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Improvement of Lead Acetate-Induced Testicular Oxidative Damage by *Vitis Vinifera* (Linn.) Seed Extract In Adult Wistar Rats

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Abstract

Introduction: Lead is the most ubiquitous hazardous toxin in the environment. It's a severe threat to public health and especially to the male reproductive system. In recent years, the usage of the antioxidant to reduce the toxicity of heavy metals has expanded globally. Antioxidants can prevent or minimise the oxidation of other molecules by ROS in a tissue or cell. Grape seeds are natural, rich sources of antioxidant compounds.

Aim/Objective: The present study was undertaken to investigate the effect of GSE (grape seed extract) against lead acetate-induced testicular oxidative damage on testis of Wistar rats.

Materials and Methods: 24 male Wistar rats were used in the study. They were split into two groups: Group I was the control group (6 rats), and Group II had 18 rats that were given LA at 50 mg/kg BW for 28 days. On the 28th day, all 18 rats were subdivided into three groups: Group II(a) (LA Cessation), Group II(b), and Group II(c) treated with GSE once a day, orally, up to the 56th day. A preliminary phytochemical analysis was conducted. The testicular weight, enzymatic and non- enzymatic oxidative stress markers were estimated.

Results: The phytochemical screening showed that bioactive compounds present in the GSE, including phenols, tannins, flavonoids, anthocyanin, glycosides, triterpenoids, and alkaloids. In addition, there was a significant decrease in SOD, CAT, GPx, and GSH levels and a significantly elevated MDA concentration. However, post-treatment with GSE significantly restored the testicular oxidative damage caused by LA in the testis.

Conclusion: We concluded that the GSE may have the potential to provide a promising therapeutic effect against LA-induced testicular toxicity.

Keywords:* *Oxidative damage; Lipid peroxidation Lyophilization; Post-treatment.

Abbreviations: MDA-Malondialdehyde; GSH-Reduced glutathione; ROS-Reactive oxygen species

1. Introduction

Heavy metals are ubiquitous environmental contaminants that occur both naturally and as a result of human activity. In that Lead (Pb) is one of the most dangerous heavy metal environmental pollutants, causing oxidative stress and endangering human health [1]. Globally, industries produce around 2.5 million tonnes of lead every year. Batteries, leaded gasoline, paints, water pipes, pesticides, and certain cosmetics contain lead. The main sources of lead exposure for humans include air, water, soil, food, and consumer items. The pathophysiology of lead is explained by the build-up of lead in diverse tissues and its interference with bio-elements [2]. According to the World Health Organization (WHO), lead poisoning caused about half a million deaths and over 9 million disability-adjusted life years in 2016 [3]. Lead acetate is a bio-toxic industrial and environmental toxin found in almost all tissues in the body, including the liver, lungs, bones, kidneys, reproductive systems, and immune system [4]. Lead exposure has recently been identified as a significant cause of testicular dysfunction and male infertility [5]. The increased content of MDA is evidence of tissue damage produced by increased free radicals in lead poisoning mechanisms [6]. Lead reduces the levels of glutathione, superoxide dismutase, and other enzymatic antioxidants. Lead's capacity to create reactive oxygen species aids its harmful action in the testes. Lead exposure has been associated with increases in membrane lipid peroxidation as well as a reduction in endogenous antioxidants. Glutathione, superoxide dismutase, and other enzyme antioxidants are all reduced [7].
A chelation agent binds with lead molecules, helping in their excretion and, as a result, reducing the lead load in the body. Chelation drugs, on the other hand, have a number of drawbacks. Succimer (nausea, vomiting, sweating, high temperature, hypertension, and tachycardia); BAL (nausea, vomiting, sweating, high temperature, hypertension, and tachycardia); Chelation using meso-2,3-dimercaptosuccinic acid (Succimer or DMSA) and D,L-2,3-dimercapto-1-propanesulfonic acid (Dimaval or DMPS); 2,3-dimercaptopropanol (British Anti Lewisite, BAL or Dimercaprol); and (EDTA) Ethylene diamine-tetra acetic acid [8,9,4].

Antioxidants have been proven to prevent or minimise the oxidation of other molecules by reactive oxygen species (ROS) in a tissue or cell organism, scavenge free radicals, and reduce their detrimental effects. Their inherent antioxidant qualities protect tissues and cells against the toxicity of reactive oxygen species (ROS) and other free radicals. These plant-based remedies have fewer adverse effects, are affordable, and are easily accessible. Current research has focused on the protective impact of antioxidant-rich plant compounds and medicinal herbs, such as curcumin, in preventing free radical-induced tissue damage [10]. The use of plant-derived polyphenol flavanone, which has no adverse effects and is abundant in nature, has piqued interest in several dietary approaches. According to the results of several animals and in vitro experiments, polyphenols are beneficial in the treatment of various health issues [11].

Current research has focused on the protective impact of plant-based products with antioxidant capabilities, such as grape seeds, for minimizing tissue damage caused by free radicals. Grapes (*Vitis vinifera*, Vitaceae) are now farmed in all over the world. Grape seed proanthocyanidin extract (GSPE) is a flavonoid polyphenolic substance derived from grape seeds. It connects +catechin, epicatechin gallate, epicatechin gallate, and epigallocatechin by C4–C6 or C4–C8 bond connections. It's available as a monomer or a polymer [12]. Grape seed has a higher antioxidant activity than vitamins C and E, beta-carotene, or monomeric flavanols like catechin. Additionally, grape seed extracts containing 39–73% proanthocyanidin have been proven to have high antioxidant activity [13]. The purpose of this study was to look into the effects of post-treatment with 80% grape seed extract (*Vitis vinifera*.L) on lead acetate-induced oxidative damage in Wistar rats.

2. Material and methods

2.1. Chemicals

Lead Acetate (Pb) purchased from Sigma Aldrich (St. Louis MO, USA; Cat No: 6080-56-4) Testosterone hormone kit, ELISA (Cat No: E-EL-0155, India), Grapes (*Vitis vinifera* L., Vitacease) were obtained from S.V. enterprises, Tenali, India and authenticated by a botanist (No: PARC/2018/36, India).

2.2. Grape Seed Extract (GSE) preparation

The grape berries were undamaged and disease-free and were cut from bunches of Vitis vinifera. After manually separating the seeds from the entire berries, the seeds were oven-dried at 30 to 40 degrees Celsius.

A laboratory mill was used to grind dried grape seeds into a fine powder. Grape (*Vitis vinifera*) seeds are pulverised (500 gms), macerated in 1000 ml of ethanol (80%), shaken daily, and kept in a refrigerator surrounded with aluminium foil. The mixture was filtered through double gauze and centrifuged at 3000 rpm for 10 minutes, after which the ethanol was evaporated using rotatory evaporator equipment (Switzerland) connected to a vacuum pump and followed by lyophilization [14], yielding 64.4 gm. Phytochemical preliminary analyses were performed [15, 16].

2.3. Experimental animal and study desgin

Rattus norvegicus male Wistar Albino rats weighing about 250 gms (2.5–3 months) and purchased from CPCSEA approved vendors. Rats were kept for 7 days as an acclimatization period before the start of the experiment. They were kept in polypropylene cages (20cm×34cm×47cm) with bedding made of sterile rice husk. All of the animals were kept in an air-conditioned room with a temperature range of 25°C ± 2°C (12:12 hrs light and dark cycles). The rats were given a conventional pellet diet and free access to water. The Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India, provided instructions for this experimental investigation (CPCSEA, 2003). This study was confirmed by the Institutional Animal Ethical Committee (IAEC), Centre for Toxicology and Developmental Research (CEFTE) Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, India under No:(IAEC/59/SRIHER/665/2019).

Table I Experimental design (24 male Wistar rats)

Group	Treatment	Number of animals (24)	Treatment period (Day)	Blood collection	Sacrifice
I	Control	6	D/w starts at1st till 56th	Day 57th	Day 57th
II	Lead acetate administered 28 days a dose of 50 mg/kg Bw (18 animals) at end of the 28th day these animals sub grouped into Group II(a), II (b) & II (c)				
II(a)	LA cessation	6	D/w starts at 29th to 56th	Day 57th	Day 57th
II(b)	GSE200mg/kg Bw	6	starts at 29th to 56th	Day 57th	Day 57th
II(c)	GSE400mg/kg Bw	6	starts at 29th to 56th	Day 57th	Day 57th

D/w: Distilled water; GSE: Grape Seed Extract; LA: Lead Acetate

All animals were administered GSE one hour after receiving lead acetate. Previous papers [7,17,18,19] were used to justify the 28-day trial period. Animals were weighed 24 hours after the last treatment, and blood samples were collected from all anaesthetized rats via the retro-orbital plexus [20]. For the determination of serum testosterone, blood samples were centrifuged and the serum separated. All of the animals were euthanized with CO2 asphyxia, and the testes were extracted, the testes being cleaned, weighed, and homogenised in an ice-cold medium containing 50 mM Tris–HCl (pH 7.4). The homogenates were centrifused for 10 minutes at 3000 RPM at 4°C, and then the supernatant was kept at –20°C to determine the oxidative stress indicators.

2.4. Behavioral and Toxicological observations

Throughout the experimental research periods, all rats were monitored daily twice for evidence of behavioural changes (head flicking, circling, lethergy and weakness) and mortality. Each rat was examined twice through the study.

2.5. Weight of the testis

The left testis was dissected out, trimmed off the extra tissues, and weighed using a digital electronic weighing balance.

2.6. Estimation of enzymatic and non-enzymatic Oxidative stress markers

Fresh tissues of the left testes were collected and washed in ice-cold saline, then homogenised in 0.1 M Tris–HCL buffer (pH 7.4) [21]. The testes homogenate samples were then taken for further analysis of the following by the respective methods: Antioxidant Enzymes - Superoxide dismutase (SOD) [22], Catalase (CAT) [23], Glutathione Peroxidase (GPx) [24], non-enzymatic antioxidant, Reduced Glutathione (GSH) [25] and metabolites of lipid peroxidation (LPO) [26].

2.7. Statistical Analysis

Data obtained from the study were summarised as means ± SEM and analysed with Graphpad Prism 9.5 Version software using one-way ANOVA with Tukey's multiple comparisons (post-hoc test), and values were significant at $p < 0.05$.

3. Results

The study was conducted to check the efficacy of GSE on lead acetate - induced testicular oxidative damage in rats.

3.1. Behavioral and Toxicological changes

In the present investigation, no mortalities were recorded. After post-lead acetate administration in group II, some clinical symptoms were seen in rats (lethargy, loss of appetite, nausea, vomiting, circling, aggression, constipation, and a ruffled coat)), as well as minor symptoms in group II(b) (aggressive behaviour); no symptoms were identified in group II(c).

3.2. Preliminary phytochemical analysis

A brown solid, lyophilized 80% Grape seed extract was obtained. The preliminary phytochemical results showed the following bioactive constituents present in the extract: flavonoids, polyphenols, saponins, tannins, triterpenoids, steroids, quinones, anthocyanidins, glycosides, and proanthocynadins.

3.3. Weight of the testis and Oxidative stress markers

Table 2 Illustrates the impact of GSE on lead acetate-administered male albino Wistar rats' weight of the testis, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and malondialdehyde (MDA). Weight of the testes in lead acetate ceased group II(a) ($p < 0.0001$) significantly declined, When compared to control (group I) rats. When compared to group II(a) weight of the testes in group II(b) & II(c) post-treatment with GSE (200 and 400 mg/kg Bw) significantly restored the weight of the testes (Table 1). When compared to control (group I) rats, in lead acetate ceased group II(a) a large rise in malondialdehyde and a significant drop in SOD, CAT, GPx, and GSH levels. When compared to group II(a) rats, post-treatment with GSE (200 and 400 mg/kg Bw) significanlty ($p < 0.05$) increased the antioxidant enzyme system and reduced the MDA production. To further investigate the molecular pattern produced by lead-induced testicular injury and subsequent oxidative stress, Whereas post- treatment of GSE in group II(b) and II (c) rats reduced the oxidant (MDA) concentration, and increased the activities of the antioxidant enzyme system compared to lead-induced testicular tissue in group II(a) rats.

Table 2. Effect on weight of the testis and Oxidative stress markers in testicular tissue

Group	Treatment	Weight of the testis (gms)	SOD (Unit/mg/mt Ptn)	CAT (nm/mt/mg ptn)	GPx (mol/mg ptn)	GSH (mcg/g tissue)	MDA (mcg/g tissue)
I	Control	1.68±0.040	8.40±0.16	8.10±0.12	30.13±0.40	16301±1242	16.63±0.82
II(a)	LA	1.31±0.040^{a}	3.77±0.06^{a}	2.98±0.09^{a}	14.17±0.76^{a}	10806±2684^{a}	38.34±2.39^{a}
II(b)	GSE 200 mg	1.48±0.016^{a,b}	4.39±0.14^{a,b}	5.15±0.25^{a,b}	20.62±0.65^{a,b}	12095±4117^{a,b}	30.10±0.63^{a,b}
II(c)	GSE 400 mg	1.63±0.042^{a,b}	6.94±0.28^{a,b}	6.87±0.17^{a,b}	27.64±0.31^{a,b}	14770±2205^{a,b}	26.00±0.41^{a,b}

Data were expressed as Standard error Mean (SEM).
a: significantly different when compared to the control group ($p < 0.05$);
b: significantly different when compared to the lead acetate-administered group ($p < 0.05$)

4. DISCUSSION

Lead is a very hazardous heavy metal that harms humans, particularly male reproductive organs [27] by imbalancing the antioxidant and reactive oxygen species (ROS) equilibrium. Environmental toxicants cause significant harm to the histomorphology of the testis [28,29]. Lead, like most divalent metals, is attached to albumin, enzymes, short peptides, cysteine, methionine, and selenomethionine in tissues through ionic (in skeletal minerals) or coordination linkages. As a result of the rapid deposition of lead, tissue or organ damage occurs. Increased quantities of reactive oxygen species (ROS) cause lipid peroxidation at the cellular level [30].

The entire study period observed for there was any behavioral and toxicological changes in various experimental group rats. There was no mortality observed in rats in the control and experimental groups, but it has been shown that some behavioral changes were found in Group II(a) (lethargy, loss of appetite,

nausea, vomiting, circling, aggression, constipation, and a ruffled coat). These similar observations were noticed in the previous experimental study [31].The symptoms frequently develop over a period of weeks to months as lead accumulates in the body. Lead's oral effects include astringency and a metallic taste [32]. A lack of appetite or weight loss is frequent with acute poisoning. Large levels of lead absorbed in a short period of time might cause shock (insufficient fluid in the circulatory system) due to water loss from the gastrointestinal tract [33].

The endogenous antioxidant enzyme system controls the cells' excessive synthesis of reactive oxygen species. GSH is the most important non-enzymatic endogenous antioxidant. This was shown to be diminished in relation to increased lipid peroxidation. It is a cellular oxidative stress marker that has long been recognized as a significant result of oxidative damage in various diseases. In the current study, LA-cessation rats (Group II(a)) showed reductions in SOD, CAT, GPx, and GSH activities and elevations in MDA concentration in tissue homogenates when compared with the untreated control rats. The present results agree with previous observations explaining that long-term exposure to lead causes oxidative damage by increasing lipid peroxidation (elevated MDA levels), inhibiting SOD, CAT, and GP_X activity, and lowering GSH levels in the testes [10,34,5,35], reduced activities of antioxidant enzymes are frequently implicated in oxidative stress. Rats exposed to LA were reported to exhibit significantly lower levels of testicular antioxidant enzymes. The present findings on antioxidant enzyme activities are in accordance with several previous studies that found significant reductions in antioxidant enzymes in the testes of rats exposed to LA; these alterations can be attributed to the numerous deleterious effects caused by the accumulation of superoxide radicals and hydrogen peroxide. Further, it has been documented that the lead ion competes with metal ions (such as Cu^{2+}, Zn^{2+}, Fe^{2+}, and Mg^{2+}) that are essential for the activity of antioxidant enzymes, resulting in a loss or decrease in antioxidant enzymes [36].

A protective effect GSE keeps GSH under control and boosts the cellular antioxidant defence system's capacity. The GSE may function by increasing GSH steady-state and rate of synthesis while protecting against oxidative stress. GSH is the main non-enzymatic antioxidant present in living organisms, both extracellular and intracellular, which works against xenobiotics and neutralises ROS production. GSH content was shown to be lower in LA-induced rats in the present research. This decrease in GSH levels might result in increased lipid peroxidation. Furthermore, LA inhibits GSH synthesis from cysteine via the -glutamyl cycle, further decreasing GSH concentration [7,37]. However, post-treatment with GSE preserved these enzymes from being down regulated. This suggests a regulatory role for GSE on the antioxidant enzymes.

The GSE has a high bioavailability of polyphenols, particularly proanthocyanidin (1332.90-95.88 mg/g of berries), polyhydroxylated flavan-3-ols, and is used to treat a variety of pathophysiological changes, such as inflammation and detoxification [38]. GSE can also be used in the treatment of cancer and weight loss attributed to metabolic complaints [39, 40, 15]. When the rats were challenged with lead acetate, post-treatment with GSE at a dosage of 400 mg/kg BW prevented MDA levels from rising. Furthermore, statistically verified restoration of antioxidant enzyme activity (SOD, CAT, GPx, and GSH) is shown in Table 2. However, treatment with GSE resulted in a marked improvement of testicular function markers. The results indicated that the GSE may stabilise the cellular membrane of spermatogenic cells and maintain their functions. In general, the high levels of phytochemical components present appear to be responsible for GSE's potent testicular protector activity. As seen in this study, the antioxidant and free radical scavenger GSE can lower the MDA level disturbed by lead acetate in the rat testis. GSE restored the antioxidant enzyme system and reversed lead acetate-induced oxidative damage.

5. CONCLUSION

The results of the present study revealed that MDA concentration was significantly increased in the lead acetate – ceased group rats and declined the antioxidant enzymes (SOD, CAT, GPx, and GSH) in the testis gradually. The reduction of antioxidant enzymes will elevate free radicals in testicular tissues and might affect the rat's reproductive fertility. GSE post-treatment demonstrated therapeutic effects by attenuating lead acetate-induced testicular oxidative damage, which could be attributed to GSE's antioxidant activity. The antioxidant enzymes (SOD, CAT, GPx, and GSH) were restored and MDA was reduced after GSE post-treatment. SOD, CAT, GPx, GSH, and MDA enzymatic activity in rats can be evaluated as indicators of heavy metal toxicity, such as lead acetate. Supplementation with grape seed extract brings remarkable recovery. The best results were achieved in group II(c). *Vitis vinifera* (grape) seeds may be beneficial in the treatment of oxidative stress-related testicular conditions. This scientific evidence supports the use of grapes (*Vitis vinifera*) in Ayurveda and the Siddha system of medicine. As a result, it's possible that the

therapeutic effect of grape seed extract (GSE) in this investigation was due to its high antioxidant content and ability to scavenge ROS.

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Author contributions
Senthil Kumar Sampath contributed to the conception and designs, acquisition, drafted the Manuscript, and revised it critically; Mahesh Babu Yallamati contributed to conception, designs, the investigation, analysis, and interpretation of the data, drafted the manuscript, and revised it critically and Janani Maheshwari.V.Vyas and Yugesh Kesavamoorthy revised it critically. All of the writers gave their final approval and agreed to be responsible for the accuracy and integrity of all parts of the work.

Statement of Animal rights
The Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India, provided instructions for this experimental investigation (CPCSEA, 2003). This study was confirmed by the Institutional Animal Ethical Committee (IAEC), Centre for Toxicology and Developmental Research (CEFT) Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, India under No:(IAEC/59/SRIHER/665/2019).

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REFERENCES

1. Kahalerras, L., Otmani, I., & Abdennour, C. (2022). Wild Garlic Allium triquetrum L. Alleviates Lead Acetate-Induced Testicular Injuries in Rats. *Biological trace element research*, *200*(5), 2205–2222. https://doi.org/10.1007/s12011-021-02818-8
2. Horton, C. J., Acharya, L., & Wells, E. M. (2019). Association between self-reported length of time in the USA and blood lead levels: National Health and Nutrition Examination Survey 2013-2016. *BMJ open*, *9*(7), e027628. https://doi.org/10.1136/bmjopen-2018-027628
3. World Health Organization. (2017). Lead poisoning and health fact sheet. *updated August*. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health
4. Sudjarwo, S. A., Sudjarwo, G. W., & Koerniasari (2017). Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats. *Research in pharmaceutical sciences*, *12*(5), 381–390. https://doi.org/10.4103/1735-5362.213983
5. Olaniyan, O. T., Ojewale, O. A., Dare, A., Adebayo, O., Enyojo, J. E., Okotie, G. E., Adetunji, C. O., Mada, B. S., Okoli, B. J., & Eweoya, O. O. (2021). Cocos nucifera L. oil alleviates lead acetate-induced reproductive toxicity in sexually-matured male Wistar rats. Journal of basic and clinical physiology and pharmacology, 10.1515/jbcpp-2020-0281. Advance online publication. https://doi.org/10.1515/jbcpp-2020-0281
6. Abdel-Emam, R. A., & Ahmed, E. A. (2021). Ameliorative effect of L-carnitine on chronic lead-induced reproductive toxicity in male rats. *Veterinary medicine and science*, *7*(4), 1426–1435. https://doi.org/10.1002/vms3.473
7. Abdrabou, M. I., Elleithy, E., Yasin, N., Shaheen, Y. M., & Galal, M. (2019). Ameliorative effects of Spirulina maxima and Allium sativum on lead acetate-induced testicular injury in male albino rats with respect to caspase-3 gene expression. *Acta histochemica*, *121*(2), 198–206. https://doi.org/10.1016/j.acthis.2018.12.006
8. Yallamati, M. B., Sampath, S. K., Mary, V., & Khan, M. Z. (2023). Ameliorative effect of Vitis vinifera (Linn.) seed extract on lead acetate induced oxidative damage on testis and sperm quality in Wistar rats. *Journal of experimental zoology. Part A, Ecological and integrative physiology*, *339*(2), 210–219. https://doi.org/10.1002/jez.2672

9. Farfel, M. R., & Chisolm, J. J., Jr (1990). Health and environmental outcomes of traditional and modified practices for abatement of residential lead-based paint. American journal of public health, 80(10), 1240–1245. https://doi.org/10.2105/ajph.80.10.124
10. Bentaiba, K., Belhocine, M., Chougrani, F., Bououdina, M., Mostari, A., Fernini, M., & Bouzouina, M. (2023). Effectiveness of Withania frutescens root extract on testicular damage induced by lead acetate in adult albino rats. *Reproductive toxicology (Elmsford, N.Y.)*, *115*, 102–110. https://doi.org/10.1016/j.reprotox.2022.12.006
11. Prasain, J. K., Peng, N., Dai, Y., Moore, R., Arabshahi, A., Wilson, L., Barnes, S., Michael Wyss, J., Kim, H., & Watts, R. L. (2009). Liquid chromatography tandem mass spectrometry identification of proanthocyanidins in rat plasma after oral administration of grape seed extract. Phytomedicine : international journal of phytotherapy and phytopharmacology, 16(2-3), 233–243. https://doi.org/10.1016/j.phymed.2008.08.006
12. Liu, M., Yun, P., Hu, Y., Yang, J., Khadka, R. B., & Peng, X. (2020). Effects of Grape Seed Proanthocyanidin Extract on Obesity. *Obesity facts*, *13*(2), 279–291. https://doi.org/10.1159/000502235
13. Odai, T., Terauchi, M., Kato, K., Hirose, A., & Miyasaka, N. (2019). Effects of Grape Seed Proanthocyanidin Extract on Vascular Endothelial Function in Participants with Prehypertension: A Randomized, Double-Blind, Placebo-Controlled Study. Nutrients, 11(12), 2844. https://doi.org/10.3390/nu11122844
14. Hala, A. H., Khattab, Z. A., Abdallah, G., & Kamel, M. (2010). Grape seed extract alleviate reproductive toxicity caused by aluminium chloride in male rats. *J Am Sci*, 6:352-361.
15. Kokate C.K, Purohit A.P and Gokhale S.B.Pharmacognosy (Text book). 2009; AppendicesA.1-A.5.
16. Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. *TheScientificWorldJournal*, *2017*, 5873648. https://doi.org/10.1155/2017/5873648
17. Sokol R. Z. (1990). The effect of duration of exposure on the expression of lead toxicity on the male reproductive axis. *Journal of andrology*, *11*(6), 521–526. https://pubmed.ncbi.nlm.nih.gov/2128299/
18. Alkhedaide, A., Alshehri, Z. S., Sabry, A., Abdel-Ghaffar, T., Soliman, M. M., & Attia, H. (2016). Protective effect of grape seed extract against cadmium-induced testicular dysfunction. *Molecular medicine reports*, *13*(4), 3101–3109. https://doi.org/10.3892/mmr.2016.4928
19. Sönmez, M. F., & Tascioglu, S. (2016). Protective effects of grape seed extract on cadmium-induced testicular damage, apoptosis, and endothelial nitric oxide synthases expression in rats. *Toxicology and industrial health*, *32*(8), 1486–1494. https://doi.org/10.1177/0748233714566874
20. Stone S. H. (1954). Method for Obtaining Venous Blood from the Orbital Sinus of the Rat or Mouse. *Science (New York, N.Y.)*, *119*(3081), 100. https://doi.org/10.1126/science.119.3081.100
21. Kempinas, W. G., & Lamano-Carvalho, T. L. (1988). A method for estimating the concentration of spermatozoa in the rat cauda epididymidis. Laboratory animals, 22(2), 154–156. https://doi.org/10.1258/002367788780864547
22. Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian journal of biochemistry & biophysics*, *21*(2), 130–132. https://pubmed.ncbi.nlm.nih.gov/6490072/
23. Sinha A. K. (1972). Colorimetric assay of catalase. *Analytical biochemistry*, *47*(2), 389–394. https://doi.org/10.1016/0003-2697(72)90132-7
24. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science (New York, N.Y.)*, *179*(4073), 588–590. https://doi.org/10.1126/science.179.4073.588
25. Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et biophysica acta*, *582*(1), 67–78. https://doi.org/10.1016/0304-4165(79)90289-7
26. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3
27.. Kolawole, T. A., Asiwe, J. N., Buduburisi, B. R., Akintade, V. A., Adebayo, O. G., Ojetola, A. A., & Dapper, D. V. (2022). Cabbage (Brassica oleracea) mitigates lead (II) acetate-induced testicular dysfunction in Wistar rats via up-regulation of Bcl-2 protein expression, pituitary-testicular hormonal axis and down-regulation of oxido-inflammatory reactions. *Andrologia*, *54*(8), e14476. https://doi.org/10.1111/and.14476

28. Kahalerras, L., Otmani, I., & Abdennour, C. (2021). Wild Garlic Allium triquetrum L. Alleviates Lead Acetate-Induced Testicular Injuries in Rats. *Biological trace element research*, 10.1007/s12011-021-02818-8. Advance online publication. https://doi.org/10.1007/s12011-021-02818-8
29. Quintanilla-Vega, B., Hoover, D. J., Bal, W., Silbergeld, E. K., Waalkes, M. P., & Anderson, L. D. (2000). Lead interaction with human protamine (HP2) as a mechanism of male reproductive toxicity. *Chemical research in toxicology*, *13*(7), 594–600. https://doi.org/10.1021/tx000017v
30. Klotz, K., & Göen, T. (2017). Human Biomonitoring of Lead Exposure. *Metal ions in life sciences*, *17*, /books/9783110434330/9783110434330-006/9783110434330-006.xml. https://doi.org/10.1515/9783110434330-006
31. Karri, S. K., Saper, R. B., & Kales, S. N. (2008). Lead encephalopathy due to traditional medicines. *Current drug safety*, *3*(1), 54–59. https://doi.org/10.2174/157488608783333907
32. Hasanein, P., Fazeli, F., Parviz, M., & Roghani, M. (2018). Ferulic acid prevents lead-induced testicular oxidative stress and suppressed spermatogenesis in rats. *Andrologia*, *50*(1), 10.1111/and.12798. https://doi.org/10.1111/and.12798
33. Shaban El-Neweshy, M., & Said El-Sayed, Y. (2011). Influence of vitamin C supplementation on lead-induced histopathological alterations in male rats. *Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie*, *63*(3), 221–227. https://doi.org/10.1016/j.etp.2009.12.003
34. Dorostghoal, M., Seyyednejad, S. M., & Jabari, A. (2014). Protective effects of Fumaria parviflora L. on lead-induced testicular toxicity in male rats. *Andrologia*, *46*(4), 437–446. https://doi.org/10.1111/and.12100
35. El-Khadragy, M., Al-Megrin, W. A., AlSadhan, N. A., Metwally, D. M., El-Hennamy, R. E., Salem, F., Kassab, R. B., & Abdel Moneim, A. E. (2020). Impact of Coenzyme Q10 Administration on Lead Acetate-Induced Testicular Damage in Rats. Oxidative medicine and cellular longevity, 2020, 4981386. https://doi.org/10.1155/2020/4981386
36. Wang, Y., Wang, S., Cui, W., He, J., Wang, Z., & Yang, X. (2013). Olive leaf extract inhibits lead poisoning-induced brain injury. *Neural regeneration research*, *8*(22), 2021–2029. https://doi.org/10.3969/j.issn.1673-5374.2013.22.001
37. El-Tantawy W. H. (2015). Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *Journal of traditional and complementary medicine*, *6*(4), 327–331. https://doi.org/10.1016/j.jtcme.2015.02.001
38. Farida, Benmeziane & Cadot, Yves. (2019). Quantitative analysis of proanthocyanidins (tannins) from cardinal grape (Vitis vinifera) skin and seed by RP-HPLC. The North African Journal of Food and Nutrition Research. 3. 201-205. 10.51745/najfnr.3.6.201-205. https://najfnr.com/home/article/view/91.
39. El-Tarras, A., Attia, H. F., Soliman, M. M., El Awady, M. A., & Amin, A. A. (2016). Neuroprotective effect of grape seed extract against cadmium toxicity in male albino rats. *International journal of immunopathology and pharmacology*, *29*(3),398–407 https://doi.org/10.1177/0394632016651447
40. Luna, L. (ed) AFIP Manual of Histological Staining Methods, 3rd Ed., New York, McGraw Hill Publications, c. 1968, p. 76.