superoxide radical and hydrogen peroxide where the latter is the substrate of catalase [47,36]. One of the possible mechanisms by which parsley can effectively enhance catalase activity suggests that parsley is a good source of iron (Fe) [48]. Cadmium decreases iron levels in the blood [49] which may stands behind decreased catalase activity because of iron demand in catalase composition; so that the administration of parsley may increase iron levels in the blood and, as a result, increase the activity of catalase. The chronic effects of cadmium on antioxidant defense system in rat's liver and kidney were studied, after 4 weeks of treatment at the dose 15 mg Cd/kg b.w. catalase activity was decreased in treated groups compared with the control [50].

References:

1. Šmirjákova, S.; Ondrašovicova, O.; Kaškova, A. and Lakticova, K. **2005**. The effect of cadmium and lead pollution on human and animal health. *Folia Veternaria*, 49(3),pp: 31-32.
2. Waisberg, M.; Joseph, P.; Hale, B. and Beyersmann, D. **2003**. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, 192,pp: 95-117.
3. Pandey, K.; Shukla, J.P. and Trivedi, S.P. **2005**. *Fundamentals of toxicology*. New central book agency (p) LTD. Delhi, Kolkata. Pp:354.
4. World Health Organization. **2007**. *Health risks of heavy metals from long-range transboundary air pollution*. World Health Organization for Europe. Germany. Pp: 130.
5. Piscator, M. **1985**. Dietary exposure to cadmium and health effects: impact of environmental changes. *Environmental Health Perspectives*, 63,pp: 127-132.
6. Galan, A.; Garcia-Bermejo, L.; Troyano, A; Vilaboa, N.E.; Fernandez, C.; de Blas, E. and Aller, P. **2001**. The role of intracellular oxidation in death induction (apoptosis and necrosis) in human promonocytic cells treated with stress inducers (cadmium, heat, X-ray). *European Journal of Cell Biology*, 80,pp: 312-320.
7. Valko, M; Morris, H. and Cronin, M.T.D. **2005**. Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, 12,pp: 1161-1208.
8. Kant, V.; Mehta, M.; Varshneya, C. and Chauhan, Sh. **2011**. Induction of oxidative stress by subacute oral exposure of cadmium sulphate in adult poultry. *Brazilian Journal of Veterinary Pathology*, 4(2),pp: 117-121.
9. Muthukumar, K. and Nachiappan, V. **2010**. Cadmium-induced oxidative stress in *Saccharomyces cerevisiae. Indian Journal of Biochemistry and Biophysics*, 47,pp: 383-387.
10. Stohs, S.J.; Bagchi, D.; Hassoun, E. and Bagchi, M. **2001**. Oxidative mechanisms in the toxicity of chromium and cadmium ions. *Journal of Environmental Pathology, Toxicology and Oncology*, 20,pp: 77-88.
11. Lopez, E.; Arce, C.; Oset-Gasque, M.J.; Cañadas, S. and González, M.P. **2006**. Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture. *Free Radical Biology & Medicine*, 40,pp: 940-951.
12. Liu, J.; Liu, Y.; Michalska, A.E.; Choo, K.H. and Klaassen, C.D. **1996**. Distribution and retention of cadmium in metallothionein I and II null mice. *Toxicology and Applied Pharmacology*, 136,pp: 260-268.
13. Anthony, C. **2005**. LDL cholesterol: bad cholesterol, or bad science? *Journal of the American Physicians and Surgeons*, 10(3),pp: 83-89.
14. Hongbao, M. **2004**. Cholesterol and human health. *Nature Science*, 2(4),pp: 17-21.
15. Dauqan, E.M.A.; Abdullah, A. and Sani, H.A. **2011**. Natural antioxidants, lipid profile, lipid peroxidation, antioxidant enzymes of different vegetable oils. *Advance Journal of Food Science and Technology*, 3(4),pp: 308-316.

16. Devasagayam, T.P.A.; Boloor, K.K. and Ramasarma, T. **2003**. Methods for estimating lipid peroxidation: an analysis of merits and demerits. *Indian Journal of Biochemistry and Biophysics*, 40,pp: 300-308.
17. Nurmi, A. **2008**. Health from herbs? Antioxidant studies on selected Laminaceae herbs *in vitro* and in human. M.Sc. thesis. Department of pharmaceutical Biology, University of Helsinki. Pp: 96.
18. Niki, E.; Yoshida, Y.; Saito, Y. and Noguchi, N. **2005**. Lipid peroxidation: mechanisms, inhibition and biological effects. *Biochemical and Biophysical Research Communications*, 338,pp:668-676.
19. Maritim, A.C.; Sanders, R.A. and Watkins, J.B. **2003**. Diabetes, oxidative stress and antioxidants: A review. *Journal of Biochemical and Molecular Toxicology*, 17(1),pp: 24-38.
20. Marnett, L.J. **1999**. Lipid peroxidation-DNA damage by malondialdehyde. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, 424,pp: 83-95.
21. Nordberg, J. and Arnér, E.S.J. **2001**. Reactive oxygen species, antioxidants and the mammalian thioredoxin system. *Free radical Biology and Medicine*, 31(11): 1287-1312.
22. Cuypers, A.; Plusquin, M.; Remans, T.; Jozefczak, M.; Keunen, E.; Gielen, H.; Opdenakker, K.; Nair, A.R.; Munters, E.; Artois, T.J.; Nawrot, T.; Vangronsveld, J. and Smeets, K. **2010**. Cadmium stress: antioxidative challenge. *Biometals*, 23,pp: 927-940.
23. Matés, J.M.; Perez-Gomez, C. and De Castro, I.N. **1999**. Antioxidant enzymes and human diseases. *Clinical Biochemistry*, 32,pp: 595-603.
24. Patra, R.C.; Rautray, A.K. and Swarup, D. **2011**. Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary Medicine International*, 2011,pp: 1-9.
25. Halliwell, B. **1999**. Antioxidant defense mechanisms: from the beginning to the end (of the beginning). *Free Radical Research*, 31,pp: 261-272.
26. Hoffmann, D. **1996**. *The complete illustrated holistic herbal*. Great Britain, USA and Australia. Pp: 256.
27. Gruenwald, J.; Brendler, T.; Jaenicke, C. and Fleming, T. (eds.). **2000**. *PDR for herbal medicines*. Medical Economics Company. Montvale, New Jersey. Pp:1244.
28. Statistical Analysis Software. **2010**. *SAS/STAT* User's Guide. Version 9.1. Volume 1. SAS. Inst. Inc. Cary. N.C. USA. Pp: 5136.
29. Eteng, M.U.; Ibekwe, H.A.; Amatey, T.E.; Bassey, B.J.; Uboh, F.U. and Owu, D.U. **2006**. Effect of vitamin C on serum lipids and electrolyte profile of albino wistar rats. *Nigerian journal of physiological sciences*, 21(1-2),pp: 15-19.
30. Stephens, N.G.; Parsons, A.; Schofield, P.M.; Kelly, F.; Cheeseman, K. and Mitchinson, M.J. **1996**. Randomized control trial of vitamin E in patients with coronary disease. Cambridge heart antoxidant study. *Lancet*, 347(9004),pp: 781- 786.
31. Kim, K.; Yeo, E.; Moon, S.; Cho, S.; Han, Y.; Nah, S. and Paik, H. **2008**. Inhibitors effects of naringenin, kaempherol and apigenin on cholesterol biosynthesis in Hep G 2 and MCF-7 cells. *Food Science and Biotechnology*, 17(6),pp: 1361-1364.
32. Afolabi, O.K.; Oyewo, E.B.; Adekunle, A.S.; Adedosu, O.T. and Adadeji, A.L. **2012**. Impaired lipid levels and inflammatory response in rats exposed to cadmium. *Experimental and Clinical Sciences Journal*, 11,pp: 677-687.
33. Al-Mzaien, A.K.A. **2009**. Protective effect of flavonoids extracted from parsley *Petroselinum sativum* on heart and liver in male rats exposed to cadmium chloride. M.Sc. thesis. College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
34. Ali, A.H. **2010**. Protective role of apigenin extracted from parsley *Petroselinum sativum* on some physiological aspects in male rabbits exposed to methionine overload. Ph.D. thesis. College of veterinary medicine, Baghdad University, Baghdad, Iraq.
35. Kelley, C.; Sargent, D.E. and Uno, J.K. **1999**. Cadmium therapeutic agents. *Current Pharmaceutical Design*, 5,pp:229-240.
36. Percival, M. **1998**. Antioxidants. *Clinical Nutrition Insights*, pp:1-4.
37. Chlubek, D.; Grucka-Mamczar, E; Birkner, E.; Polaniak, R.; Stawiaska-Pieta, B. and Duliban, H. **2003**. Activity of pancreatic antioxidative enzymes and malondialdehyde concentrations in rats with hyperglycemia caused by fluoride intoxication. *Journal of Trace Elements in Medicine and Biology*, 17(1),pp: 57-60.

38. Ozsoy-Sacan, O.; Yanardag, R.; Orak, H.; Ozgey, Y.; Yarat, A. and Tunali, T. **2006**. Effects of parsley *Petroselinum crispum* extract versus gilbornuride on the liver of streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 104(1-2),pp: 175-181.
39. Iijima, K. and Aviram, M. **2001**. Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Current Opinion in Lipidology*, 12,pp: 41-48.
40. Valko, M.; Rhodes, C.J.; Moncol, J.; Izakovic, M. and Mazur, M. **2006**. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interaction*, 160,pp: 1-40.
41. Al-Juhaimi, F. and Ghafoor, K. **2011**. Total phenols and antioxidant activities of leaf and stem extracts from coriander, mint and parsley grown in Saudi Arabia. *Pakistan Journal of Botany*, 43(4),pp: 2235-2237.
42. Vora, S.R.; Patil, R.B. and Pillai, M.M. **2012**. Oxidative stress associated alterations in lysosomal enzymes and modulatory effect of *Petroselinum crispum* [Mill] Nyman Ex A.W.Hill leaf extract on mouse brain. *International Journal of Biological and Medical Research*, 3(2),pp: 1764-1767.
43. Tandon, S.K.; Singh, S.; Prasad, S.; Khandekar, K.; Dwivedi, V.K.; Chatterjee, M. and Mathur, N. **2003**. Reversal of cadmium induced oxidative stress by chelating agent, antioxidant or their combination in rat. *Toxicology Letters* 145,pp: 211-217.
44. De Castro, M.A.C.; Neto, F.F.C.; Lima, L.M.C.; da Silva, F.M.; de Oliveira, R.J. and Zanesco, A. **2009**. Production of free radicals and catalase activity during acute exercise training in young men. *Biology of Sport*, 26(2),pp: 113-118.
45. Wronska-Nofer, T.; Wisniewska-Knypl, J.; Dziubaltowska, E. and Wyszynska, K. **1999**. Prooxidative and genotoxic effect of transition metals (cadmium, nickel, chromium and vanadium) in mice. *Journal of Trace Elements and Electrolytes*, 16(2),pp: 87-92.
46. Jurczuk, M.; Brzoska, M.M.; Moniuszko-Jakoniuk, J.; Gałazyn-Sidorczuk, M. and Kulikowska-Karpińska, E. **2004**. Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol. *Food and Chemical Toxicology*, 42(3),pp: 429-438.
47. Nagyova, A.; Galbavy, S. and Ginter, E. **1994**. Histopathological evidence of vitamin C protection against cadmium nephrotoxicity in guinea pigs. *Experimental and Toxicologic Pathology*, 46,pp: 11-14.
48. Al-Daraji, H.J.; Al-Mashadani, H.A.; Mirza, H.A.; Al-Hassani, A.S. and Al-Hayani, W.K. **2012**. The effect of utilization of parsley *Petroselinum crispum* in local Iraqi geese diet on blood biochemistry. *Journal of American Science*, 8(8),pp: 427-432.
49. Kostic, M.M.; Ognjanovic, B.I.; Dimitrijevic, S.; Zikic, S.; Zikic, R.V.; Stajn, A.; Rosic, G.L. and Zivkovic, R.V. **1993**. Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rats *in vivo* effects. *European Journal of Haematology*, 51,pp: 86-92.
50. Ognjanovic, B.I.; Markovic, S.D.; Pavlovic, S.Z.; Zikic, R.V.; Stajn, A.S. and Saicic, Z.S. **2008**. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. *Physiological Research*, 57,pp: 403-411.