Original Article

Antioxidant Effects of Walnuts on Superoxide Dismutase Levels Reduced by Lead Toxicity in Mice

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ABSTRACT

Objective: To evaluate the antioxidant effects of walnuts on lead toxicity by estimating the superoxide dismutase levels.

Study Design: Quasi Experimental Study

Place and Duration of Study: Department of Biochemistry, ANMCH, Islamabad, Pakistan in collaboration with NIH, Islamabad from November, 2015 to April, 2016.

Materials and Methods: 60 BLAB/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 along with normal diet without any supplementation. Group III was given lead acetate along with the diet supplemented with walnuts. Superoxide Dismutase (SOD) was estimated at the end of the study by xanthine oxidase method.

Results: Toxicity of lead caused decrease in the SOD in group II. Supplementation of walnut along with lead showed Increase in SOD levels in group III (p value ≤0.001) as compared to group II.

Conclusion: This study concludes that antioxidants present in walnuts decrease lead induced lipid peroxidation by increasing the levels of antioxidant enzyme SOD.

Key Words: Antioxidants, Lead Toxicity, Lipid peroxidation, Superoxide Dismutase, Walnuts

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INTRODUCTION

The pollution caused by heavy metals is considered as a major problem worldwide.[1] 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.[2]

The unsupervised use of heavy metals like lead in Pakistan is a serious threat to the human health. Toxicity of lead interferes with variety of biochemical processes, rather than disrupting a single mechanism.[3] Lead mine workers, plumbers, workers at the glass manufacturing industry, cosmetic workers and battery workers are at a greater risk of lead exposure as an occupational hazard. Around 2.5 million tons of lead is being produced per annum around the world. Chronic exposure of lead leads to its bio deposition in vital organs and it is measured as per gram weight of the tissue.[4] The proposed underlying mechanism responsible for the lead toxicity is oxidative stress which is caused by two dissident mechanisms yet parallel to each other. The first mechanism implicates the production of reactive oxygen species (ROS) whereas the other mechanism exhausts the natural antioxidant pools of the cell. These two mechanisms are associated in such a way that high levels of reactive oxygen species on one hand and the consumption of antioxidant pools on the other result in the cellular injury.[5] As lead is capable of inducing the production of ROS and also intrudes the natural antioxidant pool of humans, this property of lead results in its intense binding to thiol groups which leads to deterioration of function of antioxidant enzyme like superoxide dismutase, catalase or glutathione peroxidase.[6]

The intrinsic pool of antioxidants ascertains the vulnerability of a cell towards oxidative damage and defends the tissues against detrimental effects of reactive species at cellular level but not at plasma level.[7] The fundamental antioxidant enzymes of the body include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Superoxide dismutase is basically an antioxidant enzyme present in

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the body which plays important role in the natural defence process majorly by blocking the induction of free radical chain reactions leading to synthesis of free radicals.[8] SOD belongs to the group of metal containing enzymes which causes the disposal of superoxide anion by catalysing it to hydrogen peroxide. It is one of the endogenous antioxidant enzymes which protects against oxidative stress and has been used as an oxidative stress marker in lead poisoned individual in some recent studies.[9]

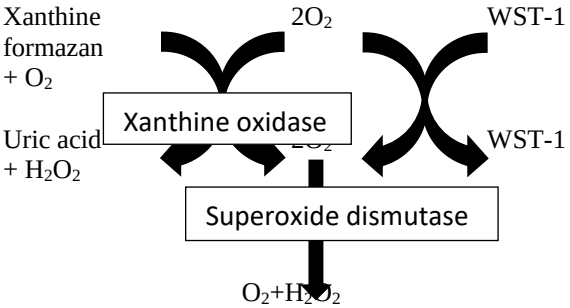
It has been observed that utilization of natural resources has increased in recent years. Among the natural antioxidants phenolic compounds are obtaining significant attention because of their advantages to human health.[10] Walnut are abundant in phenolic compounds and have beneficial role in retrenchment of oxidative stress and termination of macromolecular oxidation. Walnuts vary from all other nuts by a high content of α-linolenic acid, a vegetable n-3 fatty acid, and they also exhibit a distinct combination of nutrients and many phytochemical species like phenolic acids, flavonoids, tannins.[11] Walnut has been used in several studies as an antioxidant and its antioxidant properties are well known. It has been used as anti-neoplastic and anti-inflammatory agent in different studies.

Lead as a biological toxin has been studied with several natural antioxidants. The rationale of this study was to assess the antioxidant role of walnut on SOD decreased by lead toxicity. This study was conducted to investigate the effect of lead on SOD as a marker of oxidative stress in the tissues of mice, along with the possible valuable role of walnut in reducing the lead toxicity in the mice kernels were obtained.

MATERIALS AND METHODS

After the approval of Institutional Review Board (IRB) Isra University Islamabad; a Quasi experimental study was conducted in Department of Biochemistry, Al Nafees Medical College & hospital, Islamabad, Pakistan in collaboration with National Institute of Health Islamabad. The laboratory tests were conducted at the Multi-disciplinary laboratory 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 National Institute of Health (NIH) Islamabad. These animals were bred at the NIH and were used in the experiment. Adult healthy mice 60 days old weighing 50gms±20gms of either gender were included in study. 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 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. Mice were randomized into three groups Group I, Group II, Group III by convenience sampling technique. Each group contained 20 mice. 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.[12] 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. Group III was treated with standard mice chow, lead acetate 30mg/kg bod-y weight in drinking water. Along with Lead acetate, group III was treated with whole walnut kernels 111 g/kg diet.[13] All the samples were taken at the end of the study by intracardiac puncture. The antioxidant capacity of Super Oxide Dismutase was calculated by Super Oxide Dismutase assay kit purchased from Abcam. This kit utilizes water soluble tetrazolum-1 (WST-1) salt by xanthine oxidase and produces water soluble formazan dye (WST-1 formazan)[14]. In this assay superoxide dismutase reduces superoxide anion and has a linear relationship with the activity of xanthine oxidase. The SOD assay measures all three types of SOD (Cu/Zn, Mn, and Fe-SOD) and provides a simple, reproducible and fast tool for assaying SOD activity in serum, plasma, erythrocyte lysates, tissues etc.



All the solutions were mixed and were incubate at 37°C for 20 minutes. Absorbance was calculated for each well at 450nm. SOD percent inhibition of each sample was measured with the help of formula:

Inhibition rate % = (A blank1 – A blank3) – (A sample – A blank2) X 100
(Ablank1 – Ablank3)

Statistical Analysis: The data obtained was analysed on SPSS version 20. Descriptive studies were done. One-way ANOVA was applied followed by Post Hoc Tukey's test for multiple comparisons. Difference in mean among the control and treated groups was calculated by independent sample 't test' for two group comparisons. The difference was considered significant if p value was found ≤ 0.05.

RESULTS

We exposed group II with Lead for two months; it resulted in the significant decrease in the activity of serum superoxide dismutase when it was compared with control group Table- I. This table shows that the Serum Superoxide dismutase activity in control group was 55.53±3.84 whereas in group II it was 25.96±3.56; it showed significant decline (p value < 0.01) revealing that lead has significantly compromised the antioxidant defence enzyme in the serum of mice. Supplementation of walnuts for two months along with lead in group III was able to protect against the lipid peroxidation. This was seen as significantly increasing activity of antioxidant enzyme superoxide dismutase. Table – 2. This table shows that the serum superoxide dismutase activity of group III was 71.24±4.30 whereas in group II it was 25.96±3.56 (p value < 0.01).

Table No.1: Effect of two-month supplementation of lead acetate on serum superoxide dismutase levels in mice.

Parameter	Group I Control (n=20) Mean±SD	Group II Lead acetate (n=20) Mean±SD	p value*
Serum Superoxide dismutase % inhibition	55.53±3.84	25.96±3.56	< 0.001

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

Table No.2: Effects of two-month supplementation of lead acetate and walnuts on serum superoxide dismutase activity in mice.

Parameter	Group II Lead acetate (n=20) Mean±SD	Group III walnut (n=20) Mean±SD	p value*
Serum Superoxide dismutase % inhibition	25.96±3.56	71.24±4.30	< 0.001

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

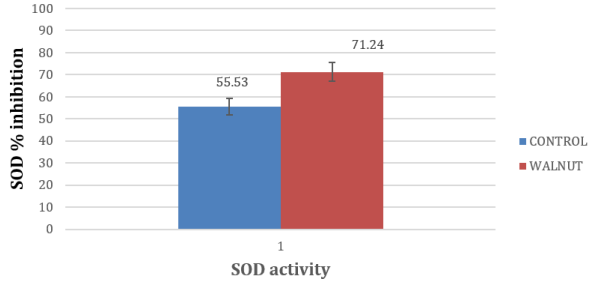


Figure No.1: Effect of two-month administration of lead acetate along with walnut on superoxide dismutase activity in mice. (p value < 0.001)**

When the superoxide dismutase activity of control group (55.53±3.84) was compared with group III (71.24±4.30) the results were highly significant (p value < 0.01) as shown in Fig – 1. This figure shows that the walnuts not only prevent the oxidative stress but also enhance the activity of serum SOD (p value < 0.01).

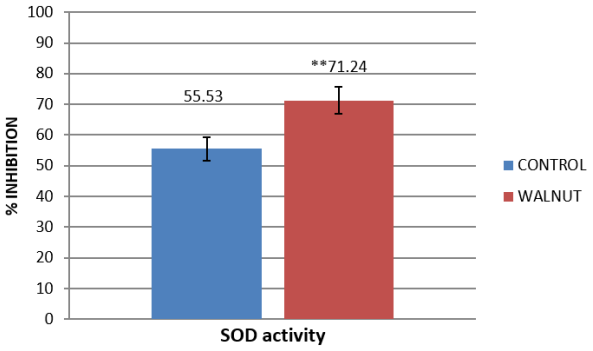


Figure No.2: Effect of two-month supplementation of walnut on superoxide dismutase activity in mice. (p value < 0.001)**

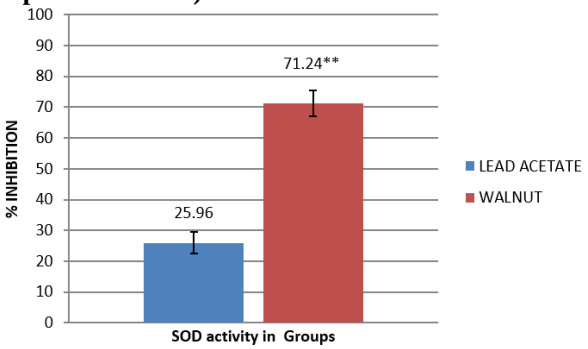


Figure No.3: Effect of lead acetate and walnut on superoxide dismutase activity after two months in mice. (p value < 0.001)**

DISCUSSION

Lead is a heavy metal and one of the ubiquitous pollutants. It is being extensively used in different industries and it induces extensive damage to human population. Despite of being noxious metal 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. Group I was given normal mice chow and distilled water for two months. Enzymatic antioxidant defence system contains SOD, a metal containing enzyme, which is one of the significant antioxidant enzyme causing dismutation of superoxide anion and halts the damage caused by this highly reactive molecule. Decrease in the levels of SOD is associated with oxidative stress. Our data of the control group generated the SOD levels in the range of 55.53±3.84. These results of the control group were in accordance with another study conducted on albino

mice.[15] Another study conducted on wistar rats also showed results similar to the results of control group of our study.[16]

In our experimental design, we exposed group II with lead acetate in drinking water. In recent few years, research studies have suggested that lead poisoning is a major concern for human population and consequences into oxidative stress. Lead is a heavy metal which binds to the thiol groups of proteins and enzymes and inhibits their activity. To check the antioxidant status of this group SOD activity was measured in the serum. SOD is one of the major enzymatic antioxidants which removes toxic substances and achieves this role with the help of its antioxidant enzymatic function. One of the studies used wistar rats to study lead toxicity. In this study rats were exposed to different heavy metals including lead acetate. After 30days the results showed significant decrease in SOD.[17] A similar study conducted on male wistar rats where they were treated with another heavy metal, cadmium for 21 days also shown marked decrease in SOD.[18]

Lead poisoning was also studied in Japanese Quails in 2017 by Abo Ismail et al. In this study birds were introduced with lead acetate in drinking water for one month. After one month of intake lead was able to attenuate all the antioxidants including SOD when compared with control group similar to the results of our study. A similar study conducted on male albino rats, showed marked decrease in SOD when they were exposed to lead for 4 weeks.[19]

Lead causes enhanced lipid peroxidation and it may cause deleterious effects to a living organism. To ensure the security of food and public health a study was conducted on cauliflower in which the seeds were germinated under lead acetate stress for 12 days and the results of this study were contrary to the results of our study The results showed increase in the activity of SOD after lead exposure in the seedlings. This contradiction in the results could possibly be due to short duration of exposure in the study mentioned above.[20]

In contrary to the results of our group II, another study was reported on human subjects by Dobrakowski M. et al in 2017. 36 male were included subjects in this study who were occupationally exposed to lead for 12 hours daily for 36 to 44 days. His results showed no decrease in SOD after lead exposure and were contrary to our results which suggested that lead causes significant decrease in SOD[21]

To investigate the beneficial role of walnut against lead toxicity, at the end of the study SOD estimation was done. The results showed significant increase in the activity of SOD when compared to control group and lead acetate group which supported our hypothesis that walnuts have antioxidant properties.

Walnuts are used as antioxidants in several studies; one such study was reported on pregnant rats. In this study pregnant diabetic rats were given walnut oil in different doses for 12 days and walnut oil was able to prevent hepatotoxicity and caused increase in SOD.[22] The antioxidant role of walnut was studied in another study conducted on rats in which oxidative stress was induced by scopolamine. Administration of walnut for 28 days was able to increase the SOD levels in rats similar to our results.[23]

Walnuts have phytochemicals which prevent oxidative stress and enhance the antioxidant status. In another study conducted on wistar albino female rats, studied the effects of walnut against oxidative stress produced by alcohol on lungs and muscle tissues. Supplementation of walnut for 50 days was able to combat oxidative stress and resulted rise in SOD.[24]

CONCLUSION

This study concludes that antioxidants present in walnuts decrease lead induced lipid peroxidation by increasing the levels of antioxidant enzyme SOD.

Author's Contribution:

Concept & Design of Study:	Roomisa Anis
Drafting:	Ayesha Shafqat, Misbah Batool
Data Analysis:	Beenish Zafar, Naveeda Zaigham , Shahzadi Ambreen
Revisiting Critically:	Roomisa Anis, Ayesha Shafqat
Final Approval of version:	Roomisa Anis

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Kobielska PA, Howarth AJ, Farha OK, Nayak S. Metal–organic frameworks for heavy metal removal from water. Coordination Chemistry Reviews. 2018;358:92-107.
2. Kumar, Amit, et al. Lead Toxicity: Health Hazards, Influence on Food Chain, and Sustainable Remediation Approaches. Int J Environ Res Publ Health 2020;17(7):2179.
3. Ahmad I, Khan B, Khan S, Khan MT, Schwab AP. Assessment of lead exposure among automobile technicians in Khyber Pakhtunkhwa, Pakistan. Science of the Total Environment 2018;633:293-9.
4. Boskabady M, Marefati N, Farkhondeh T, Shakeri F, Farshbaf A, Boskabady MH. The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. Environ Int 2018;120:404–420.
5. Pawlas N, Dobrakowski M, Kasperczyk A, Kozłowska A, Mikołajczyk A, Kasperczyk S. The level of selenium and oxidative stress in workers

chronically exposed to lead. Biological trace Element Res 2016;170(1):1-8.
6. Sies H, Berndt C, Jones DP. Oxidative Stress. Annual Review of Biochem 2017;86(1):715–748.
7. Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. Frontiers in Pharmacol 2018;9:1162.
8. Surai PF, Kochish II, Fisinin VI, Kidd MT. Antioxidant defence systems and oxidative stress in poultry biology: An update. Antioxidants 2019; 8(7):235.
9. Lopes AC, Peixe TS, Mesas AE, Paoliello MM. Lead exposure and oxidative stress: a systematic review. In Reviews of Environmental Contamination and Toxicol 2016;236:193-238.
10. 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 Molecular Sci 2017;18(1):96.
11. Sánchez-González C, Ciudad CJ, Noe V, Izquierdo-Pulido M. Health benefits of walnut polyphenols: An exploration beyond their lipid profile. Crit Rev Food Sci Nutr 2017;57(16): 3373-3383.
12. Anis R, Kausar MW, Zaigham N, Haq MBU, Humerah S, Zafar B. Antioxidant effects of walnuts on malondialdehyde levels raised by lead toxicity in mice. Isra Med J 2019;11(6): 444-448.
13. 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.
14. Peskin AV, Winterbourn CC. Assay of superoxide dismutase activity in a plate assay using WST-1. Free Radical Biol Med 2017;103:188-91
15. Desai SD, Shaik Hussain Saheb, Kusal K Das, Haseena S. Effect of Nigella Sativa Seed Powder on MDA and SOD levels in Sterptozotocine Induced Diabetis Albino Rats. J Pharm Sci Res 2015;7(4):206-9.
16. Soussi A, Gargouri M, Akrouti A, El Feki A. Antioxidant and nephro-protective effect of Juglans regia vegetable oil against lead-induced nephrotoxicity in rats and its characterization by GC-MS. EXCLI J 2018;17:492.
17. Riaz MA, Nisa ZU, Mehmood A, Anjum MS, Shahzad K. Metal-induced nephrotoxicity to diabetic and non-diabetic Wistar rats. Environ Sci Pollution Res 2019;26(30):31111-8.
18. Cadmium Induced Oxidative Stress in Wistar Rats: Ameliorative Effect of Quercetin and Embilica Officinalis Plant Extracts AJVS 2017;54(2):13-18.
19. Ameliorative effect of curcumin against lead acetate–induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. Environmental Science and Pollution Research 2020;27:10950–10965.
20. Germination and Seedling Growth of Cauliflower Under Lead Stress and Its Antioxidant Role. J Plant Growth Regul 2018;37:5–15.
21. Dobrakowski M, Boroń M, Birkner E, Kasperczyk A, Chwalińska E, Lisowska G, et al. The effect of a short-term exposure to lead on the levels of essential metal ions, selected proteins related to them, and oxidative stress parameters in humans. Oxidative Med Cellular longevity 2017.
22. 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.
23. Haider S, Batool Z, Ahmad S, Siddiqui RA, Haleem DJ. Walnut supplementation reverses the scopolamine-induced memory impairment by restoration of cholinergic function via mitigating oxidative stress in rats: a potential therapeutic intervention for age related neurodegenerative disorders. Metabolic brain disease 2018;33(1): 39-51.
24. 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.