ORIGINAL ARTICLE

IMPORTANCE OF MALONDIALDEHYDE AND SUPEROXIDE DISMUTASE LEVELS: ANTIOXIDANT ROLE OF WALNUT

Roomisa Anis, Sadaf Jafar*, Naveeda Zaigham, Beenish Zafar, Naureen Waseem, Qurat-ul-Ain Zia***

Department of Biochemistry, Al Nafees Medical College, Isra University, Islamabad, *Islamabad Medical and Dental College, Islamabad, **Islam Medical College, Sialkot, Pakistan

Background: Oxidative stress is proposed molecular mechanism in lead toxicity. Antioxidant rich plants protects against toxicity of heavy metals by neutralizing free radicals. Walnuts have proven antioxidant activity. Objective of this study was to investigate protective effect of walnuts on lipid peroxidation and antioxidant enzyme activity by measuring oxidative profile in mice. **Methods:** This experimental study was carried out from 6th Oct to 15th Dec 2018 at Biochemistry Department, Al-Nafees Medical College Islamabad, in collaboration with National Institute of Health. Sixty BLAB/C mice were divided into three equal groups. Group I was fed on standard diet, Group II was given lead acetate 30 mg/Kg in drinking water along with normal diet. Group III was given lead acetate along with diet and walnut supplementation was given as 111 gm/Kg of the diet. Malondialdehyde (MDA) and superoxide dismutase (SOD) were measured in mice blood. Data were analyzed using SPSS-20. **Results:** Lead caused an increase in MDA levels whereas activity of SOD was significantly decreased. Supplementation of walnut along with lead showed decrease in MDA in group III compared to group II ($p \leq 0.001$), although not to MDA level of control group. Activity of SOD was increased in group III ($p \leq 0.001$) as compared to control group and group II. **Conclusion:** Antioxidants in walnuts decrease lead induced lipid peroxidation and improves antioxidant defence system by increasing activity of superoxide dismutase.

Keywords: Lead, Oxidative stress, Walnuts, Malondialdehyde, Superoxide dismutase

Pak J Physiol 2019;15(4):11−3

INTRODUCTION

Lead poisoning is an accepted occupational hazard with multiple clinical implications.[1] Lead mine, smelting, plumbing, cosmetic, battery, and glass manufacturing industry workers are at a greater risk of lead toxicity. Lead toxicity interferes with variety of metabolic processes causing impedance of normal biochemical activities of the cells leading to multi-organ dysfunction. Even though the release of lead from different sources has been decreased in the environment but still exposure to this toxic metal is a huge public concern, more specifically in developing countries.[2] Pakistan is one of the countries where unsupervised use of heavy metals like lead is a serious threat to the environment. The utmost origin of lead in environment is contributed by batteries, leaded gasoline, paints, water pipes, insecticides and some cosmetics.[3] Oxidative stress is a proposed molecular mechanism in lead toxicity, which suggests that antioxidants might play a significant role in the treatment of lead poisoning.[4]

Many plants are rich in antioxidants that protect against toxicity of heavy metals by neutralizing free radicals. Among common plant foods, walnuts have proven antioxidant activity. [5]

Plumbism or lead toxicity shows non-specific sign and symptoms such as abdominal pain, constipation, irritability, and anaemia. Haemolytic anaemia and colicky abdominal pain must not be misdiagnosed for other causes. Therefore, detailed drug history including use of alternative medicine must be considered.[6] Lead causes decline even in reproductive function of male rats by causing oxidative damage and inhibiting enzymes. It causes reproductive derangements in painters, especially sperm motility and increased testosterone levels were also recorded in them.[7,8] Lead exposure affects gene expression of enzymes like superoxide dismutase, catalase and glutathione peroxidase (markers of antioxidant status) in leukocytes and erythrocytes.[9,10] β carotene reduces oxidation and improves antioxidant activity.[11] Lead causes decrease in growth of cells, increase in NO production and decreased IL-6 and TNF-α secretion in macrophages.

The present study was designed to investigate the protective effect of walnut by measuring MDA and SOD levels in experimental animals.[12]

MATERIAL AND METHODS

After approval of Review Board and Ethics Committee (IRBC), Isra University Islamabad the study was conducted in Department of Biochemistry, Al-Nafees Medical College, in collaboration with National Institute of Health Islamabad. The laboratory tests were conducted at the Multi-Disciplinary Laboratory (MDL) Al-Nafees Medical College. Malondialdehyde (MDA) –an oxidative stress marker for lipid peroxidation, and superoxide dismutase (SOD) –an antioxidant enzyme, for oxidant status were measured. This study comprised

of 60 BLAB/C mice of either gender, randomized and divided into three groups of 20 mice each. Healthy BLAB/C mice, weighing 30–50 gms, and ~60 days old were included. Mice with disease or developing disease in the course of experiment were excluded. Group I was given standard diet. Group II was given lead acetate 30 mg/Kg in drinking water along with normal diet. Group III was given lead acetate along with diet supplemented with walnut, given as 111 gm/Kg of the diet. All mice were sacrificed after 2 months and 1 ml intracardiac samples of blood were taken.

TBARS Assay kit (Item No. 10009055) from Cayman Chemicals was used to check the serum levels of MDA in mice. Superoxide dismutase assay kit from Abcam (ab65354) was used for detection of superoxide radicals. Data were analyzed using SPSS-20. Results were tabulated as Mean±SD. One-way ANOVA was applied followed by Post Hoc Tukey's test, and $p \leq 0.05$ was considered as significant.

RESULTS

Administration of lead, dissolved in water by gauge tube for two months resulted in significant increase in levels of serum malondialdehyde ($p<0.001$) whereas the level of inhibition of superoxide anion by superoxide dismutase was significantly decreased ($p<0.001$). The serum levels of malondialdehyde were significantly decreased ($p<0.001$) and the level of superoxide dismutase was significantly increased ($p<0.001$) when walnuts were given in pelleted diet for two months.

The lipid peroxidation was significantly increased ($p<0.001$) after lead administration in group II as shown by raised MDA levels, (38.06±2.99 µmol/L) when it was compared to control group (1.46±1.21 µmol/L). Lead exposure resulted in the significant decrease in the activity of serum superoxide dismutase (25.96±3.56) when it was compared with control group (55.53±3.84).

Supplementation of walnut for two month in group III was able to prevent the lipid peroxidation caused by lead intoxication by assessing the serum malondialdehyde levels. The serum malondialdehyde levels in group III (7.91±2.32), supplemented with walnuts, were although increased, but when compared with the control group (1.46±1.21), showed significant ($p<0.001$) decline in comparison to group II (38.06±2.99) which was exposed only to lead. Supplementation of walnuts was able to protect against the lipid peroxidation induced by lead by significantly increasing activity of SOD, 71.24±4.30.

When the serum superoxide dismutase activity of control group (55.53±3.84) was compared with that of group II (25.96±3.56), it showed significant decrease ($p<0.001$) revealing that lead had significantly compromised the antioxidant defence enzyme in mice. However when the serum superoxide dismutase activity of group III (71.24±4.30) was compared with control and group II (Table-1), it was significantly raised ($p<0.001$) in group III showing that walnuts improved the natural antioxidant defence system along with that walnuts were able to protect against oxidative stress caused by lead toxicity.

Table-1: Effects of two-month supplementation of lead acetate and walnuts on serum malondialdehyde and superoxide dismutase levels in mice (Mean±SD)

Parameter	Group I (n=20)	Group II (n=20)	Group III (n=20)	*p*
Malondialdehyde µmol/L	1.46±1.21	38.06±2.99	7.91±2.32	<0.001*
Superoxide dismutase (% inhibition)	55.53±3.84	25.96±3.56	71.24±4.30	<0.001*

*statistically significant

DISCUSSION

The antioxidant role of walnut was studied for oxidative stress in lung and muscle tissues. It was analyzed that alcohol induced oxidative stress in these tissues was shown by increased levels of malondialdehyde. These levels were decreased after treatment with walnut.[13] In present study similar antioxidant effects of walnut were studied in lead toxicity induced in mice.

Taurine is an antioxidant which is found in most of the nuts especially walnut. A study was conducted in Turkey in which the antioxidant effects of Taurine were studied after lead exposure. The oxidative stress parameters glutathione, malondialdehyde levels, catalase and glucose-6-phosphate dehydrogenase activities were also measured.[14] Taurine reduced malondialdehyde levels which was also found in our study with walnut. Taurine may be effective in lead poisoning along with chelating agent.

The protective effects of *Juglans regia* (walnut) oil against hepatotoxicity due to lead was confirmed. Lead toxicity raised aspartate amino transferase, alanine amino transferase and lactate dehydrogenase enzyme levels indicating hepatic inflammation. On administration of walnut oil hepatic markers and lipid profile reverted to normal. The walnut oil was found to be effective against lead toxicity.[15] Similar conclusion was drawn from our study.

Taurine is an antioxidant found in walnuts and is neuroprotective. Taurine produces perturbations in efficiency of antioxidant enzymes when given as a treatment to rats having cognitive impairment induced by chronic exposure to chlorpyrifos and lead acetate.[16] Another study reported that lead toxicity induced raised levels of alanine aminotransferases and plasma bilirubin. It also reduced the Hb, RBC, and PCV, but white blood cell count increased. D-penicillamine reduced the toxic effects of lead.[17] In the present study no drug or chelating agent was used but only the antioxidant effects of walnut were analyzed after inducing lead toxicity in mice.

Lead in specific concentration can cause poisoning in fish leading to death. Hedayati *et al*[18]

studied lead poisoning in fish. They exposed fish to low and high concentration of lead for 24, 48, and 96 hours. They concluded that heavy metal like lead affects the physiological and biochemical parameters. Our study also confirmed that lead toxicity affects mice blood parameters. In another study oxidative damage in liver and kidney was seen after administration of lead in laboratory animals which increased liver function biomarkers and changes in superoxide dismutase enzyme. The increased levels of creatinine, urea and uric acid lead to nephropathy.[19] All the toxic effects of lead were also observed in mice which were administered lead in prolong doses during our study.

Oxidative stress due to lead poisoning effects haematopoietic, renal, reproductive and central nervous system. N-acetyl cysteine, α-lipoic acid, vitamin E, quercetin and herbal extracts were used as prophylaxis against reactive oxygen species. Chelation therapy and nanoencapsulation has recently been used to treat lead poisoning.[20] In the present study lead toxicity was also induced and was treated with antioxidant effects of walnut. As lead is the earliest heavy metal, which was searched and used by man from the last 5,000 years. Use of lead is a major public concern because it has deleterious health effects. Alessia Carocci[21] reviewed the lead toxicity as we considered it in our study.

The effects of both allicin, vitamin B complex and α-tocopherol alone against lead acetate toxicity on cerebral cortex was studied immunohistochemically and ultra-structurally. It was concluded that nervous system ailments as a consequence of lead poisoning can be treated with α-tocopherol, as it has protective potential, against oxidative stress.[22] We concluded similar protective potential of walnut against oxidative stress caused by lead.

CONCLUSION

Walnuts protect against lead toxicity-induced oxidative stress by decreasing malondialdehyde, and improve the antioxidant status by increasing superoxide dismutase activity.

REFERENCES

1. Ji JS, Schwartz J, Sparrow D, Hu H, Weisskopf MG. Occupational determinants of cumulative lead exposure: Analysis of bone lead among men in the VA Normative aging study. J Occup Environ Med 2014;56(4):435–40.
2. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metal Toxicity and the Environment. In: Luch A, (Ed). Molecular, Clinical and Environmental Toxicology. Basel: Springer Basel; 2012. pp 133–64.
3. Waseem A, Arshad J, Iqbal F, Sajjad A, Mehmood Z, Murtaza G. Pollution status of Pakistan: a retrospective review on heavy metal contamination of water, soil, and vegetables. Biomed Res Int 2014; 2014:813206. doi: 10.1155/2014/813206.
4. Lopes AC, Peixe TS, Mesas AE, Paoliello MM. Lead exposure and oxidative stress: A systemic review. Rev Environ Contam Toxicol 2016;236:193–238.
5. Hayes D, Angove MJ, Tucci J, Dennis C, *et al*. Walnuts (*Juglans regia*) Chemical Composition and Research in Human Health. Crit Rev Food Sci Nutr 2016;56(8):1231–41
6. Chang SH, Yoon SB, Lee JW, Lee DJ. What caused hemolytic anemia and colicky abdominal pain? Lead! Korean J Intern Med 2013;28(4):504–6.
7. Ayinde OC, Ogunnowo S, Ogedegbe RA. Influence of Vitamin C and Vitamin E on testicular zinc content and testicular toxicity in lead exposed albino rats. BMC Pharmacol Toxicol 2012;13:17. doi: 10.1186/2050-6511-13-17.
8. Hosni H, Selim O, Abbas M, Fathy A. Semen quality and reproductive endocrinal function related to blood lead levels in infertile painters. Andrologia 2013;45(2):120–7.
9. Qasim SF, Baloch M. Lead toxicity in battery workers. J Coll Physicians Surg Pak 2014;24(Suppl 3):S284–6.
10. Kasperczyk A, Machnik G, Dobrakowski M, Sypniewski D, Birkner E, Kasperczyk S. Gene expression and activity of antioxidant enzymes in the blood cells of workers who were occupationally exposed to lead. Toxicology 2012;301:79–84.
11. Kasperczyk S, Dobrakowski M, Kasperczyk J, Ostałowska A, Zalejska-Fiolka J, Birkner E, *et al*. Beta-carotene reduces oxidative stress, improves glutathione metabolism and modifies antioxidant defense systems in lead-exposed workers. Toxicol Appl Pharmacol 2014;280(1):36–41.
12. Luna AL, Acosta-Saavedra LC, Martínez M, Torres-Avilés N, Gómez R, Calderón-Aranda ES. TLR4 is a target of environmentally relevant concentration of lead. Toxicol Lett 2012;214(3):301–6.
13. Bati B, Celik I, Turan A. Determination of antioxidant effect of Walnut (*Juglans regia L*) on lung and muscle tissue. J Nat Appl Sci East 2019;2(1):29–39.
14. Gurer H, Ozgunes H, Saygen E, Ercal N. Antioxidant effect of taurine against lead induced oxidative stress. Arch Environ Contam Toxicol 2001;41(4):397–402.
15. Soussi A, Gargouri M, El Feki A. Potential immunomodulatory and antioxidant effects of walnut *Juglans regia* vegetable oil against lead-mediated hepatic damage and their interaction with lipase activity in rats. Environ Toxicol 2018;33(12):1261–71.
16. Akande MG, Aliu YO, Ambali SF, Ayo JO. Taurine mitigates cognitive impairment induced by chronic co-exposure of male Wistar rats to chlorpyrifos and lead acetate. Environ Toxicol Pharmacol 2014;37(1):315–25.
17. Kahdim SH, Nazarmuteb, Al-Kreem ZA, Ubaid MM. The effect of penicillamine in reducing the toxic effect of lead acetate on some blood parameters, liver functions and testicular tissue in male rats. Int J Pharm Pharm Res 2016;5:22–40.
18. Hedayati A, Darabitabar F. Lethal and sub lethal impacts of lead on some hematological biochemical and immunological indices in Caspian roach. Pollution 2017;3:21–27.
19. Alya A, Ines DB, Montassar L, Najoua GH, Soloua EF. Oxidative stress biochemical alterations and hyperlipidaemia in female rats induced by lead chronic toxicity during puberty and post puberty period. Iran J Basic Med Sci 2015;18(10):1034–43.
20. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. Interdescip Toxicol 2012;5(2):47–58.
21. Carocci A, Catalano A, Lauria G, Sinicropi MS, Genchi G. Lead toxicity, antioxidant defense and environment. Rev Environ Contam Toxicol 2016;238:45–67.
22. Mustafa HN, Hussain AM. Does allicine combined with vitamin B complex have superior potentials than α-tocopherol alone in ameliorating lead acetate induced purkinji cell altrations in rats? An immunohistochemical and ultrastructural study. Folia Morphol (Warsz) 2016;75:76–86.

Address for Correspondence:

Dr. Sadaf Jafar, Associate Professor Biochemistry, Islamabad Medical and Dental College, Main Murree Road, 17-Mile, Near Barakaho, Islamabad, Pakistan. **Cell:** +92-300-9546632

Email: sadafjafar75@gmail.com

Received: 23 May 2019 **Reviewed: 8 Dec 2019** **Accepted: 29 Dec 2019**