Antioxidant effects of walnuts on malondialdehyde levels raised by lead toxicity in mice

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ABSTRACT

Objective: To evaluate the antioxidant effects of walnuts on raised levels of Malondialdehyde (MDA) by lead toxicity.
Study Design: Quasi Experimental study
Place and Duration: Department of Biochemistry, ANMCH, Islamabad, Pakistan in collaboration with National Institute of Health Islamabad. The duration of the study was six months from November, 2015 to April, 2016
Methodology: A total of 60 BALB/c mice were divided into three groups of 20 mice each. Group I was given normal standard diet. Group II was given lead acetate in drinking water with normal diet without any supplementation. Group III was given lead acetate with diet supplemented with walnuts. Levels of malondialdehyde were measured by using Thiobarbituric acid reactive substances (TBARS) method at the end of study.
Results: The data obtained from this study indicates that lead caused increase in serum Malondialdehyde (38.06±2.99.) levels in group II. Supplementation of walnut along with lead showed decrease in serum malondialdehyde levels (7.91±2.23) in group III as compared to group II.
Conclusion: Walnuts are rich in antioxidants and prevent against the lead induced oxidative stress by decreasing serum Malondialdehyde levels
Keywords: Lead Poisoning, Malondialdehyde, Walnuts

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INTRODUCTION

This is an era of modernization, urbanization and industrialization because of which humans and their environment are suffering a lot[1]. Urbanization leads to the conventional risk of infectious disease in poor population of the urban areas. According to the United Nations Human Development report, Pakistan alone will harbour the urbanization of 113 million people by 2030 contributing approximately 7.5 % of the total urbanization of the Asia[2]. The urban environment also creates the physiochemical hazards which include lead exposure, air pollution, hazards of traffic and heat wave intensification[3].

Lately environmental pollution by heavy metals has been considered as a matter of great concern[4]. Industries are the major cause of heavy metal pollution which include; refineries of metal processing, power plant which run on coal burning and petroleum products, nuclear power stations, high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants[5]. Lead is a heavy metal and one of the ubiquitous pollutants[6]. It is being extensively used in different industries for the past 8000 years but was established as toxin long after its use started[7]. Among other developing countries, unsupervised use of lead in Pakistan is a serious threat to the environment[8].

The suggested molecular mechanism underlying lead toxicity is oxidative stress which causes production of reactive oxygen

species and lipid peroxidation[9]. As a result of this lipid peroxidation, a harmful compound Malondialdehyde is produced which when combines with the DNA forms covalent attachments leading to structural changes in DNA. Moreover, interaction of MDA with the functional groups of proteins and lipoproteins results in changing their chemical behaviour possibly rendering to carcinogenesis and mutagenesis[10]. Nutritional antioxidants play a significant role in prevention against lead toxicity[11]. Use of walnuts as folk remedy is at its peak because of the antioxidant nutrients present in it. Walnut as an antioxidant nutrient contains polyphenols and ellagitannins which are capable of neutralizing oxidative stress[12]. The rationale of the present study was to evaluate the antioxidant effects of walnuts in decreasing the levels of Malondialdehyde; raised by oxidative stress due to lead toxicity. This study was conducted to investigate the effect of lead on MDA in the tissues of mice, with special reference to the possible beneficial role of walnut in ameliorating the lead – induced toxicity in the mice.

METHODOLOGY

This is a Quasi experimental study and was conducted after the approval of Institutional Review Board and ethics Committee (IRB) Isra University Islamabad (Letter No: F.2/IUIC-ANMC/EC-57/2015) in Department of Biochemistry, Al Nafees Medical College & hospital, Islamabad, Pakistan in collaboration with National Institute of Health Islamabad (NIH). Duration of study was 6 months from November, 2015 to April, 2016. The laboratory tests were conducted at the Multi-disciplinary laboratory (MDR) at Al Nafees Medical College & Hospital. Lead acetate was purchased from a local scientific shop manufactured by United Laboratory Chemical Works, Garden Town, Lahore. Walnuts (Juglans Regia) were purchased from local cultivator and seller in Mansehra District.

The BALB/c mice were procured from animal house of NIH Islamabad. These animals were bred at the NIH and were used in the experiment. The mice included in the study were adult healthy mice 60 days old weighing 50gms±20gms of either gender. Whereas mice with disease or those mice who developed disease in the course of experimentation were excluded. Mice chow was supplemented with walnuts. Walnut shells were discarded, and walnut kernels were obtained. Whole walnut kernels were stored at -20°C until grounded and added to the diet. Lead acetate was dissolved in drinking water and given by gauge as described in the grouping section.

Mice were randomized into three groups Group I, Group II, Group III using convenience sampling technique. Each group contained 20 mice. They were given standard mice chow along with the supplemented diet.

Group I served as a control group and contained 20 mice. Group I was treated with normal mice chow for two months and was given plain tap water along with 0.5 ml plain water by gauge tube Group II was treated with normal mice chow and lead acetate 30mg/kg body weight in drinking water for two months[13]. Group III was treated with standard mice chow, lead acetate 30mg/kg body weight in drinking water. Along with Lead acetate, group III was treated with whole walnut kernels 111 g/kg diet[14]. All the samples were taken at the end of the study by intracardiac puncture. To check the serum levels of MDA in mice TBARS (Thiobarbituric acid reactive substances) method was used. Thiobarbituric acid (TBA) is used to check the MDA as a product of lipid peroxidation in plasma, serum, urine, tissue homogenates and cell lysates[15]. This method required high temperature ranging between 95-100°C and acidic conditions to generate a pink coloured complex. This pink coloured complex was detected colorimetrically at wavelength of 530-540nm[16]. We calculated the average absorbance of standard and of each sample. Absorbance of the standard A was subtracted from all to get value of corrected absorbance. By plotting the corrected absorbance value against MDA concentration standard curve was generated. Results were obtained from the standard curve. SPSS version 20 was used to analyze the data obtained from the above procedure. The arithmetic means and standard deviation of mean of all samples was calculated. To observe the level of significance among groups one-way ANOVA was applied. Post Hoc Tukey's test was applied for multiple comparisons. Independent sample't test' was applied for two group comparisons to check the difference in mean among the control and treated groups. The difference was considered significant if p value was found ≤ 0.05.

RESULTS

The total number of mice included in the study were 60 (N=60). They were divided into three groups with 20 mice in each group (n=60). Administration of lead in group II significantly increased the lipid peroxidation which was calculated by estimation of serum Malondialdehyde levels as shown in Table-I. This table shows that the MDA levels in control group were 1.46±1.21 whereas the MDA levels in group II after administration of lead acetate for two months were significantly raised (p-Value < 0.01) and were 38.06±2.99.

Table-I: Effects of two-month supplementation of lead acetate on serum malondialdehyde levels in mice. (N=60)

Parameter	Group I Control (n=20) Mean±SD	Group II Lead acetate (n=20) Mean±SD	p-Value*
Serum Malondialdehyde μmol/L	1.46±1.21	38.06±2.99	< 0.001

*(p-Value of ≤ 0.05 is taken as statistically significant)

Table-II: Effects of two-month supplementation of lead acetate and walnuts on serum malondialdehyde in mice. (N=60)

Parameter	Group II Lead acetate (n=20) Mean±SD	Group III walnut (n=20) Mean±SD	p-Value*
Serum Malondialdehyde μmol/L	38.06±2.99	7.91±2.32	< 0.001

*(p-Value of ≤ 0.05 is taken as statistically significant)

The supplementation of walnut in the mice chow for two months in group III was able to prevent the lipid peroxidation caused by lead intoxication by assessing the serum Malondialdehyde levels as shown in Table-II.

This table shows that the serum Malondialdehyde levels in group III showed significant decline (p-Value < 0.01) when compared with the group II. The MDA levels in group III were 7.91±2.32 whereas the MDA levels in group II were 38.06±2.99. The MDA levels of group III were significantly increased (p-Value < 0.01) when compared to the MDA levels of control group as shown in Fig: I. This bar graph shows the MDA levels of all the three groups. When the MDA levels of group II and group III were compared with group I they showed significant increase but on comparison of group II with group III the MDA levels in group III showed significant decline (p-Value < 0.01) due to walnut administration.

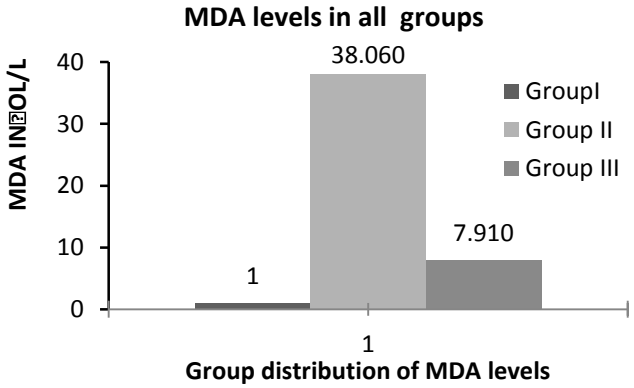

Figure-I: Effect of two-month supplementation of lead acetate and walnuts on serum Malondialdehyde levels in mice (N=60)

DISCUSSION

Exposure to heavy metals is a usual phenomenon due to environmental pervasiveness. Lead is a prevalent heavy metal toxicant which induces extensive damage to human population. Although use of lead has long been abandoned in gasoline, but it is still used in many industries. The present study was designed to investigate the protective effect of walnut on lead acetate induced oxidative stress in a mice model. Group I was given normal mice chow and distilled water for two months. After two months the oxidative stress parameter of the control group was measured; which in case of our study was MDA.

Increase in the levels of MDA is associated with oxidative stress. Our data of the control group generated the MDA levels in the range of 1.46±1.21. These results of the control group are in good agreement with several international studies[17]. One of the study conducted on humans, in which healthy subjects and subjects working in a gasoline station were enrolled. The MDA levels of the healthy subjects were in accordance to our results of the control group[18].

Higher levels of malondialdeyhide were noted in another study conducted on BALB/c mice in India in 2017. This is in accordance to the results obtained in our study in which raised levels of malondialdeyhide were found accept for the difference in the choice of metal[19].

One of the study conducted on male wister rats showed the higher levels of serum MDA in rats where lead toxicity was induced through intra peritoneal injection. The route of administration was different in the above mentioned study as compared to our study design where the route of administration of lead was oral. In comparison to this study the MDA levels were only marginally increased in the control group of our study as compared to the study conducted on male Wister rats[20].

In our experimental design group II was exposed to lead acetate in drinking water. In contemporary research, studies have suggested that lead toxicity results in oxidative stress. Introduction of lead to a biological system leads to increase in the formation of reactive oxygen species (ROS) and imbalance of the antioxidant system. A study was conducted on rats in 2016 in which they were exposed to lead for 4 weeks. The results revealed that lead has caused significantly raised levels of MDA in rats liver, which supports our results of group II that lead causes increase in the MDA levels[21].

A similar study conducted on lead also revealed that it is a neurotoxic metal which damages brain and causes increase in the MDA levels by causing increase in the lipid peroxidation as a sequel of generating highly reactive substances. In this study Sprague Dawley rats were used as experimental model which were given lead in drinking water for one month and the results of MDA were analogous to the result of lead treated group of our study[22]. Another study was done in a rat model in which rats were given lead acetate as intraperitoneal injection for 5 days. After 5 days the lead caused nephrotoxicity which was revealed by raised levels of MDA and the trends were like our study group[23].

In opposition to our results of group II, a study was reported by Dobrakowski M.*et al* in 2017. He included 36 male subjects in his study who were occupationally exposed to lead for 36 to 44 days. The daily exposure to the lead was 12 hours. His results suggested that the exposure of lead did not change the levels of MDA in his subjects. His results were contrary to our results which suggested that lead causes significant increase in MDA[24].

In our experimental model group III was administered with lead in similar doses as the group II. Along with lead this group was simultaneously given walnut as an antioxidant supplement for two months. To investigate the antioxidant effects of walnut, at the end of the study serum MDA was done. The results showed significant decrease in MDA levels.Walnuts are used as antioxidants in several studies. One such study was reported on adult male rats. Toxicity with lead is a serious and potential hazard to animal and human health and can induce oxidative stress. In this study rats were exposed to lead at a dose of 0.344 g/kg by weight. Juglans regia walnut oil was used and suggested that walnut was able to decrease the MDA and increase superoxide dismutase (SOD) [25]. A similar study conducted by Haddad *et al* in which he used a human model to demonstrate the antioxidant properties of walnut. In his study he suggested that when a fixed portion of walnuts is added to the diet it lowers MDA levels similar to our walnut group[26].

CONCLUSION

Walnuts are rich in antioxidants and prevent against the lead induced oxidative stress by decreasing serum MDA levels.

AUTHOR'S CONTRIBUTION

Anis R: Conceived idea, Designed research methodology, Data collection, Manuscript writing
Zaigham N: Literature search, Literature review
Zafar B: Data collection
Haq MBU: Statistical analysis
Humerah S: Introduction writing
Kausur MW: Manuscript final reading (supervised whole research)

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REFERENCES

1. Slovic AD, Oliveira MA, Biehl J, Ribeiro H. How can urban policies improve air quality and help mitigate global climate change: a systematic mapping review. J Urban Health. 2016;93(1):73-95.
2. UN DESA. Population division. World Urbanization Prospects: the 2011 revision. New York, NY: United Nations Department of Economic and Social Affairs, 2014. Website: [population.un.org›Publications›Files›WUP2014-Methodology] Retrieved on 20th February 2020.
3. Irfan M, Shaw K. Modelling the effects of energy consumption and urbanization on environmental pollution in South Asian countries: a nonparametric panel approach. Qual Quant. 2017;51(1):65-78.
4. Gupta VK, Nayak A, Agarwal S. Bioadsorbents for remediation of heavy metals: current status and their future prospects. Environ Eng Res 2015;20(1):1-8.
5. Karunanayake AG, Todd OA, Crowley M, Ricchetti L, Pittman JCU, Anderson R, et al. Lead and cadmium remediation using magnetized and nonmagnetized biochar from Douglas fir. Chem Eng J. 2018; 331:480-491. DOI: 10.1016/j.cej.2017.08.124.
6. Salamat N, Lamoochi R, Shahaliyan F. Metabolism and removal of anthracene and lead by a B. subtilis-produced biosurfactant. Toxicol Rep. 2018; 5:1120-1123. DOI: 10.1016/j.toxrep.2018.11.004.
7. Mesri M, Najari F, Kayal IB, Najari D. Hyper Acute Quadriplegia with Chronic Lead Toxicity; A Case Report. Emerg. 2018;6(1):1-4
8. Rehman K, Fatima F, Waheed I, Akash MS. Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem. 2018;119(1):157-184.
9. Kim BM, Rhee JS, Jeong CB, Seo JS, Park GS, Lee YM, et al. Heavy metals induce oxidative stress and trigger oxidative stress-mediated heat shock protein (hsp) modulation in the intertidal copepod Tigriopus japonicus. Comp. Biochem. Physiol C Toxicol Pharmacol. 2014; 66:65-74. DOI: 10.1016/j.cbpc.2014.07.005
10. Eid A, Zawia N. Consequences of lead exposure, and its emerging role as an epigenetic modifier in the aging brain. Neurotoxicol. 2016;56:254-261. DOI: 10.1016/j.neuro.2016.04.006.
11. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, et al. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci. 2017;18(1):96.
12. Pycia K, Kapusta I, Jaworska G, Jankowska A. Antioxidant properties, profile of polyphenolic compounds and tocopherol content in various walnut (Juglans regia L.) varieties. Eur Food Res Technol. 2019;245(3):607-616.
13. Waseem N, Butt AS. Effect of Lead acetate on area of endometrium of mice uterus and the protective role of garlic extract. Int J of Adv Res. 2013; 1 (7): 141-144.
14. Reiter RJ, Tan DX, Manchester LC, Korkmaz A, Broto FL, Hardman WE, et al. A walnut-enriched diet reduces the growth of LNCaP human prostate cancer xenografts in nude mice. Cancer Invest. 2013;31(6):365-373.
15. Lahneche AM, Boucheham R, Boubekri N, Bensaci S, Bicha S, Bentamenne A, et al. Sodium Valproate-Induced Hepatic Dysfunction in Albino Rats and Protective Role of n-Butanol Extract of Centaurea sphaerocephala L. IJPPR. 2017; 9(10); 1335-1343
16. Spickett CM, Wiswedel I, Siems W, Zarkovic K, Zarkovic N. Advances in methods for the determination of biologically relevant lipid peroxidation products. Free Radic Res 2010;44(10):1172-1202.
17. Bazrgar M, Goudarzi I, Lashkarbolouki T, Salmani ME. Melatonin ameliorates oxidative damage induced by maternal lead exposure in rat pups. Physiol Behav 2015; 151:178-188. DOI: 10.1016/j.physbeh.2015.06.040
18. Awad NA, Shanan SK, Fartosy AAJM. Effect of Lead Exposure on Malondialdehyde (MDA) Level and Some Biochemical Parameter in Workers of Gasoline Stations. RJPBCS 2014;5(6): 711-715.
19. Biwas C, Bala J, Kharb S. Effect of vitamin E supplementation on superoxide and malondialdehyde generation in acute celphos poisoning. Arch Med Health Sci 2017; 5(2):200-203.
20. Moneim AE. Indigofera oblongifolia prevents lead acetate-induced hepatotoxicity, oxidative stress, fibrosis and apoptosis in rats. PLoS One. 2016;11(7):1-18. DOI: 10.1371/journal.pone.0158965.
21. Tantawy EWH. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. J Tradit Complement Med. 2016;6(4):327-331.
22. Radad K, Hassanein K, Shraim AM, Moldzio R, Rausch WD. Thymoquinone ameliorates lead-induced brain damage in Sprague Dawley rats. Exp Toxicol Pathol. 2014;66(1):13-17.
23. Dkhil MA, Khalifa AMS, Quraishy AS, Zrieq R, Moneim AE. Indigofera oblongifolia mitigates lead-acetate-induced kidney damage and apoptosis in a rat model. Drug Des Devel Ther. 2016;10:1847-1856. DOI: 10.2147/DDDT.S105511
24. Dobrakowski M, Pawlas N, Hudziec E, Kozłowska A, Mikołajczyk A, Birkner E, et al. Glutathione, glutathione-related enzymes, and oxidative stress in individuals with

subacute occupational exposure to lead. Environ Toxicol Pharmacol. 2016;45:235-40. DOI: 10.1016/j.etap.2016.06.008.
25. 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):1261-1271.
26. Haddad EH, Chong GN, Oda K, Sabaté J. Effect of a walnut meal on postprandial oxidative stress and antioxidants in healthy individuals. Nutr J. 2014;13(1):4-13.