POST - TREATMENT EFFECT OF VİTİS VİNİFERA SEED EXTRACT ON LEAD ACETATE INDUCED OXİDATİVE DAMAGE AND TESTİCULAR HİSTO-MORPHOLOGİCAL CHANGES İN ADULT WİSTAR RATS

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Abstract

Background: Lead is the most ubiquitous hazardous toxin in the environment. It's a severe threat to public health, especially in the male reproductive system. Vitis vinifera seeds (grape seeds) are natural, rich sources of antioxidant compounds.
Aim: The present study was undertaken to investigate the effect of Vitis vinifera seed extract against the deterioration of antioxidant enzymes and testicular histomorphological changes caused by lead acetate. 24 male Wistar rats were used for the study and were divided into Group I: control (6 rats) and Group II: the remaining 18 rats were administered with LA (lead acetate) 50 mg/kg BW for 28 days; at the end of the 28th day, all 18 rats were divided equally into three groups: Group II (a): lead acetate ceased; Group II (b): rats treated with GSE (grape seed extract) 200 mg/kg BW; and Group II (c): rats treated with GSE 400 mg/kg BW orally once a day from the 29th to the 56th day. At the end of 56 days, levels of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in testicular tissue were measured in rats. Testis tissue samples were also collected for histological studies.
Results and conclusion: The current study showed that in comparison to control rats, LA-ceased group showed significantly elevated lipid peroxidation, as evidenced by a large rise in MDA levels and a significantly reduced level of the antioxidant enzymes SOD, CAT, GPx, and reduced glutathione (GSH). Additionally, severe histomorphological alternations were observed in LA-ceased rats. The post-treatment effect of grape seed extract significantly reversed the effect of lead acetate with the restoration of oxidative stress markers and the histoarchitecture of the testes. It is also a powerful natural antioxidant that shows promise as a treatment for rats with testicular injuries caused by lead acetate.

Keywords: Oxidative damage, Lipid peroxidation, Distortion of seminiferous tubule, Lyophilization, Post - treatment.

Abbrevations: GSE- Grape Seed extract; LA: Lead Acetate

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1. INTRODUCTION

Heavy metals are ubiquitous environmental contaminants that occur both naturally and as a result of human activity. Lead (Pb) is one of the most dangerous heavy metal environmental pollutants, causing oxidative stress and endangering human health (Lu J. et al., 2018). Globally, industries produce around 2.5 million metric 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 (Horton, C. J. et al., 2019).

According to the World Health Organisation (WHO), lead poisoning caused about half a million deaths and over 9 million disability-adjusted life years in 2016 (WHO, 2017). 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 (S. A. Sudjarwo et al., 2017). Lead exposure has recently been identified as a significant cause of testicular dysfunction and male infertility (Olaniyan, O. T. et al., 2021). The increased content of MDA is evidence of tissue damage produced by increased free radicals in lead poisoning mechanisms (Abdel-Emam, R. A., & Ahmed, E. A. 2021).

Lead reduces the levels of glutathione, superoxide dismutase, and other enzymatic antioxidants. It also induces apoptosis and DNA damage in spermatozoa and activates Bax and caspase3 in spermatogenic cells (Karimfar, M.H. 2016). It also inhibits sperm functioning by reducing sperm cyclic adenosine monophosphate and calcium levels and decreasing sperm protein tyrosine phosphorylation (He Y., Zou Q., et al., 2016). 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 (Abdrabou, M. I. 2019).

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-tetraacetic acid (Chisolm JJ Jr. 1990).

The use of plant-derived flavanones, which have no adverse effects and are abundant in nature, has piqued interest in several dietary approaches. According to the results of several animal and in vitro experiments, polyphenols are beneficial in the treatment of many problems. (Prasain JK et al., 2009). 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 (Liu, M. et al., 2020). 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. Odai, T. et al., 2019 The purpose of this study was to look into the effects of post-treatment with 80% *Vitis vinifera* L. seed extract (grape seed extract) on lead acetate-induced oxidative damage and changes in testicular histoarchitecture in Wistar rats.

2. MATERIAL AND METHODS

2.1. Chemicals

Lead acetate (Pb) was purchased from Sigma Aldrich (St. Louis, MO, USA; Cat No: 6080-56-4); a testosterone hormone kit, ELISA (Cat No. E-EL-0155, India); and grapes (*Vitis vinifera* L., Vitaceae) 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 berry, 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 (Hala, A., Khattab et al. 2010). The final yield of the extract was 64.4 gms. Phytochemical preliminary analyses were performed according to

the methodology followed (Kokate, C.K. et al., 2009; Gul, R. et al., 2017).

2.3. Experimental animal

Rattus norvegicus male Wistar albino rats weighing about 250 gms (2.5–3 months) were bought from CFETE for use in experiments. Rats were kept for 7 days as an acclimatisation period before the start of the experiment. They were kept in polypropylene cages (20 cm × 34 cm × 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 to 2°C (12:12 hours of 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 (CEFT), Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, India, under No. IAEC/59/SRIHER/665/2019. To investigate the efficacy of 80% Vitis vinifera seed extract (GSE) as a post-treatment for lead acetate (Pb)-induced testicular injury, rats were randomly divided into four groups of six rats each.

Table I. Experimental Study Design

Group	Treatment	Number of animals (24)	Treatment period (Day)	Blood collection	Sacrifice
I	Control	6	D/W starts at 1st until 56th.	Day 57th	Day 57th
II	Lead acetate was administered for 28 days at a dose of 50 mg/kg BW (18 animals). At the end of the 28th day, these animals were sub-grouped into Groups II (a), II (b), and II (c).				
II (a)	LA ceased	6	D/W starts from 29th to 56th	Day 57th	Day 57th
II (b)	GSE 200 mg/kg BW	6	starts from 29th to 56th	Day 57th	Day 57th
II (c)	GSE 400 mg/kg BW	6	starts from 29th to 56th	Day 57th	Day 57th

D/W: distilled water; GSE: grape seed extract; L: lead acetate

All animals were administered GSE one hour after receiving lead acetate. Previous papers (Sudjarwo SA et al., 2017; Sokol RZ, 1990; Alkhedaide A et al., 2016; Sonmez MF et al., 2016) 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 (Stone SH, 1954). For the determination of serum testosterone, blood samples were centrifuged and the serum separated. All of the animals were euthanized with CO^2 asphyxia, and the testes were extracted, with the right testis from each rat being used for histomorphological studies and the left testes being cleaned, weighed, and homogenised in an ice-cold medium containing 50 mM Tris-HCl (pH 7.4). The homogenates were spun 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. Weight of the testis

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

2.5. 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) (Kempinas, Wilma, Lamano-Carvalho, and Teresa, 1988). The testes homogenate samples were then taken for further analysis by the respective methods: antioxidant enzymes: superoxide dismutase (SOD) (Kakkar P. et al., 1984), catalase (CAT) (Asru K. Sinha, 1987), glutathione peroxidase (GPx) (Rotruck et al., 1973), non-enzymatic antioxidants: reduced glutathione (GSH) (Moren MS et al., 1979), and metabolites of lipid peroxidation (LPO) (Ohkawa H. et al., 1979).

2.6. Histo-morphology

The right testes were separated, weighed, rinsed in saline, and preserved in 10% formalin for histomorphological study (Rahim SM et al. 2013). For the histomorphological examination, testes were processed for the paraffin embedding procedure, and sections of 5 µ thickness were taken using a rotary microtome (Leica Microsystems, Germany). Sections were stained with Ehrlich alum, hematoxylin, eosin, and Vangiseins stain (Bancroft, 6th ed., 2007; Clark, G.; Luna, L., 1968). The stained sections were observed under a fluorescent microscope and photographed. Fifty tubular profiles were analysed for histomorphological examination with magnification (200x, scale bar 50 µm).

3. STATISTICAL ANALYSIS

Data obtained from the study were summarised as mean (SEM) and analysed with Graph Pad Prism

9.3 software using one-way ANOVA with Tukey's multiple comparisons (*post-hoc* test), and values were significant at $P \leq 0.05$.

4. RESULTS

In the investigation into the improvement of GSE on lead acetate-induced testicular toxicity in Wistar rats, the following results were obtained:

4.1 Mortality

No mortality was documented throughout the course of the experiment's study period.

4.2 Preliminary phytochemical analysis

A brown, solid, lyophilized 80% grape seed extract was obtained. The preliminary phytochemical results showed in the table 2 and following bioactive constituents present in the extract.

Table 2. Results of preliminary phytochemical analysis

	Componds	Tests	procedure	Indication	Result
1.	Alkaloids	Dragendroff's	1 ml extract solution + a few drops of Dragendroff's	Orange colour	present
2.	Triterpenoids	Salkowski	l ml of extract solution + one bit of thionyl chloride	Pink colour	present
3.	Tannins	Ferric chloride	5 ml extract solution + a few drops of ferric chloride	Black colour	present
4.	Saponins	Foam	1 ml extract solution +5 ml D/W shake vigorously	Formation of foam	present
5.	Flavonoids	Shinoda	1 ml of extract solution + a few drops of sodium hydroxide	Yellow colour	present
6.	flavones	Conc. H2SO4	1 ml extract solution +10% sodium hydroxide	Yellow to orange colour	present
7.	Quinones	Acid	ml extract solution + 1 ml H2SO4	Red colour	present
8.	Anthocyanin		2 ml extract solution + 2 N HCL +amonia	pink	present
9.	Phenols	Ferric chloride	).5 ml Fecl2 + 2 ml extract solution	Blue colour	present
10.	proteins	Tannic acid	ml of extract + 10% tanic acid was added.	Precipitate is not formed	abscent
11	coumarins		ml 1 ml of extract solution + 10% sodium hydroxide	No colour change	abscence
12.	Carbohydrates	Benedicts	1 ml extract solution + Bendicts reagent	Reddish colour	present
13.	Glycosides		5 ml of extract solution + 2 ml of glacial acetic acid, and one drop of 5% Fecl3 and H2S04	A brown colour ring appears.	present

4. 3. Weight of the testis and oxidative stress markers

Table 3 illustrates the impact of 80% GSE on lead acetate-administered male albino Wistar rats' testis weight, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and malondialdehyde (MDA). The weight of the testes in lead acetate ceased in group II (a) ($P \leq 0.05$) and significantly declined when compared to control (group I) rats. When compared to group II (a), the weight of the testes in groups II (b) and II (c) post-treatment with GSE (200 and 400 mg/kg BW) significantly restored the weight of the testes (Table 3). When compared to control (group I) rats, in lead acetate ceased group II (a) rats, there was 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) significantly increased the antioxidant enzyme system and reduced MDA production.

Table 3. Results of SOD, CAT, GPx, GSH, and MDA levels in testicular tissue of various experimental groups

Group	Treatment	Testis weight (gms)	SOD Unit/mg/mtptn)	CAT (nm/mt/mgptn)	GPx (mol/mg ptn)	GSH (mcg/g tissue)	MDA mcg/g tissue)
I	**Control**	1.68±0.040	8.23±0.09	8.109±0.12	30.13±0.40	16301±1242	23.80±0.55
II(a)	**LA ceased**	1.31±0.040[a]	3.77±0.06[a]	2.981±0.09[a]	14.17±0.76[a]	10806±2684[a]	36.34±0.50[a]
II(b)	**GSE 200 mg**	1.48±0.016[a,b]	4.22±0.07[a,b]	5.159±0.25[a,b]	20.62±0.65[a,b]	12095±4117[a,b]	30.83±0.33[a,b]
II(c)	**GSE 400 mg**	163±0.042[a,b]	7.27±0.07[a,b]	6.788±0.15[a,b]	27.32±0.45[a,b]	14770±2205[a,b]	26.00±0.55[a,b]

The data were expressed as the standard error mean (SEM).
[a]: significantly different when compared to the control group ($P \leq 0.05$);
[b]: significantly different when compared to the lead acetate-ceased group ($P \leq 0.05$)

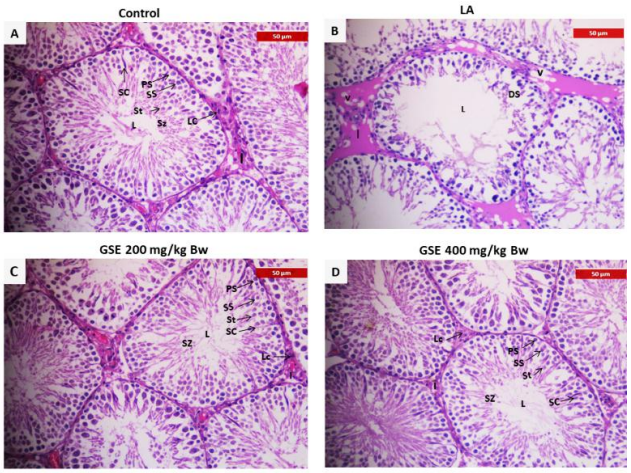

Figure 1. Histomorphological micrographs of transverse sections of the testis (H & E staining, 200X)

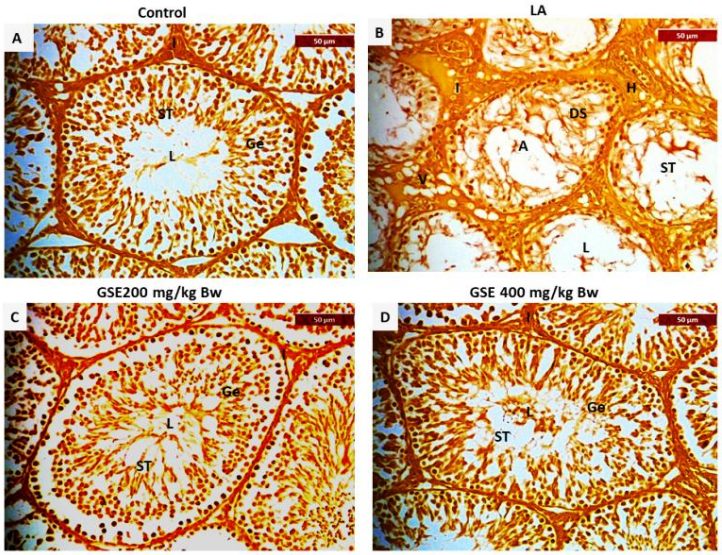

Figure 2. Histomorphological micrograph of the transverse section of the testis (Vangeinson's stain, 200X)

Figure 1 & 2. I:Interstetium, **L**:Lumen **Ps**:Primary spermatogonia, **Ss**:Secondary spermatogoea, **St**: spermatid, **Sc**:Sertoli cells, **Sz**: Spermatozoa, **Lc**: Leydig cells, **V**: Vaculation, **DS**: Distoration of germinal epethelium, **A**: Atropy of the seminiferous tuble and **H:** Hyalinization

4.5. Histomorphological study (H & E and Vangiseins stain).

A) Normal histological sections of the testis in the control group showed normal seminiferous tubules with many layers of spermatogenic cells supported by Sertoli cells and a normal interstitial space full of Leydig cells.

B) Histomorphological changes in Group II (a) testicular tissue sections revealed the most severe degree of testicular injury, with some seminiferous tubules lined by one layer of cells and the lumen packed or obliterated with necrotic debris; a change in seminiferous tubule shape; atrophy of the seminiferous tubule; various layers of spermatogenic cells severely damaged; distortion of Sertoli cells; and increased interstitial space with infiltration of various blood cells. In addition, vaculation was found in the interstitial space (VN stain) when compared with control (Group I) rats.

C) Compared to Group II (a), Group II (b), which was treated with GSE 200 mg/kg BW, had seminiferous tubules with more spermatogonia and Leydig cells and less damage to the tissue between them. Moreover, it reduced interstitial space and vaculation.

D) In Group II (c), rats were treated with GSE 400 mg/kg BW and observed the various layers of spermatogenic cells, an abundance of spermatids and Sertoli cells, and recovered the interstitial space with an increased number of Leydig cells compared to Group II (a). Moreover, the interstitial space was restored and filled with Leydig cells (H&E and VN stain).

5. Discussion

Lead is a very hazardous heavy metal that harms humans, particularly male reproductive organs (Neto, F. T. et al., 2016), by imbalancing the anti-oxidant and reactive oxygen species (ROS) equilibrium. Environmental toxicants cause significant harm to the histomorphology of the testis (Kahalerras, L. et al., 2021; Quintanilla-Vega, B. et al., 2016). 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 (Klotz, K., & Göen, T. 2017). Increased quantities of reactive oxygen species (ROS) cause lipid peroxidation at the cellular level (Diemer, T. et al., 2003).

The entire study period was observed for behavioural and toxicological changes in various experimental groups of rats. There was no mortality observed in rats in the control and experimental groups, but it has been shown that some behavioural 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 (Karri et al., 2008). Meanwhile, Dart et al., (2004) discovered that symptoms frequently develop over a period of weeks or months as lead accumulates in the body. Lead's oral effects include astringency and a metallic taste. A lack of appetite or weight loss is common 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 (Brunton et al., 2007).

The primary bioactive compounds (polyphenols, flavonoids, tannins, etc.) were detected in the preliminary phytochemical analysis. These findings are consistent with the previous findings regarding the ethanolic extract of grape seeds, which indicated that the active components help reduce the production of free radicals (Abd Eldaim MA et al., 2021). Unsaturated fatty acids are essential to human health and cannot be synthesised. They reduce excess cholesterol in the circulation, enhance the permeability of cell membranes, and protect against myocardial tissue and atherosclerosis. The antioxidants also help prevent free radical production. The amount of unsaturated fatty acids consumed by the body can also have a direct effect on the synthesis of prostaglandin, a substance with numerous effects on the body. Cholesterol binds to saturated fatty acids when there is insufficient linoleic acid, resulting in metabolic disorders. Grape seed has a high nutritional value and high development potential. The active substances ellagic acid, epicatechin, and resveratrol reduce cellular oxidative stress (Thanh LP, et al., 2022). According to the findings of the present investigation, ethanol is the more effective solvent for extracting phytochemical compounds from grape seeds. Alkaloids, flavonoids, glycosides, polyphenols, flavonoids, tannins, and sterols were identified in the ethanolic extract of grape seeds.

The phytochemicals alkaloids, flavonoids, saponins, and tannins may play a role in the

therapeutic use of grapes as bioactive agents. Flavonoids aid in preventing a microbial attack. The biological properties of grape seeds include hypoglycaemic, anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, anti-malarial, and anticholinergic properties, among others. They are primarily attributable to the substantial contribution of these secondary metabolites (Rupasinghe H. P. et al., 2003). VThen, resulting in its importance medically for grape seeds, the chemical compounds obtained are of medical importance and have many medical uses, including efficacy, antibacterial activities, antioxidant, hepatoprotective, anti-inflammatory, cancer preventive, and hypocholesterolemic properties (Felhi S. et al., 2016).

In the present experimental study, results revealed that LA-exposed rats had a significant reduction in testicular weight compared with untreated control rats. These findings are in line with earlier research showing that the distortion of seminiferous tubules and the absence of various spermatogenic layers of cells are the main causes of testis weight loss (Kahalerras, L. et al., 2021). GSE intervention, however, significantly restored these changes in groups II (b) and II (c). This suggests that the GSE removes lead from the testis by chelating it or boosting its biotransformation.

In the present study, LA causes an imbalance in the endogenous antioxidant enzyme system. 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 recognised as a significant result of oxidative damage in various diseases. In the current study, LA-ceased 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 (Dorostghoal, M. et al., 2014; Olaniyan, O. T. et al., 2021; El-Khadragy, M. et al., 2020). 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 (Wang, Y. et al., 2013).

A protective effect 80% GSE keeps GSH under control and boosts the cellular antioxidant defence system's capacity. The GSE may function by increasing the 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 (Sudjarwo, S. A. et al., 2017; El-Tantawy, W. H. 2015). However, post-treatment with GSE preserved these enzymes from being downregulated. This suggests a regulatory role for GSE in the antioxidant enzymes.

Meanwhile, Flora et al., (2012) observed that continuous exposure to lead acetate causes the production of reactive oxygen species such as singlet oxygen and hydrogen peroxide. Lead accumulation may affect mitochondrial and cytoplasmic membranes, resulting in more severe oxidative damage in tissues and the release of lipid hydrogen peroxides into the bloodstream. This causes oxidative stress, lowers antioxidant activity, and has a negative effect on the shape of the testis (Wahab, O.A. et al., 2019; Patrick, L., 2006).

Previous researchers reported histomorphological data revealing structural alterations in lead acetate-treated testicular tissue (Kahalerras, L. 2021; Offor, S. J. et al., 2019). In this study, the lead acetate-induced group had damaged testicles, a change in shape and atrophy of the seminiferous tubule, necrosis of the seminiferous tubule, a distorted series of spermatogonia cells caused by the loss of mechanical support from the Sertoli cells, and no spermatozoa. The control group did not have any of these problems. However, post-treatment with GSE prevented these enzymes from being downregulated. Histomorphological alterations were quite modest in the GSE 200 mg group, with GSE 400 mg/kg therapy restoring the deficit to a significant degree.

Farida et al., (2022) reported that 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. GSE can also be used in the treatment of cancer and weight loss attributed to metabolic complaints (El-Tarras et al., 2016; Liu, M. et al., 2016; Odai, T. et al., 2019). 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 1. However, treatment with 80 % GSE resulted in a marked improvement in 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 reduced oxidative damage and reversed lead acetate-induced histomorphological alterations.

6. CONCLUSION

In conclusion**,** the phytochemical evaluation revealed that the bioactive compounds polyphenols, flavonoids, tannins, anthocyanins, and flavonoids rich in flavonoids were present in 80% of the GSE. The histomorphological events in the testis induced by lead acetate are multi-factorial and mediated via its effects on antioxidant defence mechanisms, followed by increased lipid peroxidation, leading to elevated MDA levels and histomorphological changes, in addition to vascular and blood-testis barrier degradation, seminiferous tubule atrophy, and germinal epithelium distortion. Supplementation with grape seed extract brings remarkable recovery. *Vitis vinifera* seeds may be beneficial in the treatment of oxidative stress-related testicular conditions. This scientific evidence supports the use of *Vitis vinifera* seeds 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 reactive oxygen species (ROS).

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7. REFERENCES

1. Abdel-Emam RA, Ahmed EA. Ameliorative effect of L-carnitine on chronic lead-induced reproductive toxicity in male rats. *Vet Med Sci*. 2021;7(4):1426-1435. doi:10.1002/vms3.473. https://pubmed.ncbi.nlm.nih.gov/33724722/
2. Abdrabou MI, Elleithy EMM, Yasin NAE, Shaheen YM, Galal M. 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 Histochem*. 2019;121(2):198-206. doi:10.1016/j.acthis.2018.12.006. https://pubmed.ncbi.nlm.nih.gov/30587387/
3. Alkhedaide A, Alshehri ZS, Sabry A, Abdel-Ghaffar T, Soliman MM, Attia H. Protective effect of grape seed extract against cadmium-induced testicular dysfunction. *Mol Med Rep*. 2016;13(4):3101-3109. doi:10.3892/mmr.2016.4928. https://pubmed.ncbi.nlm.nih.gov/26935153/
4. Brunton, L.L., Goodman, L.S., Blumenthal, D., Buxton, I. and Parker, K.L. (2007): Principles of toxicology: In Goodman and Gilman, Manual of Pharmacology and Therapeutics. McGraw-Hill Professional. United States.
5. Clark, G., (ed) Staining Procedures, 3rd Ed., William & Wilkins Co., Baltimore, c 1973, p.50
6. Dorostghoal M, Seyyednejad SM, Jabari A. Protective effects of Fumaria parviflora L. on lead-induced testicular toxicity in male rats. *Andrologia*. 2014;46(4):437-446. doi:10.1111/and.12100. https://pubmed.ncbi.nlm.nih.gov/23611729/
7. El-Khadragy M, Al-Megrin WA, AlSadhan NA, et al. Impact of Coenzyme Q10 Administration on Lead Acetate-Induced Testicular Damage in Rats. *Oxid Med Cell Longev*. 2020;2020:4981386. Published 2020

Jun 2. doi:10.1155/2020/4981386. https://pubmed.ncbi.nlm.nih.gov/32566085/

8. El-Tantawy WH. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *J Tradit Complement Med*. 2015;6(4):327-331. Published 2015 Mar 27. doi:10.1016/j.jtcme.2015.02.001. https://pubmed.ncbi.nlm.nih.gov/27774414/
9. El-Tarras Ael-S, Attia HF, Soliman MM, El Awady MA, Amin AA. Neuroprotective effect of grape seed extract against cadmium toxicity in male albino rats. *Int J Immunopathol Pharmacol*. 2016;29(3):398-407. doi:10.1177/0394632016651447. https://pubmed.ncbi.nlm.nih.gov/27271977/
10. Farfel MR, Chisolm JJ Jr. Health and environmental outcomes of traditional and modified practices for abatement of residential lead-based paint. *Am J Public Health*. 1990;80(10):1240-1245. doi:10.2105/ajph.80.10.1240. https://pubmed.ncbi.nlm.nih.gov/2136329/
11. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. *Interdiscip Toxicol*. 2012;5(2):47-58. doi:10.2478/v10102-012-0009-2. https://pubmed.ncbi.nlm.nih.gov/23118587/
12. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. *ScientificWorldJournal*. 2017;2017:5873648. doi:10.1155/2017/5873648. .https://pubmed.ncbi.nlm.nih.gov/28386582/
13. Hala, A. H., Khattab, Z. A., Abdallah, G., & Kamel, M. Grape seed extract alleviate reproductive toxicity caused by aluminium chloride in male rats. *J Am Sci*. . 2010; 6:352-361.
14. Hanaa, M. A., Elshafie, M. A., Ismail, H. A., Mahmoud, M. E., & Ibrahim, H. M. Chemical studies and phytochemical screening of grape seeds (Vitis Vinifera l.). Minia J. Agric. Res. Develop. 2015;35 (2), 313-325.
15. He Y, Zou Q, Chen H, Weng S, Luo T, Zeng X. Lead Inhibits Human Sperm Functions by Reducing the Levels of Intracellular Calcium, camp, and Tyrosine Phosphorylation. *Tohoku J Exp Med*. 2016;238(4):295-303. doi:10.1620/tjem.238.295. https://pubmed.ncbi.nlm.nih.gov/27063420/
16. Horton CJ, Acharya L, Wells EM. Association between self-reported length of time in the USA and blood lead levels: National Health and Nutrition Examination Survey 2013-2016. *BMJ Open*. 2019;9(7):e027628. Published 2019 Jul 10. doi:10.1136/bmjopen-2018-027628. https://pubmed.ncbi.nlm.nih.gov/31296509/
17. Kahalerras L, Otmani I, Abdennour C. Wild Garlic Allium triquetrum L. Alleviates Lead Acetate-Induced Testicular Injuries in Rats. *Biol Trace Elem Res*. 2022;200(5):2205-2222. doi:10.1007/s12011-021-02818-8. https://pubmed.ncbi.nlm.nih.gov/34309801/
18. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys*. 1984;21(2):130-132. https://pubmed.ncbi.nlm.nih.gov/6490072/
19. Karimfar MH, Bargahi A, Moshtaghi D, Farzadinia P. Long-Term Exposure of Lead Acetate on Rabbit Renal Tissue. *Iran Red Crescent Med J*. 2016;18(2):e22157. Published 2016 Feb 6. doi:10.5812/ircmj.22157. https://pubmed.ncbi.nlm.nih.gov/27195142/
20. Karri SK, Saper RB, Kales SN. Lead encephalopathy due to traditional medicines. *Curr Drug Saf*. 2008;3(1):54-59. doi:10.2174/157488608783333907. https://pubmed.ncbi.nlm.nih.gov/18690981/
21. Kempinas WG, Lamano-Carvalho TL. A method for estimating the concentration of spermatozoa in the rat cauda epididymidis. *Lab Anim*. 1988;22(2):154-156. doi:10.1258/002367788780864547. https://pubmed.ncbi.nlm.nih.gov/2839733/
22. Klotz K, Göen T. Human Biomonitoring of Lead Exposure. *Met Ions Life Sci*. 2017;17:/books/9783110434330/9783110434330-006/9783110434330-006.xml. doi:10.1515/9783110434330-006. https://pubmed.ncbi.nlm.nih.gov/28731299/
23. Kokate C.K, Purohit A.P and Gokhale S.B.Pharmacognosy (Text book). 2009; AppendicesA.1-A.5.
24. Liu M, Yun P, Hu Y, Yang J, Khadka RB, Peng X. Effects of Grape Seed Proanthocyanidin Extract on Obesity. *Obes Facts*. 2020;13(2):279-291. doi:10.1159/000502235. https://pubmed.ncbi.nlm.nih.gov/32114568/
25. Luna, L. (ed) AFIP Manual of Histological Staining Methods, 3rd Ed., New York, McGraw Hill Publications, c. 1968, p. 76.

26. Mokhtari M, Zanboori M. The effects of lead acetate on sexual behavior and the level of testosterone in adult male rats. *Int J Fertil Steril*. 2011;5(1):13-20. https://pubmed.ncbi.nlm.nih.gov/24917919/
27. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*. 1979;582(1):67-78. doi:10.1016/0304-4165(79)90289-7. https://pubmed.ncbi.nlm.nih.gov/760819/
28. Odai T, Terauchi M, Kato K, Hirose A, Miyasaka N. Effects of Grape Seed Proanthocyanidin Extract on Vascular Endothelial Function in Participants with Prehypertension: A Randomized, Double-Blind, Placebo-Controlled Study. *Nutrients*. 2019;11(12):2844. Published 2019 Nov 20. doi:10.3390/nu11122844. https://pubmed.ncbi.nlm.nih.gov/31757033/
29. Offor SJ, Mbagwu HO, Orisakwe OE. Improvement of Lead Acetate-Induced Testicular Injury and Sperm Quality Deterioration by Solanum Anomalum Thonn. Ex. Schumach Fruit Extracts in Albino Rats. *J Family Reprod Health*. 2019;13(2):98-108. https://pubmed.ncbi.nlm.nih.gov/31988646/
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351-358. doi:10.1016/0003-2697(79)90738-3. https://pubmed.ncbi.nlm.nih.gov/36810/
31. Olaniyan OT, Ojewale OA, Dare A, et al. *Cocos nucifera L.* oil alleviates lead acetate-induced reproductive toxicity in sexually-matured male Wistar rats. *J Basic Clin Physiol Pharmacol*. 2021;33(3):297-303. Published 2021 Mar 15. doi:10.1515/jbcpp-2020-0281. https://pubmed.ncbi.nlm.nih.gov/33713589/
32. Patrick L. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. *Altern Med Rev*. 2006;11(2):114-127. https://pubmed.ncbi.nlm.nih.gov/16813461/
33. Prasain JK, Peng N, Dai Y, et al. Liquid chromatography tandem mass spectrometry identification of proanthocyanidins in rat plasma after oral administration of grape seed extract. *Phytomedicine*. 2009;16(2-3):233-243. doi:10.1016/j.phymed.2008.08.006. https://pubmed.ncbi.nlm.nih.gov/19095430/
34. Quintanilla-Vega B, Hoover DJ, Bal W, Silbergeld EK, Waalkes MP, Anderson LD. Lead interaction with human protamine (HP2) as a mechanism of male reproductive toxicity. *Chem Res Toxicol*. 2000;13(7):594-600. doi:10.1021/tx000017v. https://pubmed.ncbi.nlm.nih.gov/10898591/
35. Rahim SM, Taha EM, Mubark ZM, Aziz SS, Simon KD, Mazlan AG. Protective effect of Cymbopogon citratus on hydrogen peroxide-induced oxidative stress in the reproductive system of male rats. *Syst Biol Reprod Med*. 2013;59(6):329-336. doi:10.3109/19396368.2013.827268. https://pubmed.ncbi.nlm.nih.gov/23957393/
36. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science*. 1973;179(4073):588-590. doi:10.1126/science.179.4073.588. https://pubmed.ncbi.nlm.nih.gov/4686466/
37. Sinha AK. Colorimetric assay of catalase. *Anal Biochem*. 1972;47(2):389-394. doi:10.1016/0003-2697(72)90132-7. https://pubmed.ncbi.nlm.nih.gov/4556490/
38. Sokol RZ. The effect of duration of exposure on the expression of lead toxicity on the male reproductive axis. *J Androl*. 1990;11(6):521-526. https://pubmed.ncbi.nlm.nih.gov/2128299/
39. Sönmez MF, Tascioglu S. Protective effects of grape seed extract on cadmium-induced testicular damage, apoptosis, and endothelial nitric oxide synthases expression in rats. *Toxicol Ind Health*. 2016;32(8):1486-1494. doi:10.1177/0748233714566874. https://pubmed.ncbi.nlm.nih.gov/25614582/
40. Stone SH. Method for Obtaining Venous Blood from the Orbital Sinus of the Rat or Mouse. *Science*. 1954;119(3081):100. doi:10.1126/science.119.3081.100. https://pubmed.ncbi.nlm.nih.gov/17840528/
41. Sudjarwo SA, Sudjarwo GW, Koerniasari. Protective effect of curcumin on lead

acetate-induced testicular toxicity in Wistar rats. *Res Pharm Sci*. 2017;12(5):381-390. doi:10.4103/1735-5362.213983. https://pubmed.ncbi.nlm.nih.gov/28974976/

42. Wahab OA, Princely AC, Oluwadamilare AA, Ore-Oluwapo DO, Blessing AO, Alfred EF. Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. *JBRA Assist Reprod*. 2019;23(4):336-343. Published 2019 Oct 14. doi:10.5935/1518-0557.20190038. https://pubmed.ncbi.nlm.nih.gov/31173495/
43. Wang Y, Wang S, Cui W, He J, Wang Z, Yang X. Olive leaf extract inhibits lead poisoning-induced brain injury. *Neural Regen Res*. 2013;8(22):2021-2029. doi:10.3969/j.issn.1673-5374.2013.22.001. https://pubmed.ncbi.nlm.nih.gov/25206510/
44. 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