P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2024.126

Effectiveness of Resveratrol Supplement on Some Biochemical Criteria and Bone in Lead-Exposed Rats

Duaa Abd-Alaziz Subhi* and Baraa Najim Al-Okaily

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.
***Corresponding author:** aldorydua.a@gmail.com

Article History: 23-294 Received: 10-Sep-23 Revised: 01-Oct-23 Accepted: 14-Oct-23

ABSTRACT

The present study was designed to investigate the effectiveness of resveratrol (3,5,4′-trihydroxy-trans-stilbene) supplements in alleviating the histopathological and some biochemical criteria alterations in adult female rats exposed to lead acetate. Twenty adult female Wistar rats were divided equally into four groups; Group C served as control, group G1 was gavage lead acetate (5mg/kg BW) orally, group G2 administered lead acetate (5mg/kg BW) and resveratrol supplement at dose 87mg/kg BW and group G3 received the dosage of resveratrol supplement orally as in G2. Blood samples were collected immediately before and after 28 days of the experiment to determine serum calcium ion and phosphorus concentrations. Furthermore, specimens of femur bone were taken hold at the end of the treatment. Results of serum calcium (Ca^{+2}) and phosphorus showed a significant decrease in group G1 compared to other groups. Also, there were histopathological alterations in the bone sections characterized by a reduction of the cellular component of bone marrow tissue, visible empty lacunae, scattered osteocytes, and irregular roughened periosteum with increased in collagenous in the bony matrix compared to the control, G2, and G3 groups. Besides, a decrease in these parameters in group G2 was noticed compared to group G1. In conclusion, the results confirmed the effectiveness of resveratrol supplements in alleviating the adverse effects of lead acetate on calcium, and phosphorus and attenuating the histopathological changes of bone due to its antioxidant activities in rats.

Key words: Calcium, Femur, Histopathology, Lead acetate, Resveratrol.

INTRODUCTION

Lead (Pb) is one of the common harmful heavy metals in environmental and industrial pollution, it accumulates in soil, water, and air (Mitra et al. 2022). In some areas of Iraq, the percentage of lead was higher than the allowed range (Al-Kasser 2021; Al-Sareji et al. 2021). Nowadays almost everyone is exposed to lead in urban environments and in industrial places through inhalation (air), car exhaust gases, ingestion, water drinking, soil, dust, and dermal absorption (WHO 2011). World Health Organization recommended that the standard content of lead in water is 10µg/L (WHO 2011). Occupational and environmental exposure to lead has increased by several folds resulting in the wide range and huge volume of this element in industries, folk remedies, cosmetics, pharmacology, and nanomedicine (Ahmed and Siddiqui 2007; Ahmed and Mohammed 2022). As well as lead circulates in the peripheral bloodstream and accumulates in vital tissues such as the liver, pancreas, lungs and kidneys, or an amount of them is excreted as waste to the environment (as a pollutant). A high proportion of absorbed lead transfers to hard tissues of the body, especially in the skeletal system (bone), where it accumulates over time and remains for long periods (IHME 2017; WHO 2011). Lead toxicity affects living organisms through alterations of physiological, biochemical, and morphological modifications (Chowdhury et al. 2021; Ramírez Ortega et al. 2021; Younas et al. 2023). In physiological terms, lead is a toxic element even in few amounts and low concentrations (Levine et al. 1989). One major effect of accumulation of lead in the cells is attributed to increasing generation of reactive oxygen species (ROS) and enhancement of lipid peroxidation of the cell membrane, mitochondrial dysfunction, DNA damage, apoptosis with depletion of antioxidant defense systems of cells (Fan et al. 2020; AL-Okaily and Murad 2021; Ghazi and Al-Qaiym 2023). Furthermore, lead-induced alteration in gene expression patterns of neurotransmitters such as serotonin and dopamine (Hernández-Coro et al. 2021), cause vitamin D deficiency by disrupting the conversion of vitamin D to its biological active (Rahman et al. 2018), attacks the brain and central nervous system causing mental disorders and coma (Nawfal and Al-Okaily 2023).

Cite This Article as: Subhi DA-A and Al-Okaily BNM, 2024. Effectiveness of resveratrol supplement on some biochemical criteria and bone in lead-exposed rats. International Journal of Veterinary Science 13(4): 421-426. https://doi.org/10.47278/journal.ijvs/2024.126

Clinical signs of lead toxicity appear in domestic animals, including signs of depression and circular walking, blindness, teeth grinding, convulsive seizures, anemia, and diarrhea (Loos Scarth 2006).

Resveratrol (phytoalexin) is a phenolic compound present in wine and black grape and it has various beneficial health effects (Murad and Al-Okaily 2020), due to its antioxidant effects through the scavenger for removing ROS which depends on the arrangement of functional and bioactive groups in the nuclear structure, metal ion chelating abilities or its ability to decrease oxidation by enzyme inhibition mechanisms (Belguendouz et al. 1997). Resveratrol has various biological and pharmacological activities and its classification as a good antioxidant, anti-hyperlipidemia, anti-inflammatory, immunomodulatory, cardio-protective, anti-diabetic, and anti-carcinogenic effects (Malhotra et al. 2015; Abdulla and Al-Okaily 2022).

Therefore, the current experiment tends to explain the deleterious effects of lead acetate on some blood biochemical criteria and bone, as well as to assess the effectiveness of resveratrol supplements in ameliorating these injuries. The present study aimed to propose using an alternative antioxidant component (resveratrol) for metal toxicity in animal bodies.

MATERIALS AND METHODS

Ethical approval was granted through the local committee of animal care and use at the College of Veterinary Medicine within the University of Baghdad (Number 1507/PG) before starting this study.

Animals and Experimental Design

The current experiment has been subjected to 20 mature-healthy female Wister albino rats, aged 12-13 weeks, with an average weight of 185±15gm. Rats were housed in the College of Veterinary Medicine, University of Baghdad in plastic cages (5/cage) in a well-ventilated room and received an oral pellet diet with fulfilled requirements for rats (according to the laboratory rodent diet 5001) and drinking tap water *ad libitum* with the light cycle from 7 am to 7 pm along the experiment period at the temperature of 22-25°C and 40±5% relative humidity. The cages were cleaned weekly, and the rats were acclimatized for two weeks. After that, the 20 rats were randomly divided into four equal groups and treated daily for 28 days as detailed in Table 1. Lead acetate dose was followed as mentioned by Murad and Al-Okaily (2020) and the Resveratrol supplement dose was mentioned by Khudair and Al-Okaily (2022).

Collection of Blood Samples and Specimens of Bone

Blood samples were collected from anesthetized rats after intramuscular injections of ketamine (60mg/kg Duopharma; Malaysia) and xylazine (40mg/kg VMD; Belgium) at the baseline (day zero) and 28 days later using the heart puncture. Then serum samples were separated by centrifuging at 2500rpm for 15min for measurement of calcium and phosphorus concentration using a kit (Inorganic colorimetric method) (Liner Chemicals-Spain). Furthermore, the representative specimens of 2cm were cut from the femur bone of each rat. Specimens were fixed in neutral buffered formalin 10% for 72h. After fixation, samples of bone were decalcified with 5% nitric acid for 48h (the nitric acid solution was changed twice daily). After that all samples of bone were washed with running tap water for 30min and processed by routine histological processing method then specimens were embedded with paraffin wax to obtain blocks of paraffin. Paraffin sections were cut 4-5µm by using a rotary microtome (Histo-line company, Italy) and the paraffin sections were stained with hematoxylin and eosin (Bacha and Bacha 2012).

Statistical Analysis

The resulting data were analyzed using ANOVA two-way analysis of variance using SPSS (version 15), followed by the least significant difference (LSD) test. $P<0.05$ is considered statistically significant (Snedecor and Cochran 1973).

RESULTS

Table 2 illustrates that serum concentrations of calcium and phosphorus were significantly ($P<0.05$) reduced in lead acetate-treated rats compared with other groups. The results of the mixture-treated group (G2) showed a significant ($P<0.05$) increase in these parameters comparable to lead acetate-treated rats. A non-significantly ($P>0.05$) difference between group G3 and the control was noticed after 28 days of the experiment.

Bone sections of the control group show normal bone architecture morphology with several Haversian systems and many osteocytes (Fig. 1A). Compared to the control, sections of the Pb-acetate-treated group manifested bone histological changes characterized by a reduction of the cellular component of bone marrow tissue, visible empty lacunae and unmineralized newly formed bone with scattered osteocytes (Fig. 1B and C), other findings showed immature bone trabecular with moderate fatty replacement and irregular roughened periosteum with increased in collagenous in the bony matrix (Fig. 1D and 2A), additionally, osteoblast detachment from a bone surface with immature osteoblast was noticed. Lead acetate + resveratrol group (G2) pointed to moderate osteoblastic activity with the prominence of normal components of marrow tissues (Fig. 2B) and increased density in the periosteum (Fig. 2C) compared to G1. While sections of bone of group G3 revealed normal cellular components of bone matrix and marrow tissue with a complete surface lining of trabecular bone (Fig. 2C). In Fig. 2D, tissue with the complete surface lining of trabecular bone is observed.

DISCUSSION

A significant decrease in serum calcium and phosphorus concentrations in rats who received lead acetate was reported. These results were confirmed previously (Batra et al. 2020). It's known that bone and kidneys are the principal target organs for Pb toxicity (Sanín et al. 1998; Satarug et al. 2020). Lead interferes with Ca^{+2} in different metabolic pathways (Rădulescu and Lundgren 2019), consequently leading to dysregulation of calcium homeostasis accompanied by many disorders and functional disruptions (Upadhyay et al. 2022; Nawfal and Al-Okaily 2023). Besides, alteration of Ca+2 homeostasis

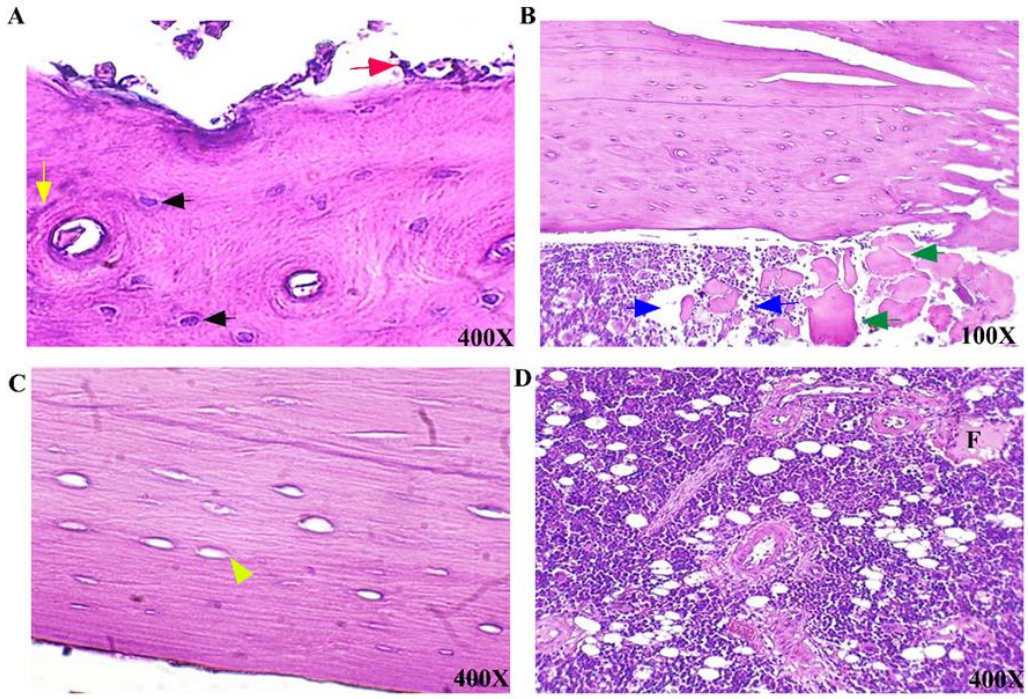

Fig. 1: A) Bone section of control (-ve) showing several haversian system and number of osteocytes seen in bone matrix (black arrows) separated by irregular collagen fibers with basophilic cement lines (yellow arrow) and active osteoblast surface lining (red arrow), B) Bone section of lead acetate group showing moderate reduction of cellular component of marrow tissue (blue arrows) accompanied with numerous bony sequestrate (green arrows), C) Bone section of lead acetate treated group showing visible of empty lacunae and absence of haversian canal (yellow arrowhead), and D) Bone section of lead acetate treated group (G1) showing vascular sinusoid with edematous fluid (F) finding and immature bone trabeculae with moderated fatty replacement of bone marrow. Stain H and E.

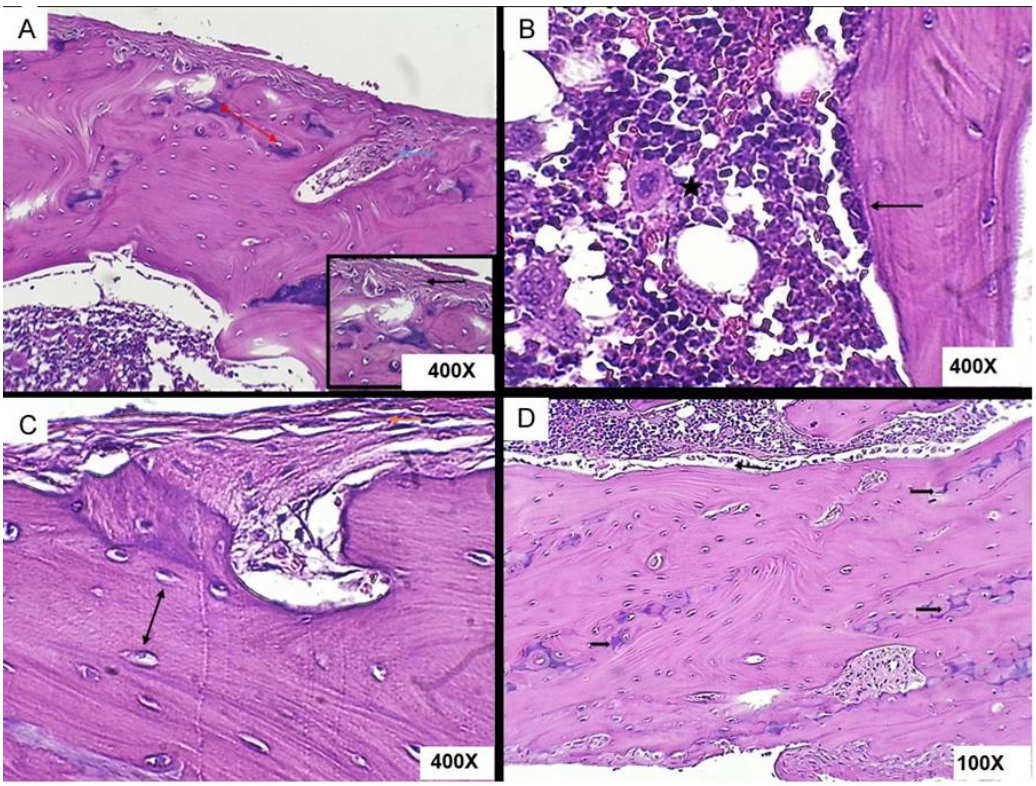

Fig. 2: A) Bone section of lead acetate treated group (G1) showing the appeared irregular roughened periosteum with moderate increased in collagenous fibers with few numbers of basophilic osteoblast (black arrow) that extend in bony cortex (blue arrow) with incomplete calcification (red arrow), B) Bone section of treated group with lead and resveratrol (G2) showing moderate osteoblastic activity lining of bone trabeculae (black arrow) with prominence normal component of marrow tissue (black star), C) Bone section of treated group with lead and resveratrol (G2) showing Thick lamellar bone containing numerous osteocyte (black arrow) with increase collagen density in the periosteum (red arrow), and D) Bone section of resveratrol treatment group (G3) showing complete endosteum lining by active osteoblast with normal cellular component of Marrow tissue (black arrow) with multiple incomplete mineralization areas (head arrow). H&E stain.

Table 1: Experimental protocol of the study

Group	Oral Treatments (mg/kg BW)	
	Lead acetate	Resveratrol supplement
Control	-	-
1	5	-
2	5	87
3	-	87

Table 2: Serum concentrations of calcium and phosphorus (mg/dL) in lead acetate-treated rats (before and after treatment).

Groups	Calcium		Phosphorus	
	Periods		Periods	
	Before	After	Before	After
Control	10.60 ± 0.19^{Aa}	10.76 ± 0.22^{Aa}	6.38 ± 0.06^{Aa}	6.28 ± 0.02^{Aab}
G1	10.90 ± 0.16^{Aa}	8.55 ± 0.16^{Bc}	6.28 ± 0.02^{Aa}	5.41 ± 0.22^{Bc}
G2	10.67 ± 0.16^{Aa}	10.20 ± 0.11^{Ab}	6.34 ± 0.02^{Aa}	6.03 ± 0.06^{Bb}
G3	10.68 ± 0.19^{Aa}	10.82 ± 0.11^{Aa}	6.33 ± 0.02^{Aa}	6.35 ± 0.08^{Aa}

Data represented as mean±SE. ^{AB} Capital letter denot a significant difference ($P<0.05$) between experimental groups (Row). ^{abc} Small letter denot a significant difference ($P<0.05$) within groups (within time) (Column).

causes disability of erythropoietin synthesis (Graziano et al. 1991), impairment of calcitriol (1, 25-DHCC) with disruption of parathyroid hormone (PTH), consequently has a more direct effect on calcium homeostasis (Dongre et al. 2013). Calcitriol has an effective role in maintaining homeostasis of calcium and phosphorus metabolism, as well as, facilitating the absorption of calcium and phosphorus in renal tubular cells (Kristal-Bouneh et al. 1998). Erythropoietin causes an increase in intracellular calcium (iCa^{+2}) concentration in erythroid progenitor cells which is attributed to the proliferation and differentiation of these cells (Zhang et al. 2022). It was pointed out that exposure to Pb caused renal tubular damage characterized by a significant excretion of Ca^{+2} (Navarro-Moreno et al. 2009).

It was also found that high circulating levels of fibroblast growth factor 23 (FGF23) are associated with high disturbances in the regulation of phosphate, vitamin D metabolism and calcium homeostasis accompanied with elevated PTH levels likely, also contribute to increase renal phosphate excretion by decreasing the expression of the renal sodium-phosphate cotransporters in the proximal tubules (Rausch and Föller 2022). So, it can be suggested that hypophosphatemia in group G1 might be due to Pb causing an increase in FGF23 production. Thus, further investigation in the future is essential to the measurement of FGF23 as a prognostic marker in lead-exposed animals. Also, lead causes changes in the expression of genes involved in calcium homeostasis related to induced oxidative stress, apoptotic, and cell death (Upadhyay et al. 2022).

In the present study, the protective effect of resveratrol on rats exposed to lead acetate was studied for the first time. Current results confirmed an improvement in serum Ca^{+2} and phosphorus concentrations in rats of group G2 compared to group G1 may be via the role of resveratrol in reducing the production of FGF23 in Pb-treated rats (Zhang et al. 2017). Zhang et al. (2020) the antioxidant properties of resveratrol or pterostilbene supplements in alleviating the damage of villus morphology and epithelial cell survivals in the jejunum of oxidative stressed young weaning piglets due to its ability to facilitate nuclear factor erythroid 2-related factor 2 signals which consequently preserves the permeability of the small intestine. This data indicates that resveratrol supplements could be a promising anti-stress supplement for the improvement of redox status (Khayoon and Al-Rekab 2020; Abdulla and Al-Okaily 2022), as well as, attenuate oxidative stress by oxalate and decreasing calcium oxalate deposition in rat kidney by inhibiting the expression of the osteogenic proteins BMP2 and OPN (Wu et al. 2021) and improve renal tubular epithelial cells (Khudair and Al-Okaily 2022). Current results documented the therapeutic importance of resveratrol in ameliorating and maintaining calcium and phosphorus homeostasis through its role in reducing the production of FGF23 in Pb-treated rats.

Results denote a marked histopathological change in the bone of rats administered lead-acetate compared to control. These results were consistent with the results of serum Ca^{+2} and phosphate concentrations in the G1 group which indicate the presence of inflammation and/or alterations of the redox state. These results are consistent with the findings of others (Jiang et al. 2021). Lead exposure could affect and suppress bone growth and development of osteoporosis (Shukla et al. 1989). A decrement in serum calcium with increased Pb in blood with scattered osteocytes, immature bone trabecular, and decreased bone mineralization is considered the main factor of osteoporosis (Alvarez-Lloret et al. 2014). Also causes the degeneration of many bone-specific proteins accompanied by apoptosis of osteoblasts leading to change in the bone microenvironment (Ma et al. 2012). Besides, lead exposure causes an increase in both P1NP and CTX which causes a decrease of the mineral/matrix ratio at the periosteum (Monir et al. 2010) and an increased collagen matrix production.

Resveratrol could have the ability in bone remodeling by reducing bone resorption via increased C-terminal telopeptides type I collagen. Some studies confirm that resveratrol could abolish bone loss in osteoporotic mice-via significantly inhibited osteoclastogenesis and reversed reducing Runx2, OCN, and type I collagen, up-regulated the level of Forkhead box protein O1 (FOXO1) and maintained the redox equilibrium in the mice (Zhao et al. 2015; Wang et al. 2017). and ameliorates the progression of osteoarthritis through inhibits the abnormal proliferation of blood vessels by downregulating the expression of vascular endothelial growth factor (VEGFA) and Angiopoiein-1 and inhibits osteoclast differentiation and reduces active bone resorption by regulating the OPG/RANKL/RANK pathway (Xiong et al. 2021). Also, resveratrol reduces osteoporosis by inhibiting osteocyte apoptosis and encouraging autophagy via AMPK/JNK1 activation in oxidative stress rats (Wei et al. 2023). Osteoporosis cause inhibition of sirtuin1 expression (one of the important roles in bone metabolism), while treatment with resveratrol cause activation the expression of this protein in osteoporotic rats (Lee et al. 2016; Wang et al. 2017).

Conclusion

Current results documented the therapeutic importance of resveratrol in ameliorating and maintaining calcium and phosphorus homeostasis and bone remodeling in rats exposed to lead may be through its role in reducing the production of FGF23 in Pb-treated rats and increased expression of some protein related to osteoporosis. The experimental results strongly indicate the protective effect

of resveratrol supplements against the toxic effect of lead on bone tissue homeostasis.

Acknowledgments

The authors would like to express their sincere thanks to the College of Veterinary Medicine, University of Baghdad for their assistance in conducting this research.

Author's Contribution

The researchers' effort was equal in accomplishing this task.

Conflict of Interest

There is no conflict of interest.

REFERENCES

Abdulla JM and Al-Okaily BN, 2022. Histomorphometric and histopathological alterations of rat testis following exposure to hydrogen peroxide: protective role of resveratrol supplement. The Journal of Veterinary Medicine 46(1): 17-23. https://doi.org/10.30539/ijvm.v46i1.1313

Ahmed M and Siddiqui MK, 2007. Low level lead exposure and oxidative stress: Current opinions. Clinica Chimica Acta 283: 57–64 https://doi.org/10.1016/j.cca.2007.04.024

Ahmed RM and Mohammed AK, 2022. Role of sodium butyrate supplement on reducing hepatotoxicity induced by lead acetate in rats. The Iraqi Journal of Veterinary Medicine 46(2): 29-35. https://doi.org/10.30539/ijvm.v46i2.1408

Al-Kasser MK, 2021. Air Pollution in Iraq Sources and Effects. In IOP Conference Series: Earth and Environmental Science 790: 012014. https://doi.org/10.1088/1755-1315/790/1/012014

Al-Okaily BN and Murad HF, 2021. Role of alpha lipoic acid in protecting testes of adult rats from lead toxicity. Iraqi Journal of Veterinary Sciences 35(2): 305-312. https://doi.org/10.33899/ijvs.2020.126814.1386

Al-Sareji OJ, Grmasha RA, Salman JM, Idowu I and Hashim KS, 2021. Street dusts contamination by heavy metals in Babylon governorate, Iraq. Journal of Engineering Science and Technology 16(1): 3528-3546.

Alvarez-Lloret P, Rodriguez-Navarro AB and Romanek CS, 2014. Effects of lead shot ingestion on bone mineralization in a population of red-legged partridge (Alectoris rufa). The Science of the Total Environment 466-467: 34–39. https://doi.org/10.1016/j.scitotenv.2013.06.103

Bacha WJ and Bacha LM, 2012. General principles of histology. In: Color Atlas of Veterinary Histology, 3rd Ed. John Wiley & Sons, pp: 60-79.

Batra J, Thakur A, Meena SK, Singh L, Kumar J and Juyal D, 2020. Blood lead levels among the occupationally exposed workers and its effect on calcium and vitamin D metabolism: A case-control study. Journal of Family Medicine and Primary Care 9(5): 2388–2393. https://doi.org/10.4103/jfmpc.jfmpc_271_20

Belguendouz L, Fremont L and Linard A, 1997. Resveratrol inhibits metal ion-dependent and independent peroxidation of porcine low-density lipoproteins. Biochemical Pharmacology 53(9): 1347–1355. https://doi.org/10.1016/s0006-2952(96)00820-9

Chowdhury KIA, Nurunnahar S, Kabir ML, Islam MT, Baker M and Islam MS, 2021. Child lead exposure near abandoned lead acid battery recycling sites in a residential community in Bangladesh: risk factors and the impact of soil remediation onblood lead levels. Environmental Research 194: 110689. https://doi.org/10.1016/j.envres.2020.110689

Dongre NN, Suryakar AN, Patil AJ, Hundekari IA and Devarnavadagi BB, 2013. Biochemical effects of lead exposure on battery manufacture workers with reference to blood pressure, calcium metabolism and bone mineral density. Indian Journal of Clinical Biochemistry 28(1): 65-70. https://doi.org/10.1007/s12291-012-0241-8

Fan Y, Zhao X, Yu J, Xie J, Li C and Liu D, 2020. Lead-induced oxidative damage in rats/mice: a meta-analysis. Journal of Trace Elements in Medicine and Biology 58: 126443. https://doi.org/10.1016/j.jtemb.2019.126443

Ghazi MA and Al-Qaiym MA, 2023. Role of *T. vulgaris* in protection DNA damage induced by lead acetate in Rats. Journal of Biotechnology Research Center 17(1): 55-65. https://doi.org/10.24126/jobrc.2023.17.1.701

Graziano JH, Slavkovic V, Factor-Litvak P, Popovac D, Ahmedi X and Mehmeti A, 1991. Depressed serum erythropoietin in pregnant women with elevated blood lead. Archives of Environmental and Occupational Health 46(6): 347–350. https://doi.org/10.1080/00039896.1991.993440.

Hernández-Coro A, Sánchez-Hernández BE, Montes S, Martínez-Lazcano JC, González-Guevara E and Pérez-Severiano F, 2021. Alterations in gene expression due to chronic lead exposure induce behavioral changes. Neuroscience and Biobehavioral Reviews 126: 361–367. https://doi.org/10.1016/j.neubiorev.2021.03.031

IHME (Institute for Health Metrics and Evaluation), 2017. GBD Compare. University of Washington. pp: 40-88.

Jiang X, Xing X, Zhang Y, Zhan C, Wu Y, Chen Y, Meng R, Jia H, Cheng Y, Zhang Y and Su J, 2021. Lead exposure activates the Nrf2/Keap1 pathway, aggravates oxidative stress, and induces reproductive damage in female mice. Ecotoxicology and Environmental Safety 207: 111231. https://doi.org/10.1016/j.ecoenv.2020.111231.

Khayoon HA and Al-Rekabi FMK, 2020. Cytotoxic Effect of Resveratrol on Colorectal Cancer Cell Line. The Iraqi Journal of Veterinary Medicine 44(1): 68-74. https://doi.org/10.30539/ijvm.v44i1.939

Khudair NT and Al-Okaily BN, 2022. Renal ameliorating effect of resveratrol in hydrogen peroxide induced male rats. Iraqi Journal of Veterinary Sciences 36(3): 571-577. https://doi.org/10.33899/IJVS.2022.130939.1898

Kristal-Bouneh E, Froom P, Yerushalmi N, Harari G and Ribak J, 1998. Calcitropic hormones and occupational lead exposure. American Journal of Epidemiology 147(5): 458–463. https://doi.org/10.1093/oxfordjournals.aje.a009471

Lee JH, Kim JS, Park SY and Lee YJ, 2016. Resveratrol induces human keratinocyte damage via the activation of class III histone deacetylase, Sirt1. Oncology Reports 35(1): 524–529. https://doi.org/10.3892/or.2015.4332

Levine MB, Stall AT, Barrett GW and Taylor DH, 1989. Heavy metal concentrations during ten years of sludge treatment to an old fieldcommunity. Journal of Environmental Quality 18 (4): 411-418. https://doi.org/10.2134/jeq1989.00472425001800040003x

Loos Scarth L, 2006. The Merck Veterinary Manual, 8th Ed, References Reviews 20: 40. Emerald Publishing. https://doi.org/10.1108/09504120610647492

Ma Y, Fu D and Liu Z, 2012. Effect of lead on apoptosis in cultured rat primary osteoblasts. Toxicology and Industrial Health 28(2): 136–146. https://doi.org/10.1177/0748233711407956

Malhotra A, Bath S and Elbarbry F, 2015. An organ system approach to explore the antioxidative, anti-inflammatory, and cytoprotective actions of resveratrol. Oxidative Medicine and Cellular Longevity 2015: 803971. https://doi.org/10.1155/2015/803971

Mitra M, Chakraborty AJ, Tareq AM, Emran TB, Nainu F and Khusro A, 2022. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity, Journal of King Saud University-Science 34: 10865. https://doi.org/10.1016/j.jksus.2022.101865

Monir AU, Gundberg CM, Yagerman SE, van der MC, Budell WC and Boskey A, 2010.The effect of lead on bone mineral properties from female adult C57/BL6 mice. Bone 47(5): 888–894. https://doi.org/10.1016/j.bone.2010.07.013

Murad HF and Al-Okaily BN, 2020. Protective effects of alpha lipoic acid against lead induced oxidative stress and DNA fragmentation to testicular function in rats. Biochemical & Cellular Archives 20(2): 5465-5474.

Navarro-Moreno LG, Quintanar-Escorza MA, Gonzalez S, Mondragon R, Cerbon-Solorzano J, Valdes J and Calderon-Salinas JV, 2009. Effects of lead intoxication on intercellular junctions and biochemical alterations of the renal proximal tubule cells. Toxicology in vitro 23(7): 1298–1304. https://doi.org/10.1016/j.tiv.2009.07.020

Nawfal AJ and Al-Okaily BN, 2023. Neurotransmitters enhancement by zingerone in brain injury-induced by lead acetate in rats (Part-II). Journal of Clinical Otorhinolaryngology, Head, and Neck Surgery 27(1): 658-673.

Rădulescu A and Lundgren SA, 2019. Pharmacokinetic model of lead absorption and calcium competitive dynamics. Scientific Reports 9(1): 14225. https://doi.org/10.1038/s41598-019-50654-7

Rahman A, Al-Awadi AA and Khan KM, 2018. Lead affects vitamin D metabolism in rats. Nutrients 10(3): 264. https://doi.org/10.3390/nu10030264

Ramírez Ortega D, González Esquivel DF, Blanco Ayala T, Pineda B, Gómez Manzo S, Marcial Quino J, Carrillo Mora P and Pérez de la Cruz V, 2021. Cognitive Impairment Induced by lead exposure during Lifespan: mechanisms of lead neurotoxicity. Toxics 9(2): 23. https://doi.org/10.3390/toxics9020023

Rausch S and Föller M, 2022. The regulation of FGF23 under physiological and pathophysiological conditions. European Journal of Physiology 474(3): 281–292. https://doi.org/10.1007/s00424-022-02668-w

Sanín LH, González-Cossío T, Romieu I and Hernández-Avila M, 1998. Accumulation of lead in bone and its effects on health. Salud Publica de Mexico 40(4): 359–368.

Satarug SC, Gobe GA, Vesey D and Phelps KR, 2020. Cadmium and lead exposure, nephrotoxicity and mortality. Toxics 8(4): 86. https://doi.org/10.3390/toxics8040086

Shukla R, Bornschein RL, Dietrich KN, Buncher CR, Berger O and Hammond PB, 1989. Fetal and infant lead exposure: Effects on growth and stature. Pediatrics 84: 604–612. https://doi.org/10.1542/peds.84.4.604

Snedecor GW and Cochran WG, 1973. Statistical Methods. 6th ed. The Iowa State University Press, pp: 238-248.

Upadhyay K, Viramgami A, Bargepole BS and Balachandar R, 2022. Association between blood lead levels and markers of calcium homeostasis: a systematic review and meta-analysis. Scientific Reports 12(1): 1850. https://doi.org/10.1038/s41598-022-05976-4

Wang X, Chen L and Peng W, 2017. Protective effects of resveratrol on osteoporosis via activation of the SIRT1-NF-κB signaling pathway in rats. Experimental and Therapeutic Medicine 14(5): 5032–5038. https://doi.org/10.3892/etm.2017.5147

Wei L, Chai S and Yue C, 2023. Resveratrol protects osteocytes against oxidative stress in ovariectomized rats through AMPK/JNK1-dependent pathway leading to promotion of autophagy and inhibition of apoptosis. Cell Death Discovery 9(1): 16. https://doi.org/10.1038/s41420-023-01331-2

WHO, 2011. Lead in Drinking-water Background document for development of WHO Guidelines for Drinking -water Quality. WHO/SDE/WSH/03.04/09/REV/1. https://cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/lead-background-feb17.pdf?sfvrsn=fc50727b_4

Wu Y, Xun Y, Zhang J, Hu H, Qin B, Wang T, Wang S and Li C, 2021. Resveratrol attenuates oxalate-induced renal oxidative injury and calcium oxalate crystal deposition by regulating tfeb-induced autophagy pathway. Frontiers in Cell and Developmental Biology 9: 638759. https://doi.org/10.3389/fcell.2021.638759

Xiong G, Yang Y and Guo M, 2021. Effect of resveratrol on abnormal bone remodeling and angiogenesis of subchondral bone in osteoarthritis. International Journal of Clinical and Experimental Pathology 14(4): 417–425.

Younas S, Tahira SA and Farooq U, 2023. Assessment of heavy metal contamination in vegetables collected from selected localities of Okara, Pakistan. Advancements in Life Sciences 10(2):167-173.

Zhang X, Yang Z, Li L, Qiao Y, Jiao H and Miao C, 2017. Prevention of injury by resveratrol in a rat model of adenine-induced chronic kidney disease. Tropical Journal of Pharmaceutical Research 16(8):2027-2032. https://doi.org/10.4314/tjpr.v16i8.37

Zhang H, Chen Y, Chen Y, Ji S, Jia P, Li Y and Wang T, 2020. Comparison of the protective effects of resveratrol and pterostilbene against intestinal damage and redox imbalance in weanling piglets. Journal of Animal Science and Biotechnology 11: 52. https://doi.org/10.1186/s40104-020-00460-3

Zhang Y, Xu Y, Zhang S, Lu Z, Li Y and Zhao B, 2022. The regulation roles of Ca2+ in erythropoiesis: What have we learned? Experimental Hematology 106: 19–30. https://doi.org/10.1016/j.exphem.2021.12.192

Zhao L, Wang Y, Wang Z, Xu Z, Zhang Q and Yin M, 2015. Effects of dietary resveratrol on excess-iron-induced bone loss via antioxidative character. The Journal of Nutritional Biochemistry 26(11): 1174–1182. https://doi.org/10.1016/j.jnutbio.2015.05.009