© 2015 IJSRSET | Volume 1 | Issue 4 | Print ISSN : 2395-1990 | Online ISSN : 2394-4099
Themed Section: Science

Effects of Broccoli on Oxidative Stress Produced by Lead Acetate in Male Albino Rats *Rattus Rattus*

Sulaf Mustafa Mohammed[*1], Mousa Jasim Mohammed[2], Ebtisam Al Aboosi[3]

*[1]Biology Department, Sulaimani University/Iraq
[2]Biology Department,Tikrit University/Iraq
[3]Canada consultation Horizons /Torento Canada

ABSTRACT

The aim of this study was to investigate the protective and adverse effects of 10% of raw broccoli on lead acetate induced oxidative stress in rats. Fifty six male rats were divided into eight groups, seven rats in each. Control group injected with distilled water, Br group were fed on 10% broccoli for five weeks ,LE6 group were injected intraperitoneally with 6 mg/kg body weight lead acetate for five weeks ,LE10 group were injected intraperitoneally with 10 mg/kg body weight lead acetate for five weeks ,Br+LE6 group were injected intraperitoneally with 6 mg/kg body weight lead acetate and feed on 10% broccoli concomitantly for five weeks , and Br+LE10 group were injected intraperitoneally with 10 mg/kg body weight lead acetate and feed on 10%broccoli concomitantly for five weeks.While LE6+Br group was injected previously with 6mg/kg of body weight lead acetate for three weeks and then fed on 10% broccoli and injected with lead acetate concomitantly for two weeks and then fed on broccoli alone for the last three weeks. Last group was LE10+Br group was injected previously with 10mg/kg of body weight lead acetate for three weeks and then fed on 10% broccoli and injected with lead acetate concomitantly for two weeks and then fed on broccoli alone for the last three weeks. Our result revealed that the SOD% and total glutathione increased significantly ($p<0.05$)in group Br and decreased significantly ($p<0.05$) in group LE6and LE10 in dose-dependent manner compare with group C .while the levels increased significantly in all Broccoli treated groups.Moreover, the results revealed that the broccoli was more effective in the groups that injected with lead acetate and fed on broccoli concomitantly. In another hand, the level of MDA µmol/ml increased significantly ($p<0.05$) in group LE6 and LE10 compare with group C while decreased in all Broccoli treated groups. In conclusion, broccoli at a dose of 10% exhibited a protective effect on lead acetate induced oxidative stress in rats, also it had ability to alleviate and adverse the Pb toxicity by the improvement of the antioxidant system.

Keywords: antioxidant, oxidative stress, lead acetate, broccoli.

I. INTRODUCTION

The contacts of human beings with metal compounds that have no physiological activity, even at low concentrations, are correlated with high levels of toxicity [1]. Lead is one of these heavy metals that pronounced by its toxicity to human being,lead-induced oxidative stress by enhance the production of free radicals or ROS has ben identified as the primary contributory agent in the pathogenesis of lead poisoning[2].ROS are the by-products of many degenerative reactions in many tissues. They will affect the regular metabolism and damaging the cellular components ,because this molecule has one or more unpaired electrons, making it highly reactive with other molecules. ROS can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions and destroy the living cells. The shift in the balance between oxidants and antioxidants in favor of oxidants was termed “oxidative stress”. [3].The ability of lead for the production of reactive oxygen species result in DNA strand breaks and replace zinc in DNA binding proteins[4].Cells have developed various antioxidant defense systems against free radical attacks by antioxidants.Antioxidants are molecules present in cells

that prevent these reactions by donating an electron to the free radicals without becoming destabilized themselves. The primary antioxidant enzymes contained in mammalian cells, which are thought to be necessary for life in all oxygen metabolizing cells are, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) [5].Accordingly, interest has recently grown in the role and usage of natural antioxidants like fruits and vegetables as a strategy to prevent oxidative damage in various health disorders with oxidative stress [6]. Broccoli belongs to the cabbage family, *Brassica oleracea* var. broccoli . Brassicaceae or Cruciferae, it resembles the Cauliflower.Raw, green broccoli is a source of multiple vitamins and minerals, including calcium, magnesium, potassium, iron, zinc, and selenium, as well as carotene, thiamine, riboflavin, niacin, folate, and vitamins C and K.However, content varies widely and the bioavailability of compounds may be low. Flavonoids have been described [7]. Cruciferous vegetables, including broccoli, are being investigated for a potential role in the prevention and treatment of cancer. A Recent study focused on using of broccoli for oxidative stress treatment, a relatively short dietary treatment with broccoli sprouts can strongly protect the heart against oxidative stress and cell death caused by ischemia-reperfusion [8].

However, limited information is available about the influence of broccoli treatments on the oxidative stress produced by lead acetate. The aim of the present study was to evaluate the effect of broccoli on reducing oxidative stress in male rats.

II. METHODS AND MATERIAL

A. Experimental animals

Fifty-six healthy adult male Wistar Rat aged 8-10 weeks were Ben purchased from the Agriculture College/ Tekret University and Science College/ Salah Alden University.Male Wistar rats (180-230 g) were Ben randomly housed in plastic cages in groups of 4-3. The cages were cleaned and sterilized weekly with 70% ethanol. Each cage was embedded with wooden shelve and maintained under controlled temperature conditions (25 ±2 °C) with a 12-hour Light /dark cycle [9]. During the period of the experiment abnormal and sick rats were excluded. Water and Standard diet was locally prepared and consist of available constituents that fulfill the dietary rat requirements. The rats were acclimatized for two weeks.

B. Lead acetate

Lead acetate was ben obtained from the faculty of science and educational science/Sulaimani University for the preparation of two stock solution 10g/L and 6 g/L.

C. Broccoli

Scientific Name(s): *Brassica oleracea*
Common Name(s): Broccoli

A large quantity of Broccoli was Ben purchased from supermarket (Zara) at the sulaimani city. Broccoli has ben washed with tap water and then dried. Dry Broccoli was grinding with using a grinder (WAHL James MARTIN) into a fine powder. The powder used for the Preparation of Standard diet containing 10% Broccoli.

D. Experimental design

After two weeks acclimation period, the forty tow adult male rats that included in this study were divided into six groups of 7 rats each as follow:

1) Group C (Control): The Animals were injected intraperitoneally (I.P) with normal saline using an insulin syringe, ones each a week for five weeks respectively.The animals were feed with standard diet and water *ad libitum* along the period of the experiment.

2) Group LE6 : The Animals were injected intraperitoneally (I.P) with 6mg/Kg body weight of lead acetate, using insulin syringe, ones each a week for five weeks respectively, and were feed with standard diet and water *ad libitum* Along the period of the experiment .

3) Group LE10: The Animals were injected intraperitoneally (I.P) with 10mg/Kg body weight of lead acetate , using insulin syringe, ones each a week for five weeks respectively, The rat were given standard diet and water *ad libitum* during the period of experiment .

5) Group Br+LE6: The Animals were injected intraperitoneally (I.P) with 6mg/Kg body weight of lead acetate using an insulin syringe, ones each a week for five weeks respectively and the rats were fed with a standard diet contained 10% Broccoli and water *ad libitum* along the period of the experiment

6) Group Br+LE10 : The Animals were injected intraperitoneally (I.P) with 10mg/Kg body weight of lead acetate using insulin syringe, ones each a week for five weeks respectively, The rat were given standard diet and water *ad libitum* during the period of experiment .

7) Group LE6+Br : The animals were injected previously with 6mg/kg of body weight lead acetate ones each a week for three weeks and then fed on 10% broccoli and injected with lead acetate concomitantly for two weeks and then fed on broccoli alone for the last three weeks

8) Group LE6+Br: The animals were injected previously with 10mg/kg of body weight lead acetate, ones each a week for three weeks respectively, and then fed on 10% broccoli ,and injected with lead acetate concomitantly for two weeks and then fed on broccoli alone for the last three weeks.

E. Blood Collection

All the fasted animals have ben sacrificed after chloroform anesthesia at the end of experiment time.Heart puncture took blood samples and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min by using Centrifuge and micropipette then serum were stored in the deep freeze (-45C°).

F. The biochemical assays

1) Assay for SOD activity: SOD % in serum was measured using an SOD Assay Kit-WST (Dojindo), monitoring the decrease in the rate of superoxide-mediated reduction of nitroblue tetrazolium at 450 nm using a spectrophotometer**.**

2) Determination of serum MDA: In the presence of heat and acid, MDA reacts with TBA to produce a pink colored end product. The intensity of color at 532 nm corresponds to the level of lipid peroxidation in the sample the level of serum MDA was determined spectrophotometrically**.**

3) Determination of total serum Glutathione: The content of reduced GSH and oxidized GSH in the serum were determined by using OxiSelect ™ Total Glutathione Assay Kit(Cell BIOLABS, INC.). This kit a quantitative assay for measuring the total glutathione content within a sample (GSH/GSSG). Glutathione reductase reduces oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH. Subsequently, the chromogen reacts with the thiol group of GSH to produce a colored compound that absorbs at 405 nm.The total glutathione content in unknown samples is determined by comparison with the predetermined glutathione standard curve.

G. Statistical Analysis

Analysis of data was performed by using SPSS (version15).Results were expressed as mean ±S.E. Statistical differences were determined by Duncan Post Hoc test for multiple comparisons after ANOVA.

III. RESULTS AND DISCUSSION

Lead is known to produce oxidative damage, ROS production can be used as a marker of oxidative stress Said et al., (2005)[10]. Several antioxidant enzymes and molecules have been used to evaluate lead-induced oxidative damage in animal and human studies. Reduced glutathione (GSH) and glutathione disulfide (GSSG) concentrations, as well as modifications in superoxide dismutase (SOD) activity, are the most frequently used markers in tissues or blood [11].The present study revealed that lead acetate -induced oxidative stress as indicated by significant changes in serum biochemical parameters, including increased MDA and decreased total glutathione and superoxide dismutase (SOD)% in lead acetate treated groups LE6 and LE10 compared to control group, fig. (1-3).

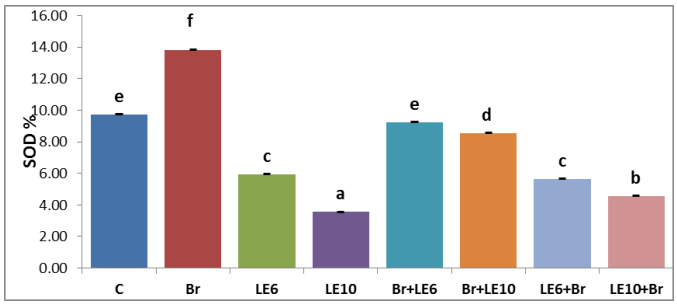

Figure 1 : Mean ± S.E for the effect of Lead acetate, broccoli and their combination in serum SOD% in rats

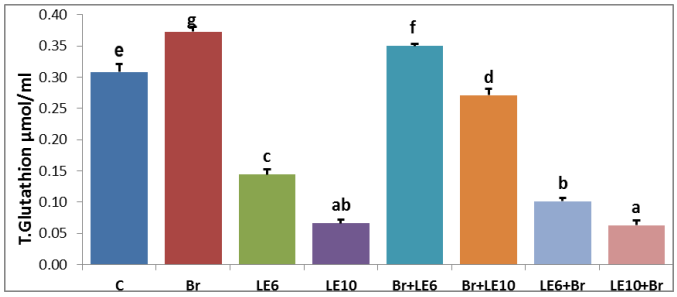

Figure 2 : Mean±S.E for the effect of Lead acetate, broccoli, and their combination on serum T.glutathion µmol/ml in rats

MDA is a marker of oxidative stress. GSH can act as a nonenzymatic antioxidant, by direct interaction of sulfhydryl (SH) groups with ROS, or it can be involved in enzymatic detoxification reaction for ROS, as a cofactor or a coenzyme, because it is a tripeptide containing cysteine that has a reactive SH group with reductive potency[12].

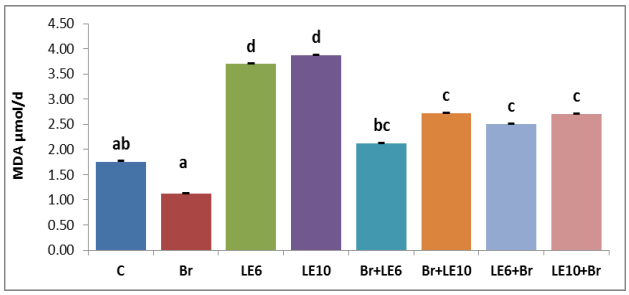

Figure 3 : Mean ±S.E for the effect of Lead acetate, broccoli and their combination on serum MDA µmol/ml in rats

The conclusion of significant($P<0.05$) decrease in total glutathione in LE6 and LE10 group fig.(2) may be due to high ability of lead to bind with SH group of glutathione to decrease its level and lead-induced oxidative stress and this is the possible explanation of increase MDA significantly in these groups fit (3) [13]. High affinity of lead for SH groups or metal cofactors in antioxidant enzymes and molecules, results in a reduction in another antioxidant enzyme activities, such as SOD fig.(1), which form important line of defense against ROS and decrease in their activities contribute to oxidative stress in the tissues[14]. The toxicity effects of lead acetate are confirmed by previous study of Elgawish and Abdelrazek (2014)[15] they proved that superoxide dismutase (SOD) was reduced in lead acetate treated rats compared to the other groups.Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects [16,17,18]. Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation [19] and brain thiobarbituric acid-reactive substances and altered the antioxidant defense system [20].Similar effects were also reported in the hepatic tissues[21]. Some recent studies confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity [22]. Based on the observation that free radical was generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy [23] In the present study, to see the effects of broccoli, a potent antioxidant,on lead acetate induced oxidative stress we examined level of SOD and total glutathione and level of MDA lead acetate treated rats. Interestingly, we found that as showed in figure (1-3)broccoli could, at least partly, attenuate oxidative stress by increasing total glutathione level and SDO% and decreasing lipid peroxide level in lead acetate-treated rat serum Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. [24].In Several previous studies herbs used to treat or protect against lead toxicity like using of cinnamon [15].And *Ocimum basilicum* are well known to contain flavonoids and have a strong antioxidant effect that is beneficial for serum antioxidant levels, leading to improved sperm health parameters via the reduction of oxidative stress [11], Many natural antioxidants, whether consumed before or after or in concomitant with lead acetate ,can confer some level of protection. In addition to beneficial effects accrued from established antioxidants, such as, vitamin C and E, and their derivatives, vitamin A, beta carotene, protection is also conferred by several novel molecules, including, flavonoids, epigallocatechin and other polyphenols [11]. Previuos study of [7]showed that Raw, green broccoli is a source of multiple vitamins and minerals, including calcium, magnesium, potassium, iron, zinc, and selenium, as well as carotene, thiamine, riboflavin, niacin, folate, and vitamins C and K,and flavonoids.the therapeutic properties of broccoli attributed to its chemical constitutes and antioxidant activity that are beneficial for managing and preventing several illnesses [25] so it seems likely that broccoli can protect and adverse the oxidative stress of lead acetate in rats.

IV. CONCLUSION

It can be concluded that the diet that contain 10% broccoli may protect and adverse the toxicity of lead acetate in male rats. This is might be due to its chemical composition that acts as chelating agents to lead in one hand and a rich source of antioxidants that prevent oxidative stress of lead acetate. However, further research is required to throw some more lights on the subject by different corners.

V. REFERENCES

[1]. Clarkson, T.W. , Magos, L., Myers, G.J (2003)"The toxicology of mercury - current exposures and clinical manifestations". *N Engl J Med*(Oct 2003), 349: 1731-1737P.C.

[2]. Hsu and Y.L. Guo. (2002). Antioxidant nutrients and lead toxicity. *toxicology*(Oct 2002),180:33-44.

[3]. Birben , E. Sahiner,U.M., Sackesen, C. , Erzurum, S. , and Kalayci, O. .(2012). Oxidative Stress and Antioxidant Defense. REVIEW ARTICLE. *World Allergy Organization.*5:9–19.

[4]. Kumar, M.N. Prasad, V. Mohan Murali Acharya, B.B. Panda,((2013)." Elucidation of lead-induced oxidative stress in Talinum triangular roots by analysis of antioxidant responses and DNA damage at the cellular level".*Environ. Sci. Pollut.Res. Int*. 20:4551–4561.

[5]. Kinnula VL.(2005) Production and degradation of oxygen metabolites during inflammatory states in the human lung. Curr Drug Targets Inflamm Allergy. 2005;4:465–470.WAO JournalJanuary 2012 Oxidative Stress.

[6]. Khaki, A., Bayatmakoo, R., Nouri,M. and Khaki, A.A.(2013). Remedial Effect of Cinnamon Zeylanicum on serum anti-oxidants levels in male diabetic Rat, *Life Science Journal*;10:4.

[7]. Fimognari C, Lenzi M, Hrelia P.(2008)" Interaction of the isothiocyanate sulforaphane with drug disposition and metabolism: pharmacological and toxicological implications". Curr Drug Metab. 2008;9(7):668-678.).

[8]. Akhlaghi M and Bandy B.(2010). Dietary Broccoli Sprouts Protect Against Myocardial Oxidative Damage and Cell Death During Ischemia. *Plant Foods Hum Nutr.* 65,3:193–199 *(*must be added to the thesis.

[9]. Subramanian, P., Sivabalan, S., Menon, V.P., Vasudevan, K. (2000)"ر Influence of chronic zinc supplementation on biochemical variables and circadian rhythms in Wistar rats". *J Nutr Res*.20:425-413.

[10]. Said, T.M., Aziz, N., Sharma, R.K., Lewis-Jones, I., Thomas, A.J. and Agarwal, A(2005). A novel association between sperm deformity index and oxidative stress-induced DNA damage in infertile male patients.*Asian J. Androl*., 7(2): 121-126).

[11]. Khaki A, Fathiazad F, Nouri M, Khaki AA, Abassi MN,Ahmadi P, Jabarikh H . (2010). Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. *Phytother. Res.* J., 24.9: 1285-1291.

[12]. Sivaprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and 2, 3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. J Nutr Biochem. 2004;15:18–23.

[13]. Kasperczyk, S., Błaszczyk, I., Dobrakowski, M., Romuk, E., Kapka-Skrzypczak, L., Adamek, M., Birkner, E., 2013.Exposure to lead affects male biothiols metabolism. *Annals of Agricultural and Environmental Medicine*, Vol 20, No 4, 721–725.

[14]. Antonio-Garcia MT, Massó-Gonzalez EL. Toxic effects of perinatal lead exposure on the brain of rats: Involvement of oxidative stress and the beneficial role of antioxidants. Food Chem Toxicol. 2008;46:2089–95.

[15]. Elgawish, R.A. and Abdelrazek, H.M.A. (2014)."Effects of lead acetate on testicular function and caspase-3expression with respect to the protective effect of cinnamonin albino rats" . Toxicology Reports 1, 795–801.

[16]. Pande M, Flora S (2002). Lead induced oxidative damage and its response to combined administration of lipoic acid and succimer in rats. Toxicology, 177: 187-196

[17]. Auman JT, Chou J, Gerrish K, Huang Q, Jayadev S, Blanchard K, Paules RS (2007). Identification of genes implicated in methapyrilene-induced hepatotoxicity by comparing differential gene expression in target and nontarget tissue. Environ. Health Perspect.

[18]. Waters M, Stasiewicz S, Merrick BA, Tomer K, Bushel P, Paules R, Stegman N, Nehls G, Yost KJ, Johnson CH (2008). CEBS –Chemical Effects in Biological Systems: a public data repository integrating study design and toxicity data with microarray and proteomics data. Nucleic acids research, pp. 892-900.

[19]. Upasani C, Khera A, Balaraman R (2001). Effect of the lead with Vitamins E, C, or spirulina on malondialdehyde: conjugated dienes and hydroperoxides in rats. Indian J. Exp. Biol., 39-: 70.

[20]. Adanaylo V, Oteiza P (1999). Lead intoxication: antioxidant defense and oxidative stress in rat brain. Toxicology, 135: 77-85.

[21]. Sandhir R, Gill K (1995). Effect of lead on lipid peroxidation in liver of rats. Biol. Trace Elem. Res. 8: 91-97.

[22]. Gurer H, Ercal N (2000). Can antioxidant be beneficial in the treatment of lead poisoning? Free Radic. Biol. Med. 29: 927-945.

[23]. Khaki A (2010). Protective effect of quercetin against necrosis and apoptosis induced by experimental ischemia and reperfusion in rat liver. AJPP, 4(1): 022-026.

[24]. Feng R, He W, Ochi H (2001). A new murine oxidative stress model associated with Senescence. Mech. Ageing Dev., 122:547-559.
[25]. Fratianni , F. , Cardinale, F. Cozzolino, A., Granese, T. , Pepe.S., Riccardi, R. , Spigno, P. , Coppola, R. , Nazzaro, F.(2014). "Polyphenol Composition and Antioxidant Activity of Two Autochthonous Brassicaceae of the Campania Region, Southern Italy." Food and Nutrition Sciences, 5, 66-70).