THE EFFECT OF PICEATANNOL ON THE REPRODUCTIVE SYSTEM IN MALE RATS EXPOSED TO LEAD

PhD Dissertation

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Department of Biology

Programme in Molecular Biology

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Eskişehir

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Institute of Graduate Programs

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FINAL APPROVAL FOR THESIS

This thesis titled THE EFFECT OF PICEATANNOL ON THE REPRODUCTIVE SYSTEM IN MALE RATS EXPOSED TO LEAD has been prepared and submitted by Tayeb Mohammed BELKHIR in partial fulfillment of the requirements in "Eskişehir Technical University Directive on Graduate Education and Examination" for the Degree of PhD in Biology Department has been examined and approved on 09/08/2024.

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ABSTRACT

THE EFFECT OF PİCEATANNOL ON THE REPRODUCTİVE SYSTEM İN MALE RATS EXPOSED TO LEAD

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Reproductive system issues, which are often the result of environmental or molecular factors, could directly impact future generations. Recent research highlights that exposure to heavy metals, such as lead (Pb), can negatively affect the reproductive system and sperm quality. To mitigate this damage, studies have focused on natural antioxidants. This study explores the regulatory effects of piceatannol (PIC), a phenolic acid antioxidant, on the reproductive system of male rats exposed to Pb toxicity. A study was conducted using 24 male Sprague Dawley rats, weighing between 200-225 g, divided into 4 groups (n=6). Pb acetate was administered intraperitoneally at a dose of 20 mg/kg/day for 28 days. From the 15th day, PIC was administered at 5 mg/kg/day for 14 days. On day 28, sperm count, morphology, and oxidative stress markers such as ALAD, SOD, CAT, T-GSH, and MDA were analyzed, along with FSH, LH, and testosterone levels. DNA damage was measured using single-cell gel electrophoresis, and testis tissues were examined histologically. The experiments found that PIC helped restore antioxidant levels, reduced MDA, and normalized testosterone and FSH levels. DNA damage caused by Pb exposure was significantly reduced in the Pb+PIC group, becoming similar to control levels. Histological analysis also revealed tissue structure resembling the control group. Overall, PIC demonstrated a protective effect on the reproductive system by reducing lipid peroxidation, preventing DNA damage, and normalizing hormone levels in Pb-exposed male rats.

Keywords: Lead, PIC, Reproductive toxicity, Oxidative stress.

ÖZET

KURŞUN VERILEN ERKEK SIÇANLARDA ÜREME SISTEMI ÜZERINE PICEATANNOL'ÜN ETKISI

Tayeb Mohammed BELKHIR

Biyoloji Anabilim Dalı

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Danışman: Doç. Dr. Volkan KILIÇ

Üreme sistemi sorunları, genellikle çevresel veya moleküler faktörlerin bir sonucu olarak ortaya çıkar ve doğrudan gelecek nesilleri etkileyebilir. Son araştırmalar, kurşun (Pb) gibi ağır metallere maruz kalmanın üreme sistemi ve sperm kalitesi üzerinde olumsuz etkileri olabileceğini vurgulamaktadır. Bu zararı en aza indirmek için doğal antioksidanlara yönelik çalışmalar yapılmaktadır. Bu çalışma, kurşun toksisitesine maruz kalan erkek sıçanların üreme sistemi üzerinde bir fenolik asit antioksidanı olan piseatanolün (PIC) düzenleyici etkilerini incelemektedir. Çalışma, 200-225 g ağırlığında 24 erkek Sprague Dawley sıçanı kullanılarak ve 4 gruba (n=6) ayrılarak gerçekleştirildi. Sıçanlara 28 gün boyunca günde 20 mg/kg dozunda intraperitoneal olarak kurşun asetat uygulandı. 15. günden itibaren 14 gün boyunca günde 5 mg/kg PIC verildi. 28. günün sonunda sperm sayısı, morfolojisi ve ALAD, SOD, CAT, T-GSH ve MDA gibi oksidatif stres belirteçlerinin yanı sıra FSH, LH ve testosteron seviyeleri analiz edildi. DNA hasarı tek hücre jel elektroforezi ile ölçüldü ve testis dokuları histolojik olarak incelendi. Deneyler, PIC'in antioksidan seviyelerini geri kazandırdığını, MDA'yı azalttığını ve testosteron ile FSH seviyelerini normale döndürdüğünü gösterdi. Pb maruziyetinin neden olduğu DNA hasarı, Pb+PIC grubunda önemli ölçüde azalarak kontrol seviyelerine yaklaştı. Histolojik analiz de doku yapısının kontrol grubuna benzer olduğunu ortaya koydu. Genel olarak, PIC'in kurşun maruziyetine uğrayan erkek sıçanlarda üreme sistemi üzerinde koruyucu bir etki gösterdiği bulundu.

Anahtar Sözcükler: Kurşun, PIC, Reprodüktif toksisite, Oksidatif stres.

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Tayeb Mohammed BELKHIR

09/08/2024

STATEMENT OF COMPLIANCE WITH ETHICAL PRINCIPLES AND RULES

I hereby truthfully declare that this thesis is an original work prepared by me; that I have behaved in accordance with the scientific ethical principles and rules throughout the stages of preparation, data collection, analysis and presentation of my work; that I have cited the sources of all the data and information that could be obtained within the scope of this study, and included these sources in the references section; and that this study has been scanned for plagiarism with "scientific plagiarism detection program" used by Eskişehir Technical University, and that "it does not have any plagiarism" whatsoever. I also declare that, if a case contrary to my declaration is detected in my work at any time, I hereby express my consent to all the ethical and legal consequences that are involved.

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GLOSSARY OF SYMBOLS AND ABBREVIATIONS

ATP	:	Adinosine Triphosphate
BSA	:	Bovine Serum Albumin
CAT	:	Catalase
EDTA	:	Ethylenediamine tetraacetic acid
G	:	Gram
GPx	:	Glutathione Peroxidase
GR	:	Glutathione Reductase
GSH	:	Glutathione
GSSG	:	Glutathione disulfate
GST	:	Glutathione S Transferase
i.p	:	Intraperitoneal
i.v	:	Intravenous
Kg	:	Kilogram
LDL	:	Low Density Lipoprotein
LPO	:	Lipid Peroxidation
LPS	:	Lipopolysaccharides
Mg	:	Milligram
mL	:	Milliliter
mM	:	Millimolar
NADPH	:	Nicotinamide Adenine Dinucleotide Phosphate
PBS	:	Phosphate Buffer Saline
Pb	:	Lead
Pic	:	Piceatannol
PCNA	:	Proliferating cell nuclear antigen
PUFA	:	Polyunsaturated fatty acid
MDA	:	Malondialdehyde
ROS	:	Reactive oxygen species
rpm	:	Round per minite
SCGE	:	Single Cell Gell Electrophoresis
SOD	:	Superoxide Dismutase
TCA	:	Tricarboxylic acid

TNF- α	:	Tumor Necrosis Factor- Alpha
v.v	:	Volume:Volume
Vit E	:	Vitamin E
μM	:	Micromolar

1. INTRODUCTION

Heavy metals have become a significant problem worldwide due to the intensive increase in industrialization and environmental pollution, leading to a growing number of research studies (Shirazi et al., 2022). Heavy metals, which can accumulate in numerous regions of the body and cause various deformities, especially contribute to the formation of reactive oxygen species (ROS) and free radicals, leading to oxidative stress and potentially fatal tissue and organ failures. Another significant issue with heavy metals is their inability to degrade or be destroyed, similar to organic pollutants, as evidenced by studies (Fonesca et al., 2006). Lead (Pb), a naturally occurring element found in geological materials, exhibits a high capacity for rapid dissemination in air, soil, and water environments. Pb is a commonly encountered environmental pollutant known to accumulate in organisms, leading to physiological and biochemical deformities (Maruldalı et al., 2010).

Partiularly, accumulatin in food sources and airborne transmission make it more easily transferable to humans. The duratin of exposure to Pb also directly correlates with the occurrence of these deformatins. The tisues where Pb accumulates most are sof tisues in young to middleaged individuals and bones in older individuals, with the liver, kidneys, and male reproductie system tisues being the most affcted organs (Kavas, 2008).

Similar to the toxic effct mechanisms of many metals, Pb exerts its toxic effct by inducing oxidatie stress. The oxidatie stress metabolism attmpts to compensate for this situatin through various antixidant enzymes. Endogenous antixidant mechanisms such as SOD, CAT, GPx, and GSH in the organism try to cope with reactie oxygen species; however, when the level of generated free radicals exceeds the body's antixidant capacity, oxidatie stress occurs. Free radicals affct all essentil compounds in cells, including lipids, proteins, DNA, carbohydrates, and enzymes. They disrupt aerobic respiratin in mitochondria (Soyer et al., 2006). Additinally, reactins causing cell membrane damage, such as lipid peroxidatin, occur when enzymes are insuffient. Unsaturated bonds of membrane cholesterol and fatt acids react with free radicals to produce peroxidatin products. Lipid damage is irreversible (Karahan, 2015).

One of the most fundamental factors ensuring the contiuity of life is reproduction Therefore, reproductie system health holds great importance for the contiuity of future generatins. Issues such as infertiity are increasing due to more frequent and intense exposure to various agents nowadays. Male-related infertiity problems manifest

themselves with conditions such as decreased sperm motility, morphological abnormalities, decreased sperm count, and pathological issues in testicular tissue (Aral, 2008). Factors causing these deformations that affect sperm quality can be genetic or environmental.

These environmental factors may include poor nutrition, poor air quality, heavy labor, smoking and alcohol consumption, medication use, and certain diseases like obesity. Studies have shown that heavy metals and Pb affect the reproductive system, disrupt sperm quality, and hormonal balance (Shirazi, 2022). In an experimental study, female rats were given Pb acetate in drinking water until the 21st day of lactation over a total of 120 days; male rats born from these females showed a decrease in testi volume and weight, seminiferous tubule diameters, and germinal epithelium height, and upon reaching puberty, a decrease in average sperm density and testosterone levels were observed (Dorostghoal et al., 2011).

ROS (reactie oxygen species) generated due to heavy metal stress causes oxidative stress and affects the reproductive system. They impair sperm quality and lead to consequences such as infertility. In addition to endogenous antioxidants that work to regulate this stress, studies are being conducted on reducing/preventing damage with externally obtained natural antioxidants (Ekici et al., 2016). Piceatannol, a compound derivative of resveratrol, is found occurring naturally in a diverse range of fruits, which encompass red grapes, white grapes, passion fruit, and highbush blueberries. (Matsui, Y et al, 2010). Researchers suggest that piceatannol demonstrates a diverse array of biological effects, including its potential as an antitumor agent, its antibacterial properties, and its role as an antioxidant. (Piotrowska, H et al, 2012). This study investigates the effects of piceatannol, a polyphenolic compound, on the reproductive system of male rats exposed to lead. Through histological, biochemical, and DNA analyses, it aims to assess any protective effects of piceatannol against lead-induced damage.

1.1. Heavy Metals and The Mechanism of Toxicity

Heavy metals, characterized by their high atomic mass and density, are naturally present in the environment and owe their widespread distribution to a diverse array of industrial, residential, agricultural, medical, and biotechnological uses (Tchounwou, P. B., 2012). The generation of reactive oxygen derivatives and the occurrence of oxidative stress reactions represent two significant factors contributing to heavy metal toxicity

(Flora, S. J. S et al., 2008). Metals within biological systems can trigger reactions leading to the oxidation of polyunsaturated fatty acids. Additionally, they can engage with nuclear proteins and DNA, inducing oxidative damage to intracellular biological molecules (Juan, C. A., et al., 2021). Endogenous antioxidants serve as the primary defense mechanism against cellular damage inflicted by reactive oxygen derivatives. This defensive system comprises key elements such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and reduced glutathione (GSH). They play pivotal roles as primary antioxidants in combating free radicals, particularly targeting the continuous generation of superoxide anion radicals (O2•) through the mitochondrial energy production pathway (MEPP) within normal human metabolism (Ighodaro, O. M. and Akinloye, O. A., 2018). Within the body, superoxide dismutase (SOD) decomposes superoxide anions (O2•) into hydrogen peroxide (H2O2) and separate oxygen radicals. Subsequently, glutathione peroxidase (GSH-Px) transforms H2O2 into water (H2O) and oxygen (O2) (Birben. E et al., 2012). During the GSH-Px reaction, glutathione (GSH) is consumed, and glutathione reductase (GR) converts it into its reduced form, known as the "active form." However, if the production rate of reactive oxygen intermediates surpasses the capacity of antioxidant systems to eliminate them, oxidative stress ensues (Galván-Arzate, S., et al., 2005).

1.2. General Characteristics and Sources of Lead

Pb is a light bluish, bright silvery metal. It is an element with an atomic number of 82 and an atomic mass of 207.19. It melts at 327.5 degrees Celsius and boils at 1740 degrees Celsius. In nature, it has four stable isotopes with mass numbers of 204, 206, 207, and 208. It is located in the 14th group of the 6th period of the periodic table. The concentration of lead in the Earth's crust is 16 µg/g. However, due to industrial activities, its amount in soil has risen above 5000 µg/g, in water above 10 µg/L, and in the air above 10 µg/m3 (Ciğerci, 2005).

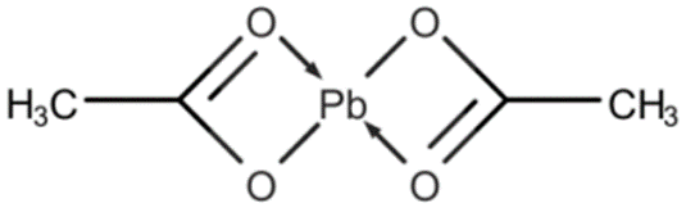

Figure 1.1. *Structure of acetate lead (Pb(C2H3O2)2)*

The permitted maximum limits for lead in foods are 6 ppm for solid foods and 1 ppm for liquid foods. The most concerning metals worldwide are arsenic, lead, cadmium, and mercury, listed in order. Pb is among naturally occurring metals. The most commonly encountered ores are the sulfur mineral galena (PbS) and its oxidized products cerussite (PbCO3) and anglesite (PbSO4). Among these minerals, galena is the most important. It is generally found in association with sphalerite (ZnS), silver, and pyrite (FeS2).

Lead (Pb) occurs naturally as a metal and typically forms compounds by bonding with two or more elements. When exposed to air and water, lead reacts to produce lead sulfate, lead carbonates, or lead oxide, serving as a protective layer against corrosion. It can interact with both acidic and basic substances, and due to its low melting point and position above hydrogen in the electromotive series, lead exhibits distinct properties. While lead is naturally present in the environment, human activities have significantly contributed to its increased levels (Shahid, M., et al, 2015). Lead enters the atmosphere through various sources such as lead mining, industrial processes using lead compounds and alloys, vehicle emissions, and the combustion of fossil fuels (Violante, A., et al, 2010). Lead is removed from the atmosphere through rainfall, where it is deposited onto soil or surface water. Additionally, lead is utilized as a pesticide in the cultivation of vegetables and fruits (Gall, J.E., et al., 2015). The disposal of waste products containing lead, the removal of lead-based paints from structures like bridges and buildings, and the disposal of damaged batteries from industries contribute to the accumulation of lead in municipal landfills. Lead binds tightly to soil particles and is predominantly found in the top layer of soil (Gupta, N., et al., 2008).

Lead enters water bodies or lakes when soil particles containing lead are washed away by rainwater. This leads to the transfer of lead to animals and plants from air, water, and soil, perpetuating a continuous cycle (Abadin et al., 2007).

The main sources of Pb exposure include drinking water, food, tobacco smoke, industrial processes, and household sources. Pb's industrial sources include gasoline, house paint, plumbing pipes, lead bullets, batteries, tin cans, toys, and faucets, used in over 900 professional industrial fields (Thurmer K, 2002). Pb is released into the atmosphere through industrial processes, mining activities, and vehicle exhausts. Therefore, it can enter the soil and be taken up by water bodies that plants can absorb, leading to human exposure to Pb through food or drinking water (Wani AL, 2011).

1.3. Effects of Lead on Human Health

Pb is an environmental pollutant. Despite the low amounts absorbed, prolonged exposure to Pb can accumulate in the human body system, resulting in lead poisoning or toxicity. Lead has a half-life of around 30 days in the blood, after which it diffuses into soft tissues such as the kidneys, brain, and liver and then distributed to bones, teeth and hair as lead phosphate (Engwa et al., 2019).

In general, Pb is transported by red blood cells to the liver and kidneys upon intake, where it binds to red blood cells immediately upon absorption, forming a major part of its toxic effects, and is mostly distributed as phosphate salts to teeth, bones, and hair (Y.Ming-Ho, 2005). Pb stored in bones and teeth in adults can be released back into the bloodstream, especially in situations of calcium stress and deficiency. A portion of Pb can also be stored in various soft tissues such as the liver, pancreas, brain, prostate, and testes (Sharma et al., 2011). Pb exposure, particularly affecting fetuses, embryos, and children more severely, triggers immune modulation, oxidative, and inflammatory mechanisms, leading to neurological, respiratory, nephrological, gastrointestinal, and cardiovascular disorders. Additionally, Pb interacts with various enzymatic steps. It interferes with synthesis by inhibiting enzymes such as delta-aminolevulinic acid dehydratase (ALAD) and heme synthetase, leading to functional loss. This reduces erythrocyte production by inhibiting porphyrin and heme synthesis, leading to the accumulation of aminolevulinic acid and protoporphyrin, resulting in toxic effects (Shaik et al., 2014).

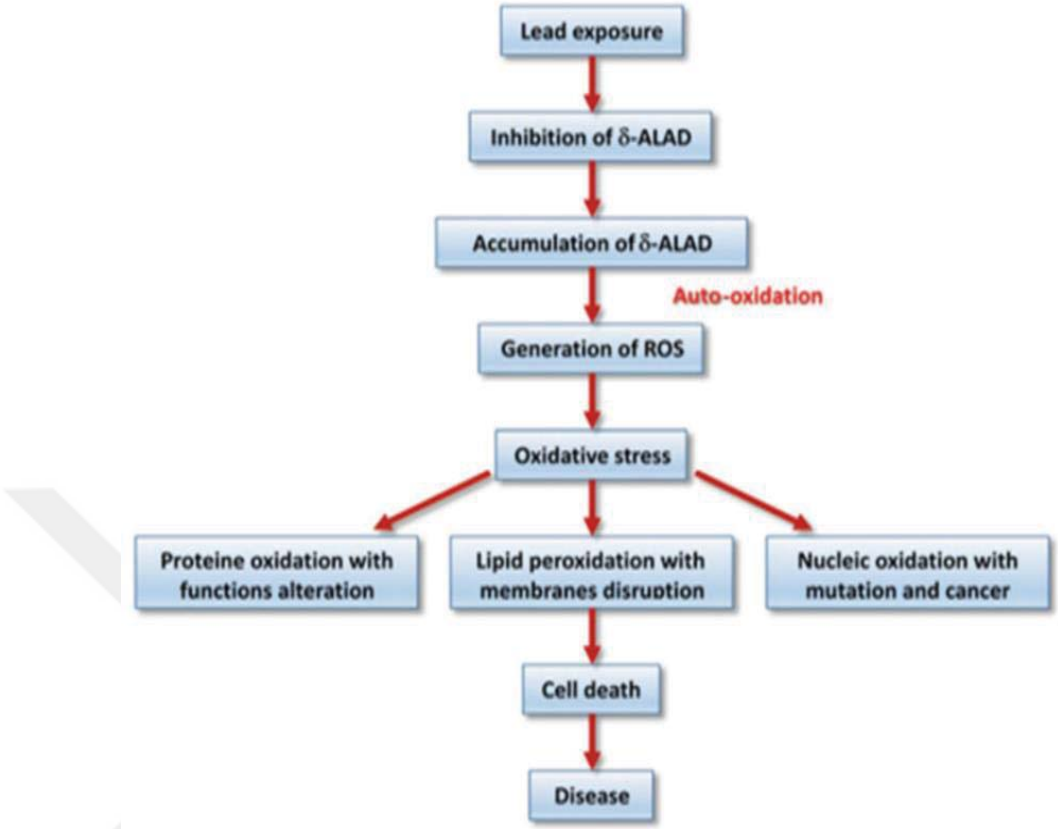

Figure 1.2. *Possible mechanism for lead-induced oxidative stress and cell death*

Pb reacts with enzyme proteins' sulfhydryl (-SH) groups, leading to their damage. It interacts with the sodium-potassium ATP pump, increasing cellular fragility. Through these mechanisms, it can disrupt the balance of the oxidant-antioxidant system and induce inflammatory responses in various organs (Andjelkovic, 2019). Exposure to Pb can alter physiological functions in the body and is associated with many diseases (Joseph, 2005). The international threshold for lead poisoning in blood is 10 µg/dl (Burki, 2012). Accumulation of Pb in cells disrupts mitochondrial function, inducing oxidative stress. This is a dominant cause of reduced cellular defense mechanisms (Santhosh Kumar R, 2018).

Lead poisoning leads to the production of Reactive Oxygen Species (ROS) like hydroperoxide, hydrogen peroxide, and singlet oxygen. Pb generates these free radicals, causing oxidative stress and cellular damage to the body cells. Oxidative stress occurs when there's an imbalance between ROS and antioxidant defenses in the body. Oxidative

stress induces cell and tissue damage, raising the risk of health issues such as cardiovascular disease and cancer (Flora, S.J., 2011).

Increased production of ROS leads to a reduction in antioxidant defenses, resulting in oxidative stress and subsequent cell damage. Lead additionally disrupts the function of other antioxidant enzymes such as superoxide dismutase and catalase (Violante, A., et al., 2010).

Various antioxidant enzymes and molecules have been used to assess oxidative damage caused by Pb. Reduced glutathione (GSH) and glutathione disulfide concentrations, as well as glutathione peroxidase, superoxide dismutase, and catalase activities, are the most commonly used (Solliway BM, 1996). Glutathione plays a crucial role in balancing ROS levels in the body. Approximately ninety percent of glutathione in cells exists in a reduced form, while the remaining ten percent is oxidized, serving as an antioxidant defense system. Glutathione stabilizes ROS and is converted back to its reduced form by glutathione reductase after being oxidized to glutathione disulfide (Sardar, K. et al., 2013).

Lead binds to the sulfhydryl group of glutathione, making it inactive and inhibiting the replenishment of GSH, thereby increasing oxidative stress (Batool, Z. et al., 2017). Even a small deposition of lead in the human body disrupts cellular function and adversely affects an individual's health. Pb is a highly toxic metal in humans and other mammals. This heavy metal can increase oxidative activity and trigger tissue damage by disrupting structures and reducing levels through interaction with endogenous antioxidant enzymes, and by disrupting membrane integrity via lipid peroxidation (Oteiza PI, 2004). Numerous studies have demonstrated that Pb induces damage by causing ROS formation in the liver, kidneys, brain, lungs, endothelial tissue, testes, and sperm (De Rosa M, 2003).

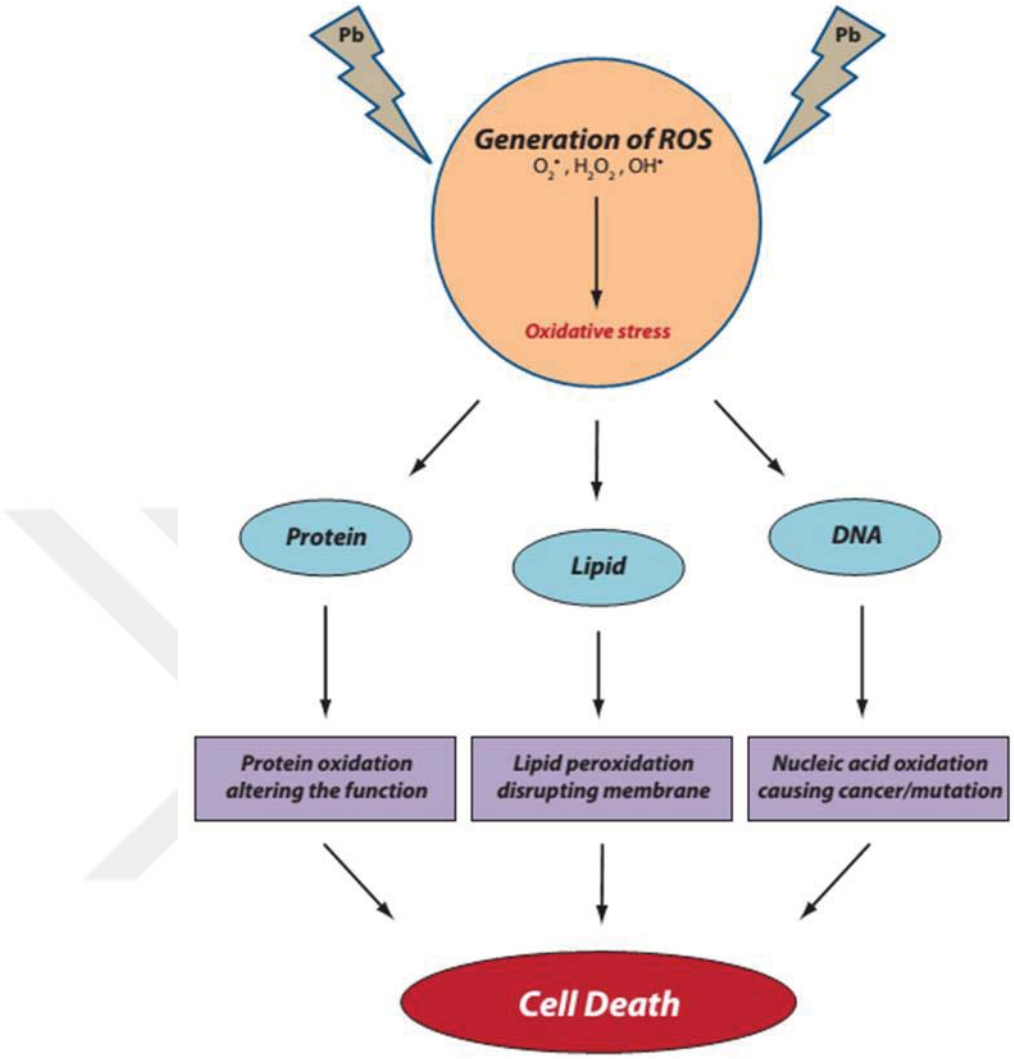

Figure 1.3. *Possible mechanism and targets for lead-induced oxidatie stress.*

Exposure to lead can happen through inhalation, ingestion, or skin contact. Direct contact with lead or lead-based compounds through the mouth, nose, eyes, or skin cracks can elevate lead levels. In adults, approximately 35–40% of inhaled lead dust settles in the lungs, with about 95% entering the bloodstream (Merill et al., 2007). Upon ingestion of inorganic lead, approximately 15% is absorbed, although this rate is higher in children, pregnant women, and individuals with deficiencies in calcium, zinc, or iron (Karri et al., 2008). A certain quantity of lead, typically sequestered in tissues such as bones, teeth, hair, or nails, is deemed non-toxic due to its limited availability to other tissues. The half-life of lead in these tissues leads to its release into the bloodstream long after the initial exposure (Patrick, 2006). Extended exposure to lead over many years results in a much

slower clearance process. This is attributed to the gradual buildup of lead in bones, which is gradually released over an extended period (Grant, 2009).

In addition to bones, teeth, and blood, lead is stored in various other tissues in the body, including the brain, spleen, kidneys, liver, and lungs (Dart et al., 2004). Slight amounts of lead are excreted through feces, while minor amounts are eliminated through hair, nails, and sweat (Rubin & Strayer, 2008). Lead's impact has been extensively researched even at the cellular level. Heavy metals like lead generate reactive radicals, causing harm to cell structures such as DNA and cell membranes (Kosnett, 2006). Lead disrupts enzymes involved in vitamin D synthesis and those responsible for preserving cell membrane integrity. Moreover, it interferes with DNA transcription processes. Lead's interference with cell membrane maintenance renders red blood cells more fragile, leading to anemia. There are also suggestions that lead might affect the permeability of blood vessels and the synthesis of collagen. The reduced activity of immune system cells, like polymorphonuclear leukocytes, results in decreased immune function.

Lead poisoning contributes to anemia primarily because it disrupts the function of a crucial enzyme called delta-aminolevulinic acid dehydratase (ALAD). This enzyme is vital for the production of heme, a cofactor present in hemoglobin (Patrick et al., 2006).

Lead interference with heme precursors like aminolevulinic acid has been observed to accumulate, potentially harming neurons both directly and indirectly (Fujita et al., 2002). A recent discovery revealed that the accumulation of organic lead in the liver can lead to oxidative imbalance and protein impairment, potentially causing endoplasmic reticulum stress and subsequent liver injuries (Fang et al., 2014). In a recent study, researchers investigated the combined effects of lead exposure and a high-fat diet (HFD). They found that both lead and HFD contributed to the suppression of osteoblastogenesis and changes in progenitor cell differentiation, leading to increased osteoclastogenesis and adipogenesis. Moreover, researchers noted an increase in PPAR-γ activity and inhibited β-catenin activity in MC3T3 cells exposed to lead and non-esterified fatty acids in vitro. They concluded that lead and HFD contributed to selective deficit bone accrual, potentially involving reduced Wnt signaling and alterations in progenitor cell activity (Beier et al., 2015).

1.4. Free Radicals and Oxidative Stress

A free radical refers to a molecule or atom containing one or more unpaired electrons, which often renders them highly reactive. Consequently, free radicals have the

potential to induce organ and tissue damage by altering the structure of proteins, nucleic acids, and lipids (Di Meo, S. and Venditti, P., 2020). Under normal conditions, minimal quantities of reactive oxygen species (ROS) such as the hydroxyl radical (OH•), superoxide (O2•–), and hydrogen peroxide (H2O2) are produced during oxygen metabolism. The primary origins of reactive oxygen and nitrogen species include the mitochondrial electron transport chain and oxidase enzymes, such as NAD(P)H oxidases, xanthine oxidases, lipoxygenases, cyclooxygenases, cytochrome P450 enzymes, and uncoupled nitric oxide synthases (V. Goncharov, N., et al., 2015). Reactive oxygen species (ROS) participate in numerous biological processes, including the modulation of transcription factors, signaling pathways, and direct engagements with various intracellular molecules (Vaziri, N. D., and Rodríguez Iturbe, B., 2006). Additionally, reactive oxygen species (ROS) generated by activated leukocytes and macrophages exhibit strong antibacterial properties (Papayannopoulos, V., and Zychlinsky, A., 2009). Typically, the antioxidant defense system, comprising various endogenous enzymes and dietary antioxidant compounds, effectively neutralizes ROS produced during metabolism. Nonetheless, in numerous pathological conditions, the rate of ROS formation surpasses the capability of the antioxidant defense system to counteract it, leading to oxidative stress (Rao, P. S. et al., 2011). In such circumstances, ROS attacks biomolecules, disrupting redox-sensitive signal transduction pathways and transcription factors, resulting in numerous impairments and dysfunctions (Trachootham, D., et al., 2008). There is growing recognition that elevated levels of ROS could serve as a crucial element in the development of various chronic diseases, encompassing cardiovascular disease, atherosclerosis, cancer, diabetes, Parkinson's disease, Alzheimer's disease, and liver-related disorders (Shankar, K., and Mehendale, H. M., 2014).

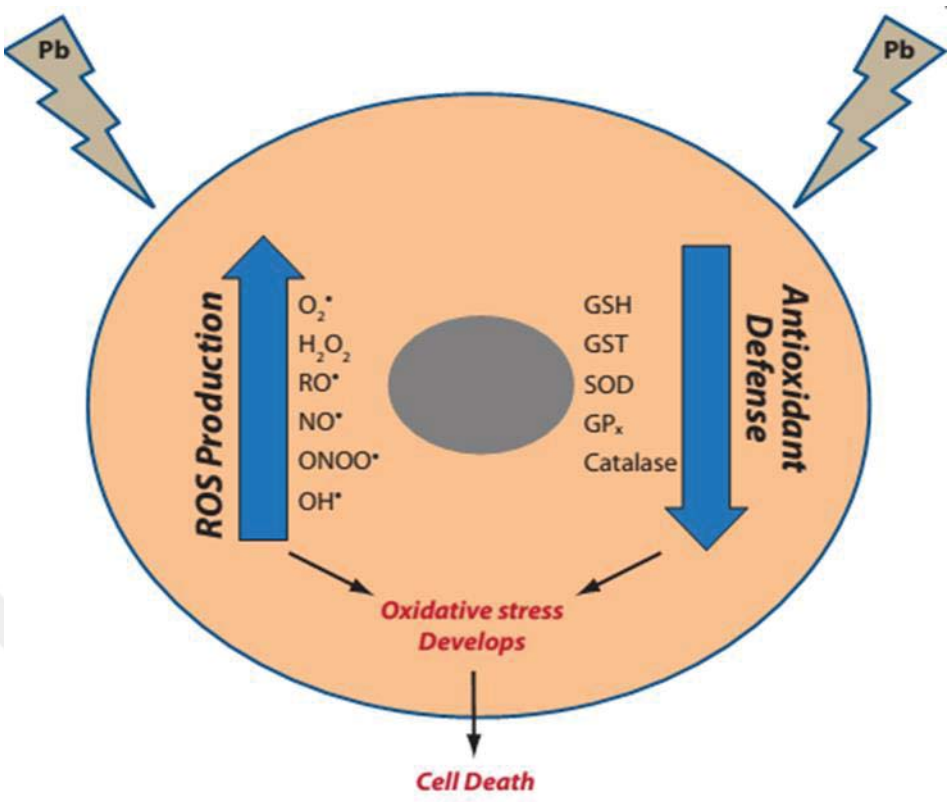

Figure 1.4. *Mechanism underlying the development of oxidative stress in a cell on lead exposure.*

1.5. Antioxidant Defense Systems/ Mechanisms.

Aerobic organisms, which rely on oxygen for energy production, have developed antioxidant defense systems consisting of both enzymatic and non-enzymatic antioxidants. These systems efficiently mitigate the harmful impacts of ROS (Birben, E. et al., 2012). Ensuring adequate levels of antioxidants and proper localization of antioxidant enzymes are integral aspects of regulating antioxidant capacity (Sies, H., 1993). Key antioxidant enzymes that combat superoxide radicals comprise SOD, CAT, and GSH-Px (Reddy, R. D. and Yao, J. K., 1996). Key antioxidant enzymes that combat superoxide radicals comprise SOD, CAT, and GSH-Px (Reddy, R. D. and Yao, J. K., 1996). These enzymes work in concert to prevent the oxidation of lipids by superoxide and hydroxyl radicals. SOD transforms superoxide into hydrogen peroxide, which is then eliminated by CAT into water and oxygen, preventing the formation of hydroxyl radicals. Alongside CAT, GSH-Px serves as a prominent intracellular antioxidant enzyme, oxidizing reduced GSH into glutathione disulfide (GSSG), thereby converting peroxides and hydroxyl radicals into non-toxic forms. The enzyme (GR) glutathione reductase reverses GSSG back into GSH (Wu, J. Q., 2013).

1.5.1. Enzymatic endogenous defense systems

1.5.1.1. Superoxide dismutase (SOD)

Superoxide dismutase, a metalloprotein, was first identified in 1968. Superoxide dismutases (SODs) are common enzymes that facilitate the conversion of superoxide to molecular oxygen and peroxide, safeguarding the cell against the detrimental effects of aerobic respiration (Perry, J. J et al., 2010). Depending on the metal co-factor they contain, SODs can be categorized into four groups: FeSOD, MnSOD, Cu/ZnSOD, and NiSOD (Sen, S., and Chakraborty, R. 2011). FeSOD and NiSOD represent bacterial enzymes, with FeSOD predominantly localized within chloroplasts. CuZnSODs, on the other hand, are typically distributed throughout the extracellular matrix and cytoplasm of eukaryotic cells. MnSOD, located across bacteria and eukaryotes, is primarily situated within the mitochondrial matrix of eukaryotic cells, where it encounters a notable production of superoxide radicals (Azadmanesh, J. & Borgstahl, G. E., 2018). The predominant form of superoxide dismutase is Cu/Zn-SOD, typically situated within the cytoplasm and possessing a molecular weight of 32 kDa. While Cu/Zn-SOD activity can be detected in various tissues, its highest concentrations are often observed in the liver and kidney, organs known for their significant metabolic functions (Okado-Matsumoto, A., and Fridovich, I. 2001).

1.5.1.2. Catalase (CAT)

Catalase comprises a hemoprotein structure consisting of four subunits, each containing a heme group and an associated NADPH molecule (Majumder, D. et al., 2017). Due to its crucial role in neutralizing byproducts of lipid metabolism, catalase is commonly localized within intracellular organelles such as peroxisomes. Liver cells, characterized by a higher abundance of peroxisomes compared to other organs, rely on catalase to convert hydrogen peroxide produced during lipid metabolism into water (H2O) and oxygen (O2). While hydrogen peroxide itself is not a reactive radical and does not directly interact with biological substances, its significance lies in its ability to catalyze the formation of the highly reactive hydroxyl radical (OH) via the Fenton reaction, facilitated by Fe and Cu ions (Gutteridge, J. M. 1994).

1.5.1.3. Glutathione peroxidase (GPx)

Discovered initially in 1957 as an enzyme within erythrocytes safeguarding hemoglobin from oxidative damage, glutathione peroxidase (GPx) is a vital endogenous

enzyme responsible for catalyzing the conversion of hydrogen peroxide (H2O2) into water (Lubos, E., et al., 2011). The enzyme effectively stops lipid peroxidation reactions, protecting cells from oxidative stress (Ighodaro, O. M., and Akinloye, O. A., 2018). In mammalian tissues, there are four primary selenium-dependent GPx isozymes identified: GPx1, GPx2, GPx3, and GPx4. These are primarily situated in the liver, gastrointestinal tract, plasma, and kidneys, respectively (Margis, R et al., 2008). GPx1 regulates the insulin signaling pathway, while GPx2 plays a dual role in cancer formation, influencing both its initiation and progression stages. GPx3 is membrane-associated, potentially accounting for its peroxidative activity despite low plasma GSH levels. GPx4, on the other hand, governs apoptosis (Brigelius-Flohé, R., and Maiorino, M., 2013). Glutathione peroxidases facilitate the conversion of hydroperoxides (ROOH) by glutathione (GSH). This reaction results in the production of water, alcohol (ROH), and glutathione disulfide (GSSG). Subsequently, the enzyme glutathione reductase is accountable for regenerating GSH from GSSG within the cell (Mannervik, B., 1985).

1.5.2. Non-enzymatic defense systems

Polyphenols serve as natural antioxidants and are present in various sources such as fruits, vegetables, essential oils, cereals, tea, and beverages (Zhang, H. and Tsao, R., 2016). Research has revealed that plants contain approximately 8000 isolated and identified phenolic chemical compounds (Singh, B. et al., 2017). Consuming polyphenols has been associated with a reduced risk of various conditions including heart disease, cancer, atherosclerosis, diabetes, osteoporosis, and neurological diseases (Scalbert, A., et al., 2005). While it's challenging to predict the precise impact of polyphenol consumption on disease prevention in humans, the widely acknowledged mechanism involves polyphenols acting as antioxidants, either scavenging or limiting the generation of free radicals (Chiva-Blanch, G., and Visioli, F., 2012). Polyphenols act as robust antioxidants, effectively neutralizing reactive species by donating either an electron or a hydrogen atom. They work to restrain oxidation levels by impeding or deactivating the formation of free radical precursors. Specifically, in lipid peroxidation chain reactions, they typically function as direct radical scavengers, known as chain breakers. By providing free radicals with an electron, they neutralize and convert them into stable, "less reactive" radicals, thereby terminating the chain processes (Tsao, R., 2010)..

1.5.2.1. Piceatannol (PIC)

Piceatannol (trans-3,3',4,5'-tetrahydroxystilbene) is a hydroxylated derivative of resveratrol found in foods like berries and passion fruits (A.M. Rimando, et al., 2004; S. Sano et al., 2011). In wines, this dietary polyphenol can reach concentrations similar to those of resveratrol (F. Buiarelli et al., 2007). Piceatannol is well-known for its strong antioxidant properties in solution and has played a role in demonstrating that resveratrol is not the most effective radical scavenger among natural polyphenols (M. Murias et al., 2005; S. He and X. Yan, 2013). Piceatannol has also been shown to have prooxidative characteristic (M. Ruweler et al., 2009; M. Kucinska et al., 2014) and greater metabolic stability compared to resveratrol (Y. Setoguchi et al., 2014).

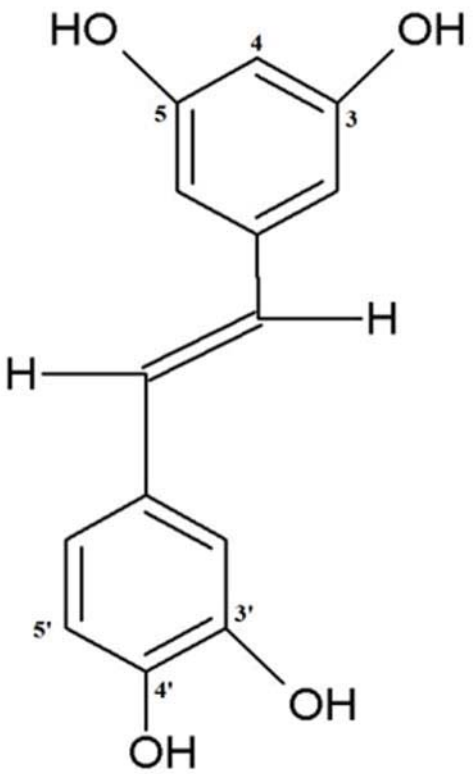

Figure 1.5. *Molecular structure of Piceatannol*

The potent antioxidant properties of piceatannol have paved the way for its diverse pharmacological applications. Widely utilized as an antimicrobial, antifungal, and antiallergenic agent, piceatannol showcases promising pharmacological attributes for addressing numerous chronic ailments. Its multifaceted biological roles and diverse mechanisms of action underscore its significance in therapeutic interventions. It demonstrates anticancer, antioxidant, antileukemic, and anti-inflammatory activities.

Piceatannol is also effective against diseases such as breast cancer, prostate cancer, colon cancer, leukemia, lymphoma, and melanoma (Piotrowska H et al, 2012).

Piceatannol's antioxidant properties originate from the hydroxyl groups within its stilbene rings. It effectively scavenges lipid peroxyl radicals and demonstrates stability in its semiquinone radical form. The antioxidant capability of piceatannol is validated by its capacity to inhibit melanin production in B16 melanoma cells (Piotrowska H et al, 2012; Wittgen HG and van Kempen LC., 2007).

Piceatannol demonstrates anticancer effects by inhibiting the proliferation of various tumor cells, including lymphoma, leukemia, breast cancer, prostate cancer, and colon cancer. It hinders cancer cell growth and induces apoptosis. This proapoptotic impact may arise from mitochondrial potential loss, caspase activation, and cytochrome C release (Piotrowska H et al, 2012). Piceatannol's proapoptotic effects have been observed in several cancer types, such as skin, prostate, bladder, and breast cancers. It demonstrates cytotoxic activity against prostate cancer cells and inhibits JAK1 (Janus kinase) activity, suggesting its potential as a therapeutic agent for prostate cancer (Piotrowska H et al, 2012). In colon and liver cancer, piceatannol has been found to induce cell cycle arrest, leading to the accumulation of cells in the G1 and S phases (Wolter F et al, 2002). In bladder cancer, piceatannol has been reported to induce apoptosis and inhibit the progression of cells from G0/G1 phase (Kuo PL and Hus YL 2008).

Piceatannol, a naturally occurring polyphenolic compound, exhibits diverse biological activities and potential therapeutic benefits. Its structural features, including an additional hydroxyl group compared to resveratrol, contribute to its enhanced efficacy. However, thorough investigation into aspects such as bioavailability, metabolic pathways, and potential toxicity in humans is crucial for its clinical application. Moreover, Piceatannol is garnering increasing scientific interest due to its biological activity and bioavailability compared to resveratrol. It shows promising potential for addressing health-related diseases. However, further research is essential to substantiate its efficacy in disease prevention and treatment.

1.5.2.2. Other exogenous antioxidants

Mostly taken through diet or dietary supplements, carnitines from animal or plant sources, E vitamin, C vitamin, and carotenoids are exogenous antioxidants. E vitamin is a vital antioxidant molecule localized in the cell membrane. It is suggested to inhibit lipid

peroxidation and scavenge free radicals produced during the monovalent reduction of molecular oxygen and during the normal activities of oxidative enzymes. The production of these radicals results in phospholipid peroxidation in sperm mitochondria, leading to low motility (Palamanda J.R., 1993). Vitamin C has a high efficacy in clearing ROS (Brzozowski T., 2001). In a study involving 30 infertile but healthy males, daily supplementation of 200 mg and 100 mg of vitamin C increased sperm count by 112% and 140%, respectively. It has been stated that the concentration of vitamin C in seminal plasma is 10 times higher than in serum (Dawson E.B., 1987). Carotenoids are naturally found in fruits and vegetables. They are responsible for the yellow, red, and orange pigments in plants (Rao A.V., 2006). The most important compound in the carotenoid family is lycopene. Research results are being discussed more in testicles on any subject. This could be due to the antioxidant factor in spermatogenesis (Zini A., 2010).

1.6. Reproductive System

The male reproductive system is designed to produce male gametes and transmit them to the female reproductive system through supportive fluids and testosterone synthesis. Paired testes (the site of testosterone and sperm production), scrotum (partition for testis localization), epididymis, vas deferens, seminal vesicles, prostate gland, bulbourethral gland, ejaculatory duct, urethra, and penis are parts of the male reproductive system. The male reproductive system primarily functions in the production, nourishment, and temporary storage of male gametes (spermatozoa) via spermatogenesis (Oyovwi Mega Obukohwo, 2021). It produces androgens and estrogens via steroid genesis and, most importantly, is associated with the mating organ (penis) facilitating the entry of semen containing spermatozoa into the female genital system through copulation (Stevens, 2005). The male reproductive system is divided into four main parts. Figure 1-1 depicts a representation of the male reproductive system.

1. Testes

2. Accessory ducts: Including epididymis, vas deferens, and ejaculatory duct

3. Accessory glands: These glands are internal reproductive organs that nourish sperm cells and lubricate the canal system with fluids. They include seminal vesicles, bulbourethral glands, and prostate glands (Cowper's glands).

4. Supportive structures containing scrotum and penis.

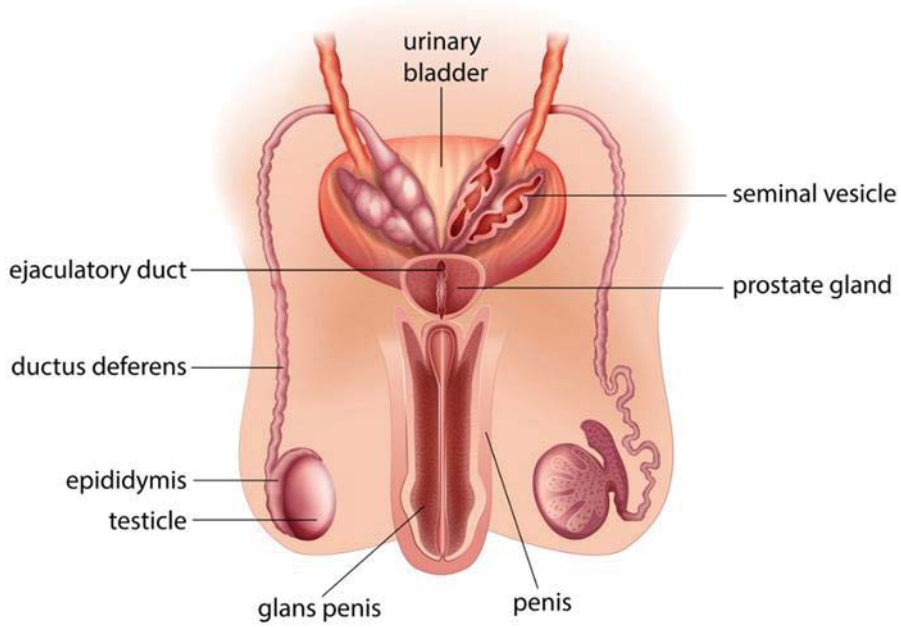

Figure 1.6. *Typical Structure of the Male Reproductive System, (Mescher, 2016)*

Spermatozoa formed in the testis are functionally immature and attain functional maturity as they pass through the epididymis. The absorptive and secretory behavior of the epididymal epithelium helps maintain a specific intraluminal environment necessary for sperm maturation (Cooper, (2011)).

Male reproductive organs are functionally organized for the following purposes (Stevens, 2005):

Spermatogenic function; for sperm production

Maintenance and transportation of sperm (male reproductive cells and protective seminal fluid)

Sperm ejaculation function; is to expel sperm into the female reproductive system

Hormonal function; for the production and secretion of male sex hormones such as testosterone.

1.6.1. Spermatogenesis

The cellular divisions and developmental changes occurring in the seminiferous tubules of the testes are referred to as spermatogenesis and consist of two main parts. In Part 1, spermatogenesis begins with spermatogonia, which undergo mitotic divisions of stem cells to produce spermatocytes in the early stages, followed by meiosis to reduce the chromosome count to produce spermatids. In Part 2, spermiogenesis occurs, where

spermatids undergo metamorphic changes to become spermatozoa (Gribbins, 2005). Figure-6 depicts the process of spermatogenesis, while Figure-7 illustrates the microscopic anatomy of seminiferous tubules.

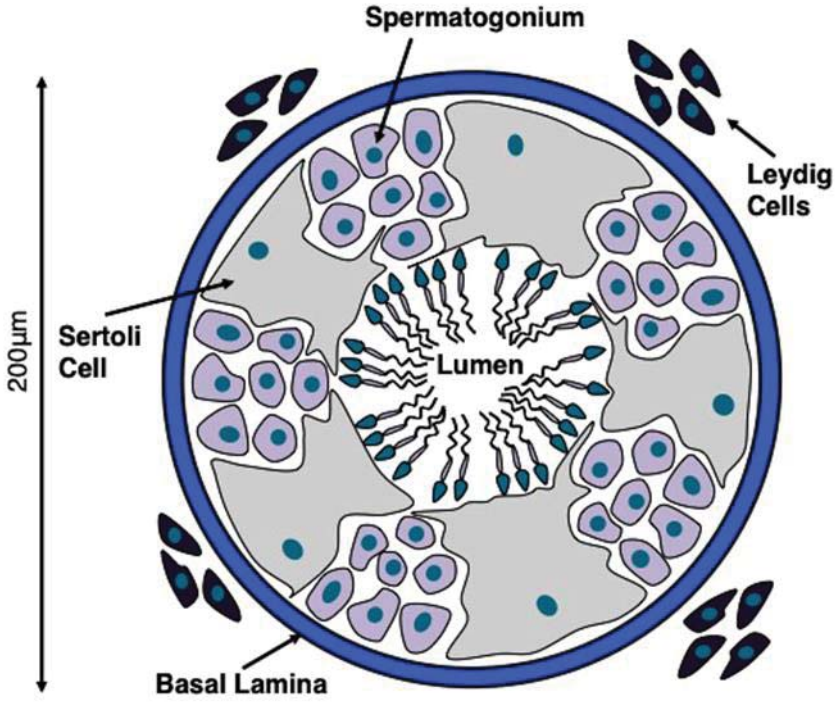

Figure 1.7. *Demonstration of the Microscopic Anatomy of Seminiferous Tubules, (Gribbins, 2005)*

Spermatogenesis is a highly organized but complex process that typically continues throughout life (Ohmura, 2003). Spermatogenesis is divided into three main phases: spermatocytogenesis, meiotic division, and spermiogenesis. The process involves (1) primary spermatocytes, which usually undergo self-renewal and multiply, (2) secondary spermatocytes, (3) spermatids, and (4) spermatozoa, located on the basal membrane of seminiferous tubules (Nakamoto, 2004). Spermatogonia undergo mitotic division to produce primary spermatocytes, one of the pair of primary spermatocytes undergoes meiotic division to generate secondary spermatocytes. When spermatogonia (diploid primary spermatocytes) complete the first meiosis, two haploid daughter cells known as secondary spermatocytes will be produced. At the end of the second meiotic cell division, each of the two secondary spermatocytes generates two haploid spermatids (Ridge & Dubuque, 2004).

The most common causes of male infertility are associated with spermatogenesis. Semen analysis (evaluation of sperm count, semen volume, sperm morphology, and functional parameters) is one of the first laboratory tests commonly performed for infertile couples, as it is relatively easy, non-invasive, and inexpensive. Research

conducted on men exposed to Pb occupationally has shown that as seminal plasma or blood Pb concentrations increase, typically above >40μg/dl levels, but sometimes even at <10μg/dl levels, multiple sperm parameters are affected.

Numerous studies on the reproductive system of male animals have documented Pb as a toxic substance for testicular tissue and functions.

1.6.2. Cell types

1.6.2.1. Spermatogonium

Studies on spermatogonial stem cells conducted to date have mostly been carried out on experimental animals, with the majority of information obtained from studies conducted on mice. Primordial germ cells, originating from the epiblast and migrating from the yolk sac, reach the gonad during the 10th to 12th days of embryonic development. Germ cells in mice gonads, derived from these primordial germ cells, are termed gonocytes on the 13th day of embryonic development. These gonocytes in postnatal testes form the origin of spermatogonial stem cells. After birth, gonocytes migrate to the basal membrane of the seminiferous tubule and differentiate into spermatogonial stem cells. By the 6th day after birth, gonocytes differentiate into type A spermatogonia, and by the 8th day, type B spermatogonia are formed (Bellvè et al., 1977).

There are two main types of spermatogonium cells. These are named as Type A and Type B. Type A spermatogonium, which forms on the 6th day after birth, can continue its life in three different ways:

It takes on the task of preserving the stem cell pool by proliferating through mitotic division.

It differentiates and transforms into Type B, from which other cell structures of spermatogenesis develop.

It undergoes controlled cell death.

1.6.2.2. Spermatids

When Type B spermatogonium cells develop and reach a certain size, they become suitable for meiotic division, they stop proliferating and differentiate into primary spermatocytes. . The cells formed as a result of the meiosis division 1 of these cells are called secondary spermatocytes. The two secondary spermatocytes derived from each primary spermatocyte proceed through meiotic division II to produce four spermatids (Alberts B, et al, 2002).

1.6.2.3. Sperm

Primary spermatocytes undergo meiotic division I to become secondary spermatocytes. Following meiotic division II, secondary spermatocytes produce haploid spermatids. These spermatids then undergo morphological changes to become mature spermatozoa, which enter the lumen of the seminiferous tubule. The sperm then move to the epididymis, a coiled tube on the surface of the testis, where they continue to mature and are stored (Alberts B, et al., 2002).

1.6.2.4. Sertoli cells

Sertoli cells, found in the seminiferous tubules of the testes, were first described by the Italian physician Enrico Sertoli in 1865 and are named in his honor (Griswold MD, 2018). These cells, along with spermatogonia, are crucial for sperm production. Sertoli cells are large, closely connected cells located near the basolateral area of the seminiferous tubule. Also known as sustentacular cells of Sertoli, they play a vital role in nurturing and supporting the development of primary spermatogonia (Duan P, et al., 2017).

Sertoli cells play a crucial role in spermiogenesis, aiding in the production of viable sperm. They secrete essential molecules such as androgen-binding protein (ABP), inhibin B, and activin, which support spermatogenesis either directly or through hormonal feedback mechanisms. Sertoli cells also respond to pituitary hormones like follicle-stimulating hormone (FSH), initiating and sustaining spermatogenesis by supporting the adjacent spermatogonia (Ravel C and Jaillard S, 2011). Their absence in the testes can lead to male infertility despite normal sperm production (Chojnacka K et al., 2016).

1.6.2.5. Leydig cells

Leydig cells are located in the connective tissue surrounding the seminiferous tubules within the testes. During fetal development, these cells produce high levels of androgens, such as testosterone or androstenedione, which are crucial for the development of male reproductive organs and brain masculinization. After birth, androgen production declines significantly but rises again as adult Leydig cells develop from stem cells. In adults, Leydig cells respond to luteinizing hormone (LH) by producing cyclic AMP (cAMP), which enhances cholesterol transport to mitochondria. There, cholesterol is converted to pregnenolone by the enzyme CYP11A1, and subsequently to testosterone through further enzymatic processes in the mitochondria and endoplasmic reticulum (Zirkin et al., 2018).

1.6.3. Reproductive hormones

Reproductive hormones have a crucial and intricate role in regulating spermatogenesis and sperm development. Testosterone, the main male sex hormone, is produced and released by Leydig cells in the testes, stimulated by luteinizing hormone (LH).

Over the past 30 years, numerous studies have examined the endocrine regulation of spermatogenesis (O'Donnell et al., 2017). The initiation and maintenance of normal spermatogenesis are primarily regulated by the gonadotropins LH (luteinizing hormone) and FSH (follicle-stimulating hormone). The hypothalamus produces and releases gonadotropin-releasing hormone (GnRH), which controls the secretion of LH (luteinizing hormone) and FSH (follicle-stimulating hormone) from the pituitary gland. LH then targets Leydig cells in the testes to promote the production of androgens, such as testosterone. These androgens act on receptors in the seminiferous epithelium, supporting and stimulating spermatogenesis and aiding in the maturation of germ cells within the seminiferous tubules (Corenblum & Boyd, 2017).

FSH directly targets Sertoli cells to aid in spermatogenesis. While both androgens and FSH are essential for this process, successful spermatogenesis also depends on the collaboration of growth factors, signaling molecules, and various internal mechanisms (O'Donnell et al., 2017).

Testosterone regulation is controlled by the release of GnRH (gonadotropin-releasing hormone) and gonadotropins. Elevated blood levels of testosterone inhibit the release of LH from the pituitary gland. In addition, Sertoli cells produce inhibin B and follistatin, which suppress the release of FSH from the pituitary, whereas activin stimulates it (Woldemeskel, 2017).

1.7. The Relationship Between Lead and The Reproductive System

The endocrine system maintains the homeostasis of body systems through hormones, which can travel long distances in the body and often have potent effects. Endocrine-disrupting chemicals are substances that alter the course of endocrine systems in a way that adversely affects the organism itself or its offspring (Marcoccia D, 2017). These chemicals can be found in various everyday products and items such as food, water, plastics, shampoos, clothing, toothpaste, soaps, fertilizers, paper, textiles, carpets, kitchenware, bedding, toys, cosmetics, and deodorants (Joensen UN, 2009). Androgens (such as testosterone) are particularly important for the development of male secondary

characteristics and also support the spermatogenesis process; hence, the endocrine system is especially important for male reproductive development.

Decreases in male reproductive health especially sperm count, and testosterone are associated with an increase in various endocrine-disrupting chemicals such as heavy metals (Nordkap L, 2012). The group of endocrine-disrupting chemicals includes different substances such as dioxins, bisphenol A, and heavy metals (Duffus, 2002). In one study, it was reported that average semen Pb concentrations of 2 μg/dl were inversely proportional to serum testosterone in men occupationally exposed to Pb (Alexander BH, 1998).

Suppression of testicular testosterone levels and increased levels of steroid-binding globulin due to increased Pb exposure duration have been demonstrated in rats exposed to Pb for 30 days (Rodamilans M, 1988). However, there are reports of increased serum testosterone concentrations between men with relatively low (median 5 μg/dl) and relatively high (greater than 40 μg/dl) blood Pb levels (Gustafson A, 1989). These findings suggest that besides direct inhibition of androgen biosynthesis in Leydig cells, Pb-induced disruptions in testosterone secretion in the reproductive hormonal axis may involve other hormonal feedback pathways, such as reflex deficiency in response to plasma testosterone (Wiebe JP, 1983).

The results of experimental studies in rats indicate that the effects of Pb cover multiple sites on male reproductive hormones, but a major part of these disorders likely occurs in the hypothalamic-pituitary-testosterone (HPT) axis. For instance, depending on the levels and duration of exposure to Pb, signals within and between the hypothalamus and pituitary gland in mice appear to be disrupted by Pb (Ronis MJ, 1996).

2. MATERIAL AND METHOD

2.1. The Laboratory Animal Foundation and The Establishment of Experimentation Groups

2.1.1. Experimental animals

This thesis study adhered to the guidelines established by the Experimental Animals Ethics Committee (Decision No: 2018-52) of Anadolu University for all experiments involving animals. Male Sprague Dawley rats, with an average weight ranging from approximately 200 to 300 grams and aged between 8 to 12 weeks, were utilized in the experiments. Before commencing the experiments, the animals underwent a 5-day acclimatization period to laboratory conditions. During this period, they were housed in cages with eight animals per cage, maintained at a room temperature of 24 ± 2°C, with an ambient humidity level of 55 ± 10%.

The rats were kept in a 12-hour light-dark cycle and had free access to standard rat food and water during the entire experiment. This study aims to find the best dosage of lead acetate (Pb(C2H3O2)2), a known genotoxic substance, by reviewing relevant academic papers. Adlibmoradi et al. (2015) used a single dose of lead acetate at 20 mg/kg/day. In a thorough analysis of published literature by Lihong Yang et al. (2017), it was found that the recommended dosage of piceatannol is 5 mg/kg, which has notable pharmacological effects.

On the 28th day, the animals were dissected, and 1.5 mg/kg of urate anesthesia was administered to the rats before dissection. The right epididymis was gently milked to collect sperm, which was then transferred to a petri dish containing DMEM/Ham's F12 solution at 37 degrees Celsius. The cauda epididymis was also placed in a separate petri dish with the same medium, and the vascular and adipose tissues were carefully removed. The collected parts were evaluated for sperm concentration, motility, and morphology. Additionally, some of the testicular tissue underwent histological processing for tissue follow-up and single-cell gel electrophoresis. Another portion was promptly frozen in liquid nitrogen and stored at -80°C for subsequent biochemical analysis. Further details regarding injections and grouping are provided below.

2.1.2. Experimental groups

The experimental groups were organized into four groups, each comprising eight rats. A repeated-dose intraperitoneal toxicity study was conducted over a duration of 28 days for the experimental groups, as outlined below:

Group 1 (Control): The animals were intraperitoneally administered physiological saline (0.9%) daily for a period of 28 days.

Group 2 (Pb): Lead acetate (Pb(C2H3O2)2) was intraperitoneally administered at a dose of 20 mg/kg/day for a duration of 28 days.

Group 3 (Pb+piceatannol): Lead acetate was administered intraperitoneally (i.p.) at a dosage of 20 mg/kg/day for a span of 14 days. After this period of 14 days, on the day 15, Piceatannol was administered intraperitoneally at a dosage of 5 mg/kg/day for the next 14 days to complete 28th days.

Group 4 (PiC): The animals were given physiological saline (0.9%) for a period of 14 days. Starting from the 15th day onwards, Piceatannol was administered at a dosage of 5 mg/kg/day for an additional 14 days.

2.1.3. Sample collection from experimental animals

After 24 hours following the last dose of lead acetate (Pb) and Piceatannol (PIC), the rats' weights were recorded. Subsequently, the rats were euthanized under intraperitoneal anesthesia using 80 mg/kg of ketamine, and blood was collected from the right ventricle of the heart. Plasma samples were prepared by first allowing the blood samples from the rats to stand at 2–8°C overnight. Subsequently, the samples were centrifuged at 1000 x g for 15 minutes. These plasma samples were then stored at -20°C in 200 µl aliquots for later analysis. They were used to determine the levels of hormones involved in the spermatogenesis process, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone.

Following blood collection, the right testis and right epididymis tissues of the rats were removed. The left testis and left epididymis tissues were treated with phosphate buffer solution (PBS) containing NaCl (8 g/L), KCl (0.2 g/L), KH2PO4 (0.2 g/L), and Na2HPO4 (1.14 g/L) at a pH of 7.4. This treatment aimed to remove any blood and other contaminants from the tissues. After cleaning, the testis and epididymis tissues were weighed, and their weights were documented. The left testis tissues were divided into equal portions and stored at -20°C for subsequent examination of parameters such as glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT), which are crucial in assessing oxidative stress. The right testis tissues were fixed for histopathological examination following cleaning with PBS. The cauda tissues from the right epididymis were utilized to evaluate sperm parameters.

2.2. Assessment of Sperm Samples

2.2.1. Collection and evaluation of semen samples

The spermatozoa extracted from the right epididymis immediately after euthanizing the rats were transferred to a Petri dish containing DMEM/Ham's F-12 solution at 37°C. The cauda epididymis was then placed in a new Petri dish containing 1 ml of the same medium, and any blood vessels and adipose tissue were carefully removed. Approximately 0.5 cm of the cauda epididymis was further placed in another Petri dish containing 1 ml of the same medium, allowing the spermatozoa to float out from the tissue for a duration of 1 minute.

2.2.2. Assessment of sperm concentration and motility

Five microliters of a concentrated spermatozoa solution were carefully placed onto a Leja slide. A negative phase-contrast objective (4×) was used on the microscope along with a phase-contrast enhancer to observe sperm motility and concentration using the sperm analyzer.

2.2.3. Assessment of sperm morphology

A volume of 5 µl of the sperm sample was withdrawn from the fresh sample and transferred onto a slide. Using another slide at a 45° angle, the sample was spread evenly across the slide. The prepared slides were then left to air-dry at room temperature. The dried slides were stained with eosin for morphological examination (Mukherjee A et al., 1988). After staining, the slides were rinsed thoroughly with distilled water and left to dry at room temperature. Once completely dry, coverslips (24 x 60 mm) were carefully placed over the slides and secured with plastic wrap, preparing them for morphological examination. For each rat, a total of 200 spermatozoa were analyzed. Morphological analysis involved identifying head and tail disorders according to criteria outlined in the literature.

2.3. Single Cell Gel Electrophoresis in Sperm (SCGE/Comet) Method

The procedure involved covering frozen slides with 1% normal melting temperature agarose gel (NMA), prepared with PBS. Subsequently, 8 µl of sperm samples from rat epididymis were mixed with 72 µl of 1% low-melting temperature agarose gel (LMA) and applied to the slide, covered with a coverslip. Following a 5-minute incubation, the coverslip was removed, and the slides were immersed in lysis buffer containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10), 1% Triton X-100, and 40 mM dithiothreitol for 24 hours. Then, 0.1 mg/ml of proteinase K and 4 mM Lis (Lithium 3,5-

diiodosalicylate) were added to the lysis buffer, and the samples were further incubated at 37°C for 24 hours. Following the procedure, the slides underwent three 5-minute washes in distilled water to eliminate salts and detergents. Next, they were positioned on a horizontal electrophoresis unit and immersed in TBE buffer (10 mM Tris, 0.08 M Boric Acid, and 0.5 M EDTA, pH 8.2) for 20 minutes. Electrophoresis was conducted at 25 V for 25 minutes. After the electrophoresis, the slides were rinsed with distilled water and subsequently air-dried. Afterwards, the slides were stained with Sybr green stain. The samples were then imaged using a Leica DM6000 B fluorescence microscope (Leica, Germany) and analyzed using BS 200 ProP, BAB Imaging System (BAB Imaging System, Ankara, Turkey). An average of 100 cells from each preparation was counted for analysis (Kilic, V., 2019).

2.4. Biochemical Measurement

The biochemical analyses were performed using samples obtained from testicular tissue.

2.4.1. Determination of lipid peroxidation (MDA)

Lipid peroxidation was quantified by measuring malondialdehyde (MDA) levels using the method described by Ohkawa et al. (1979).

Principle: The peroxidation of fatty acids with three or more double bonds results in the production of malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA). This reaction forms a pink-colored compound that can be quantified using spectrophotometer at 532nm.

2.4.2. Superoxide dismutase (SOD) activity quantification

The determination of SOD (Superoxide Dismutase) activity in testis samples was conducted following the method described by Nebot et al. (1993), which had been previously standardized. The SOD 525 method relies on measuring the enzyme-dependent auto-oxidation rate of 5,6,6a,11b-tetrahydro-3,9,10,-trihydroxybenzo[c]fluorene (BXT-01050) in an aqueous alkaline solution. This measurement is based on the chromophore generated by the reactions.

1 unit of enzyme activity is defined as the quantity of enzyme necessary to catalyze the auto-oxidation of 1 micromole of 5,6,6a,11b-tetrahydro-3,9,10,-trihydroxybenzo[c]fluorene (BXT-01050) within 1 minute, under conditions of 37°C and pH 8.8.

2.4.3. Catalase (CAT) activity determination

CAT (Catalase) activity was assessed using the method described by Beers and Sizer (1952). This method relies on measuring the reduction in absorbance of hydrogen peroxide at a wavelength of 240 nm during a reaction catalyzed by the CAT enzyme, which is monitored through spectrophotometric analysis. The rate of decrease in absorbance per minute is directly proportional to the enzyme's activity. 1 unit of enzyme activity is defined as the quantity of enzyme required to decompose 1.0 micromole of hydrogen peroxide within 1 minute, under conditions of 25°C and pH 7.0.

2.4.4. Total glutathione (GSH) level measurement

The determination of total glutathione (GSH) was conducted using the Cayman glutathione assay kit (Item No. 703002). This assay employs an enzymatic recycling method that has been meticulously optimized, utilizing glutathione reductase to quantify GSH levels. During the assay, the sulfhydryl group of 10-12 GSH molecules reacts with DTNB (5,5'-dithio-bis-2-(nitrobenzoic acid)) to yield yellow-colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide GSTNB, formed between GSH and TNB, undergoes recycling of GSH through reduction by glutathione reductase, resulting in increased TNB production. This recycling reaction is directly proportional to the concentration of GSH in the sample. Absorbance measurement allows for an accurate estimation of GSH levels. GSH is prone to oxidation to form the disulfide dimer GSSG, particularly during the reduction of hydroperoxides by glutathione peroxidase. GSSG is then converted back to its reduced form, GSH, by glutathione reductase, predominately found in biological systems.

2.5. Aminolaevulinic Acid Dehydrogenase (ALAD) Level Measurement

ALAD activity in erythrocytes, spleen, liver, kidney, and brain was assessed using a colorimetric assay. Samples were incubated with ALA for 60 minutes at 37°C. The reaction was halted with trichloroacetic acid/HgCl2 and mixed thoroughly. After centrifugation, the supernatant was incubated with a modified Ehrlich reagent. The enzymatically synthesized porphobilinogen (PBG) was quantified, with an absorption coefficient of 6.2 x 104 molar at 555 nm, resulting in an Ehrlich-PBG colored salt (Mauzerall and Granick, 1956).

One unit (U) of enzyme activity is defined as the amount of enzyme that produces 1 μmol of PBG per hour at 37°C. Enzyme activity in erythrocytes or other tissues is typically expressed as U/ml packed cells or U/mg protein (Wiley et al., 1999).

2.6. Determination of Serum FSH, LH and Testosterone Levels

This ELISA kit operates on the Competitive-ELISA principle. The microplate included in the kit is pre-coated with T. During the reaction, T in the samples or standards competes with a fixed quantity of T on the solid phase support for binding sites on the Biotinylated Detection Antibody specific to T. Any excess conjugate and unbound sample or standard are washed away from the plate. Avidin conjugated to Horseradish Peroxidase (HRP) is then added to each well and incubated. Next, a TMB substrate solution is introduced to each well. The enzyme-substrate reaction is stopped by adding a stop solution, and the resulting color change is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The concentration of T in the samples is determined by comparing the optical density (OD) of the samples to a standard curve. Blood samples collected from the animals were stored at 2-8°C overnight. Afterward, they were centrifuged for 15 minutes at 4°C and 1000 x g to separate the serum. Hormonal analyses were conducted using commercially available kits following the manufacturer's instructions.

2.7. Histologic Analysis

The dissected tissues were initially placed in a mounting solution containing 4% paraformaldehyde with a pH range of 7.2-7.3. Subsequently, the tissues were sectioned into pieces with a flat surface and immersed in the fixative solution for 24 hours at +4°C. Following fixation, the tissues underwent dehydration using a graded alcohol series (70-80-90-95-100%) to remove water content, followed by a xylene series (100-100%) to achieve transparency. Finally, the dehydrated tissues were embedded in paraffin by surrounding them with liquid paraffin within blocks. Following embedding, the tissue blocks were kept in an oven overnight to ensure complete paraffin infiltration and subsequently allowed to freeze at room temperature. Using a Thermo Shandon microtome device, 5 μm thick sections were obtained from the blocks. These sections were then stained with hematoxylin and eosin and subjected to histopathological analysis using a Leica DM 6000B light microscope at magnifications of 40X, 63X, and 100X according to the standard protocol outlined by Bancroft and Gamble (2008).

2.8. Immunohistochemical Analysis

The 5 μm thick sections obtained from the tissue blocks designated for histological assessment were labeled with specific primary antibodies targeting PCNA (proliferating cell nuclear antigen) (Cell Signaling Technology 2586S), followed by the application of an appropriate secondary antibody (Streptavidin HRP-conjugated secondary antibody). These sections go through deparaffinization through xylene and alcohol series, followed by antigen retrieval. After that, a peroxide block was applied for 10 minutes, and the sections were washed with distilled water to remove any residual substances. The tissues underwent antigen retrieval by boiling in citrate buffer and were subsequently washed with PBS. Blocking of nonspecific binding was achieved using 2% BSA. The primary antibody, appropriately diluted, was then applied to the tissues and allowed to incubate. The slides, left at room temperature for one hour, were washed with PBS and then incubated with diluted, secondary antibodies compatible with the primary antibodies.

After incubation for one hour at room temperature in the dark, the slides were washed again and subjected to DAB staining. Next, they were washed with PBS and counterstained with Hematoxylin Mayer, followed by a wash with tap water. The slides were then dehydrated with alcohol and sealed using Consul Mount. Finally, the prepared slides were examined under a Leica DM 6000B fluorescence microscope at 63x magnification.

2.9. Statistical Analysis

The data were expressed as mean ± standard deviation. Statistical comparisons were performed using One-way ANOVA followed by post-hoc Dunnett's T3 test. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was conducted using the SPSS Statistics V26 2019 program.

3. RESULTS

3.1. The Variations in Body Weights and Organ Relative Weights

At the end of the study period, the subjects were euthanized, and their organs were dissected. The testes and epididymal tails were excised and weighed. The organ relative weights (organ weight/body weight × 100) were measured for each rat in treated and control groups.

Table 3.1. *Testis, and Epididymis Weights of the Groups.*

Experimental Group	Relative testis weight (g/100g Body weight)	Relative epididymis weight (g/100g Body weight)
Control	0.53 ± 0.01	0.31 ± 0.004
Lead	0.44 ± 0.01 (a)	0.24 ± 0.14 (a)
Lead+Piceatannol	0.50 ± 0.015 (b)	0.265 ± 0.002 (b)
Piceatannol	0.54 ± 0.005 (b)	0.309 ± 0.005 (b)

3.2. Activity Measurement Results (ALAD)

The graph below illustrates the findings of ALAD activity. In the Pb group, the level of ALAD activity has been significantly reduced relative to the control group (~39%). in the Pb+PIC group ALAD level decreased significantly (~13%) compared to the control group, in contrast, in the PIC Group, the level of ALAD has been found to significantly increase (~30%) over the Pb group.

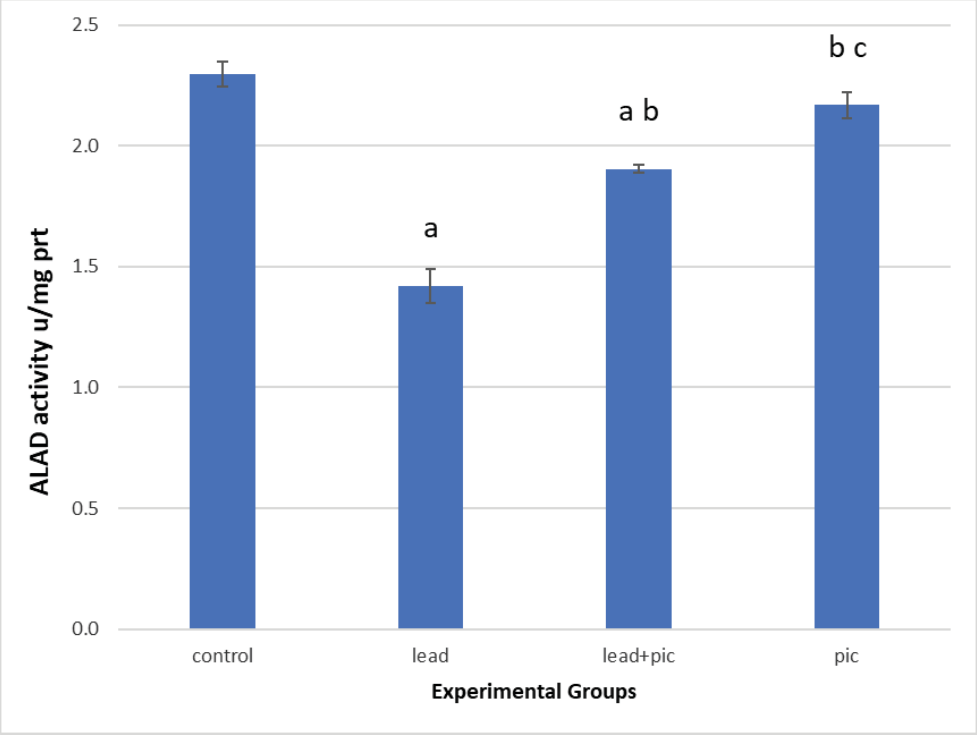

Figure 3.1. *The level of ALAD activity in the experimental groups*

Figure 3.1. Column graph of ALAD activity results indicating significant differences when compared with (a) the Control group, (b) the Pb group, and (c) the Pb+PIC group ($P < 0.05$) mean ± SD Control: 2.31±0.006; Pb: 1.43±0.008; Pb+ PIC: 1.94±0.009; PIC: 2.20±0.009.

3.3. Catalase (CAT) Activity Measurement Results

The graph of CAT activity results is as follows: CAT activity in the Pb-treated group significantly decreased compared to the control (~59%). However, CAT activity in the Pb+PIC group, while significantly decreased compared to the control (~18%), is approximately twice as high as that in the Pb group.

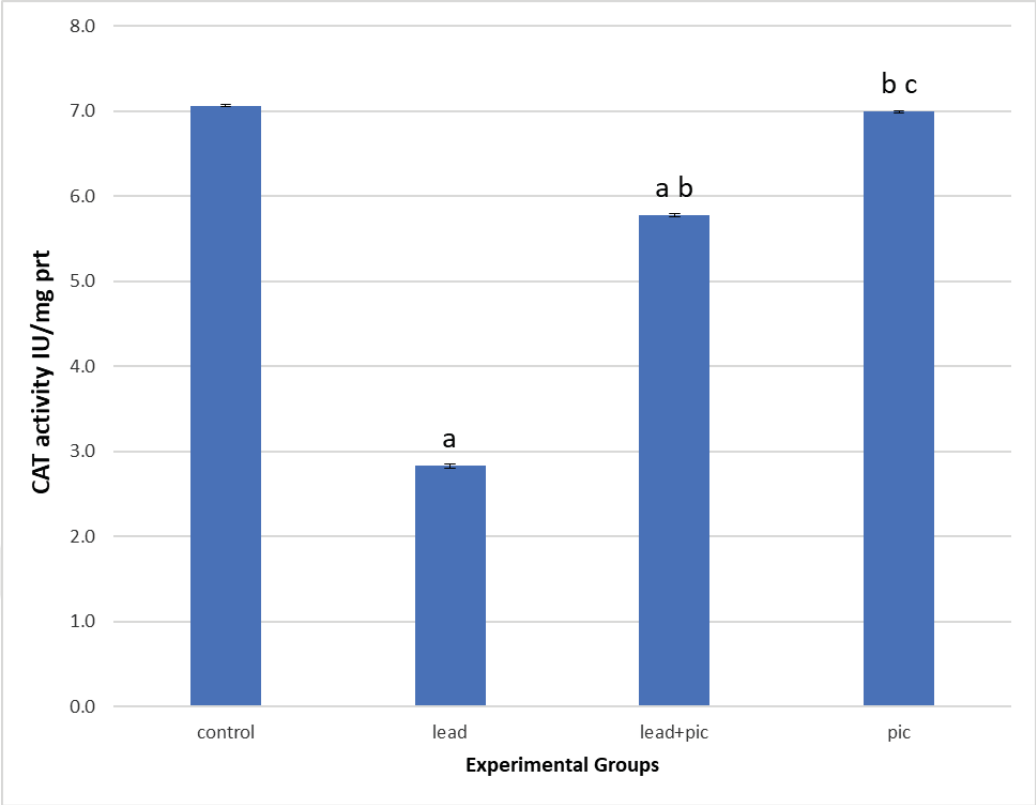

Figure 3.2. *The CAT activity U/mg prt in the experimental groups*

Figure 3.2. illustrates the graph of CAT activity results: with (a) Control group, (b) Pb group, and (c) Pb+PIC group, indicating significant differences ($P < 0.05$). The average values are presented with standard deviation (SD): Control: 70.4±3.8; Pb: 27.8±4.2; Pb+ PIC: 57.5±4.4; PIC: 69.7±2.

3.4. The Malondialdehyde Level in The Testis Tissue

Based on the findings of our studies, high levels of lipid peroxidation were anticipated in the Pb group. A significant increase was observed compared to the control group, exceedingly more than twice the control levels. In the Pb + PIC group, however, the MDA level showed a significant increase compared to the control (~30%), but it was lower than that in the Pb group. No significant difference was observed between the PIC group and the control.

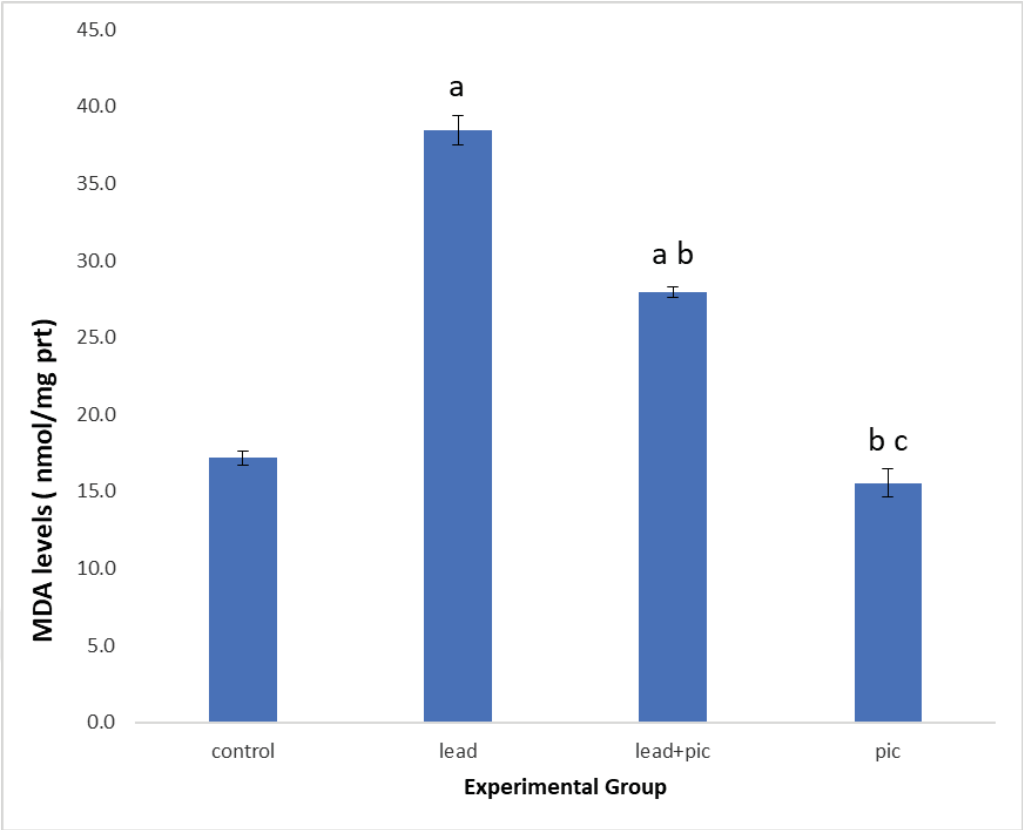

Figure 3.3. *MDA levels nmol/mg prt in the experimental group*

Figure 3.3. Column graph of MDA levels results (a) compared with the control group, (b) the Pb group, (c) the Pb+PIC group, indicating significant differences ($P < 0.05$) mean ± SD Control: 18.17±0.99; Pb: 38.92±1.38; Pb+ PIC: 27.45±1.00; PIC: 19.07±1.07.

3.5. SOD Activity Results

The findings of SOD activity results are as follows; SOD is significantly decreased in the Pb group compared to the control (about 1.5 times). Although the SOD activity in the Pb+PIC group is closer to the control level, it is significantly lower than the control (~17%). There is no significant difference between the PIC group and the control.

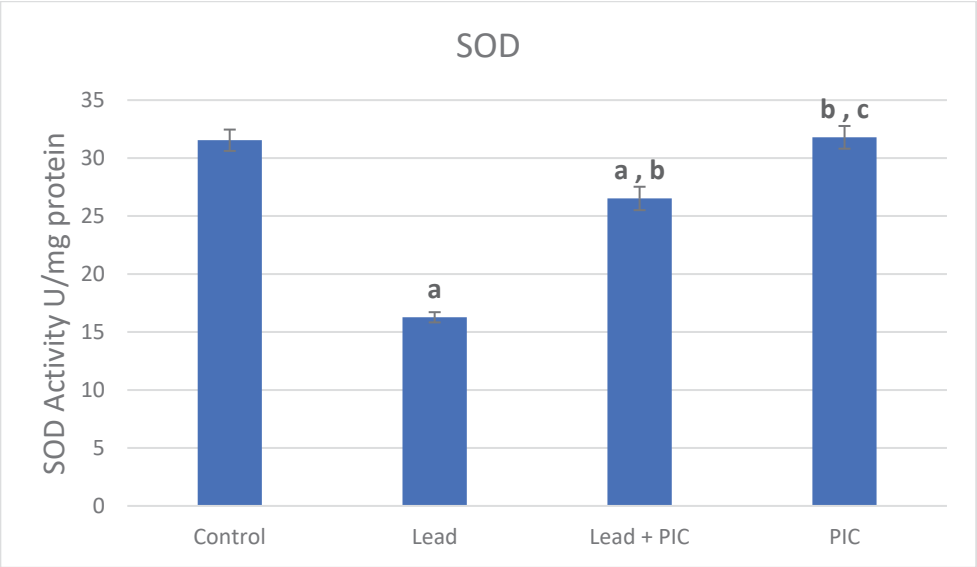

Figure 3.4. *SOD activity U/mg prt*

Figure 3.4. The SOD activity results indicated significant differences ($P < 0.05$) when compared with (a) Control group, (b) Pb group, (c) Pb+PIC group ($P < 0.05$) mean ± SD Control: 34.09±1.8; Pb: 19.74±1.68; Pb+ PIC: 29.93±1.97; PIC: 33.37±1.2.

3.6. Total Glutathione (T-GSH) Level Measurement Results

The T-GSH findings showed a decrease by approximately half (~49%) in the Pb-treated group compared to the control. However, in the Pb+ PIC group, the T-GSH level reduced by about 15% compared to the control.

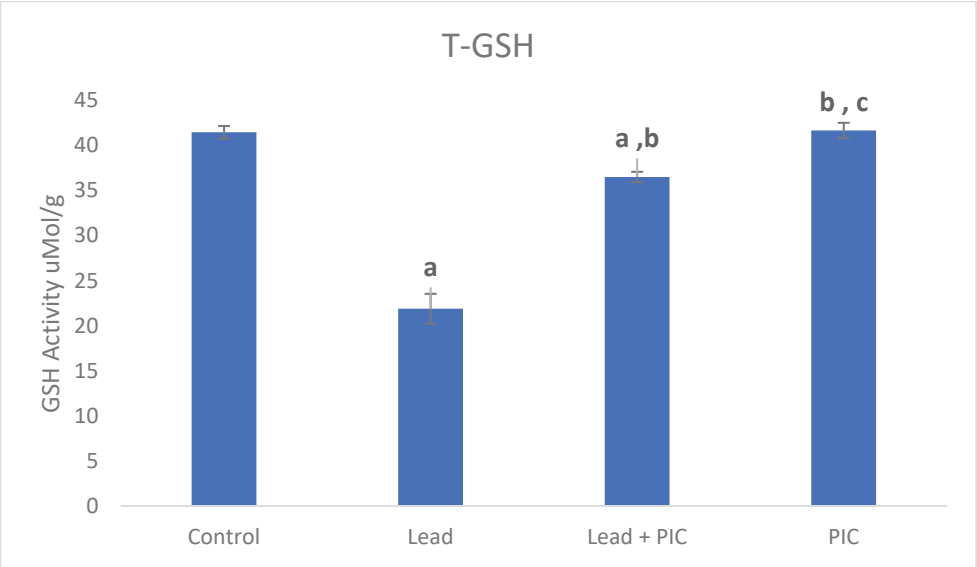

Figure 3.5. *Total GSH (U/mg prt)*

Figure 3.5. The T-GSH activity results are shown in the bar graphs (a) Control group, (b) Pb group, (c) Pb+PIC group and (P<0.05) mean ± SD Control: 43.63±1.23; Pb: 22.00±1.36; Pb+ PIC: 36.71±1.46; PIC: 44.10±1.79

3.7. Assessment of Serum Hormone Levels

3.7.1. Testesterone

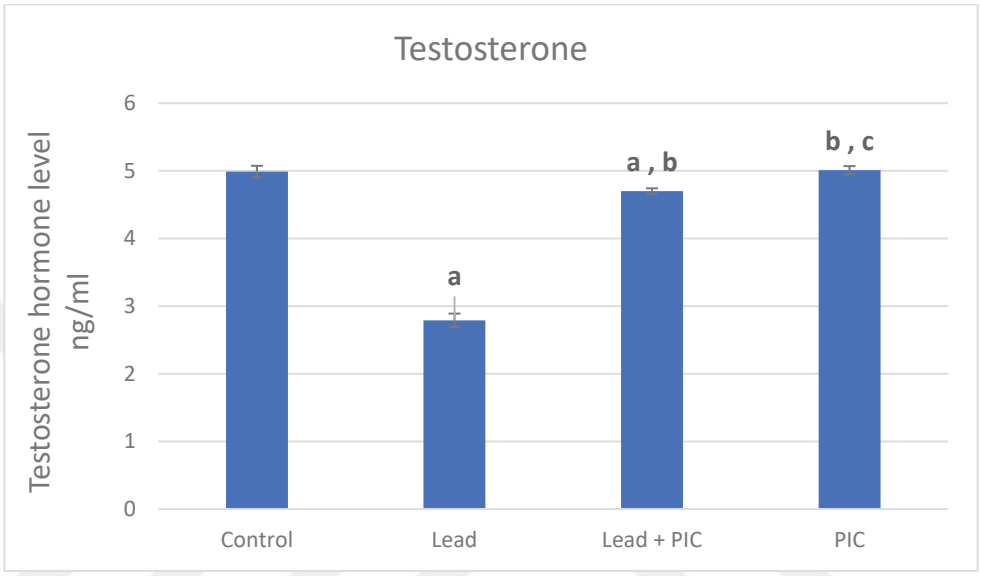

Figure 3.6. *testosterone activity (ng/ml)*

Figure 3.6. The graph of testosterone level results indicating significant differences between (a) the control group, (b) the Pb group, and (c) the Pb+PIC group (P <0.05) mean ± SD Control: 5.41±0.22; Pb: 3.14±0.27; Pb+PIC: 4.70±0.18; PIC: 5.43±0.27.

3.7.2. FSH

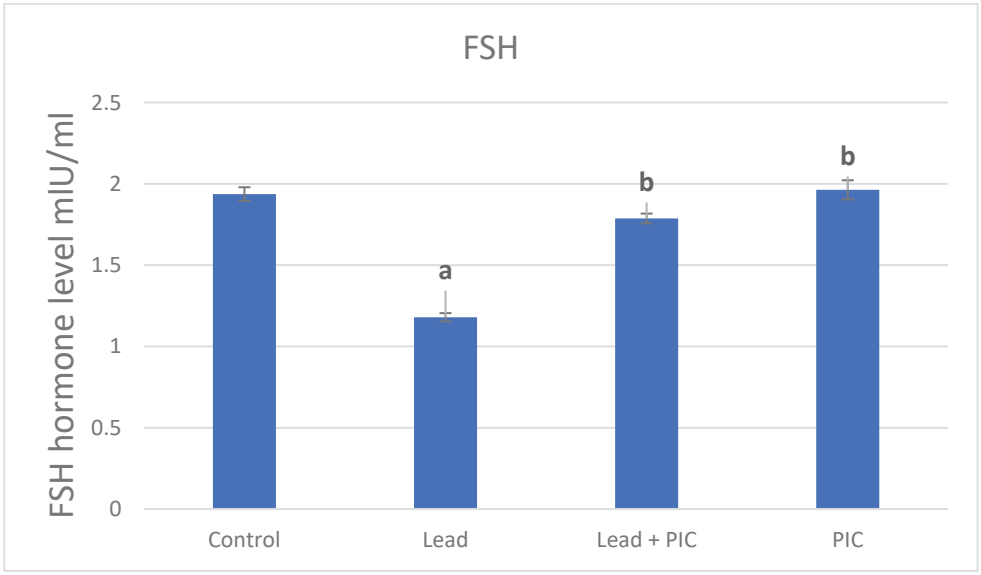

Figure 3.7. *FSH activity (ng/ml)*

Figure. Column graph of FSH level results indicating significant differences between (a) the control group, (b) the Pb group, and (c) the Pb+PIC group ($P < 0.05$) mean ± SD Control: 1.47±0.05; Pb: 1.23±0.03; Pb+PIC: 1.36±0.03; PIC : 1.45±0.05.

3.7.3. LH

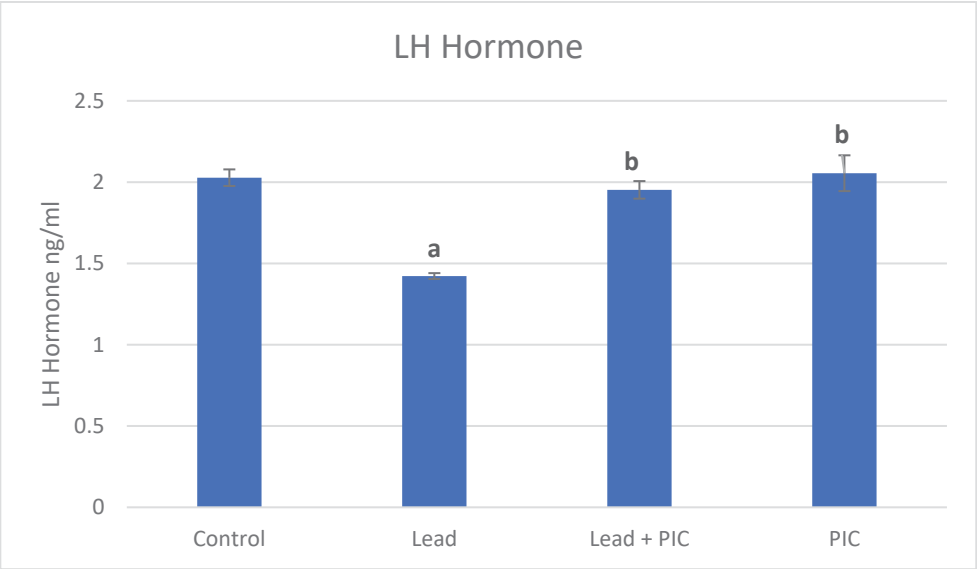

Figure 3.8. *LH activity (ng/ml)*

Figure 3.8. Column graph of LH level results indicating significant differences between (a) the control group, (b) the Pb group, and (c) the Pb+PIC group ($P < 0.05$) mean ± SD Control: 2.03±0.06; Pb: 1.90±0.07; Pb+PIC: 1.98±0.04; PIC: 2.05±0.09.

3.8. Single Cell Gel Analysis (SCGE-Comet Method) DNA Damage Results

DNA damage findings revealed by SCGE analysis showed that DNA damage in sperm of the experimental animal groups represent head and tail moments. The control group shows a normal structure (Fig). Significant DNA damage was observed in the Pb-treated group 7 times more compared to the control group (Fig).

DNA damage was reduced by approximately half (~56%) in the Pb+PIC group compared to the Pb-treated group. A significant reduction in DNA damage was observed in the Pb+PIC treated group.

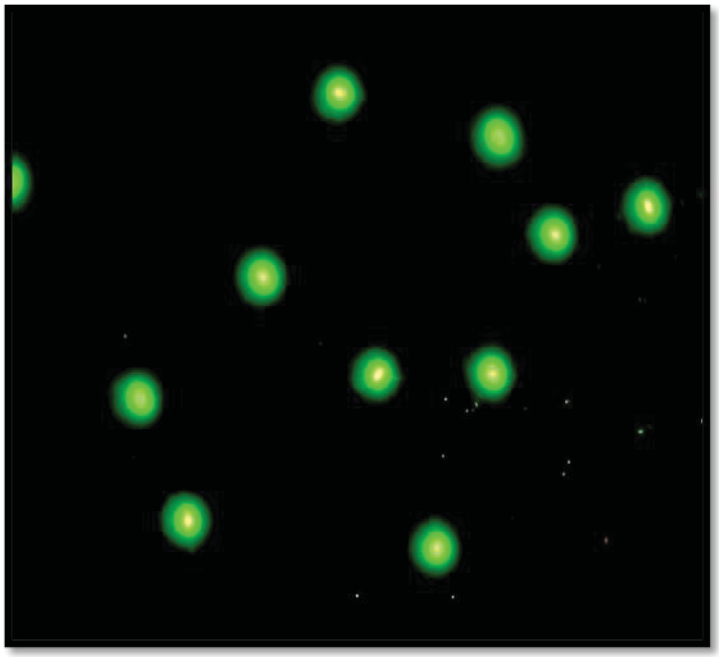

Figure 3.9. *Control group.Single-cell gel electrophoresis imaging. Scale 20 μm*

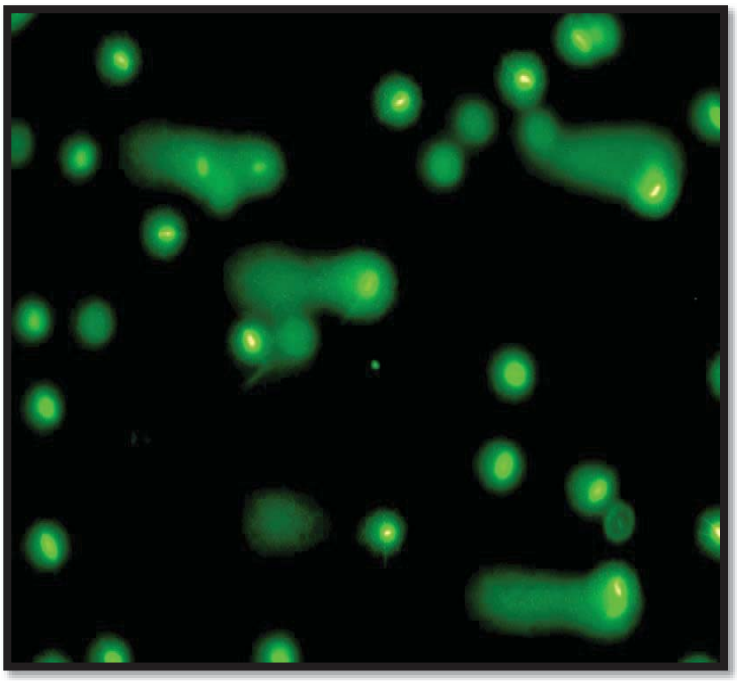

Figure 3.10. *Pb group.Single-cell gel electrophoresis imaging. Scale 20 μm*

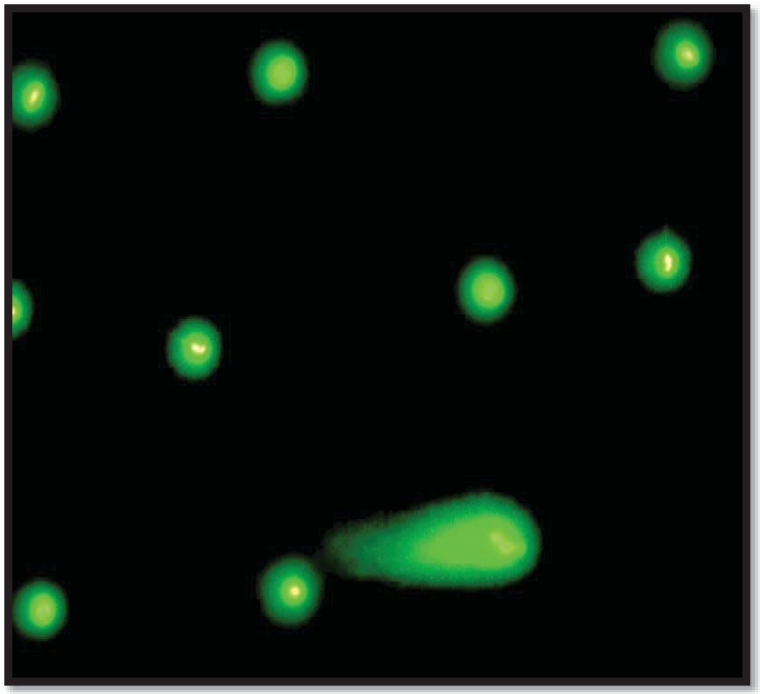

Figure 3.11. *Pb+PIC group. Single-cell gel electrophoresis imaging. Scale 20 μm*

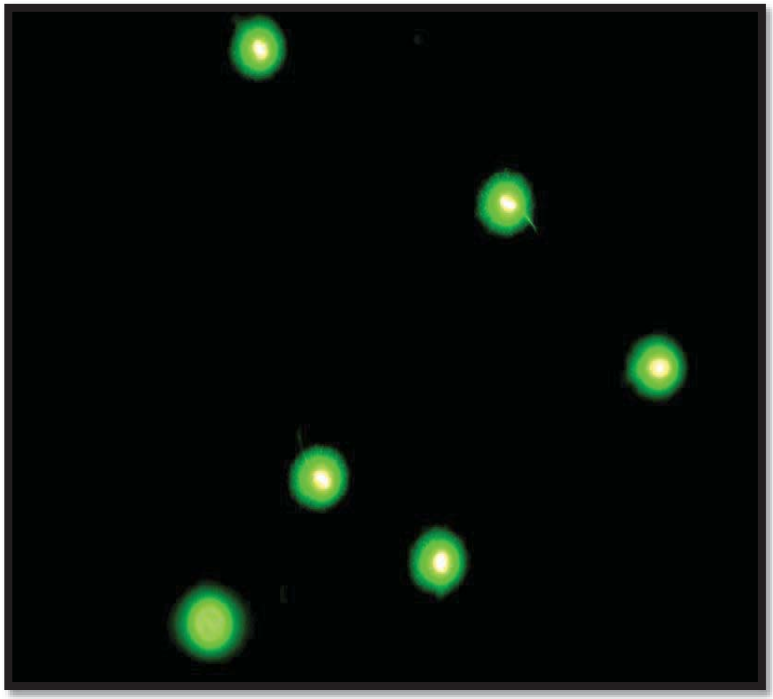

Figure 3.12. *PIC group. Single-cell gel electrophoresis imaging. Scale 20 μm*

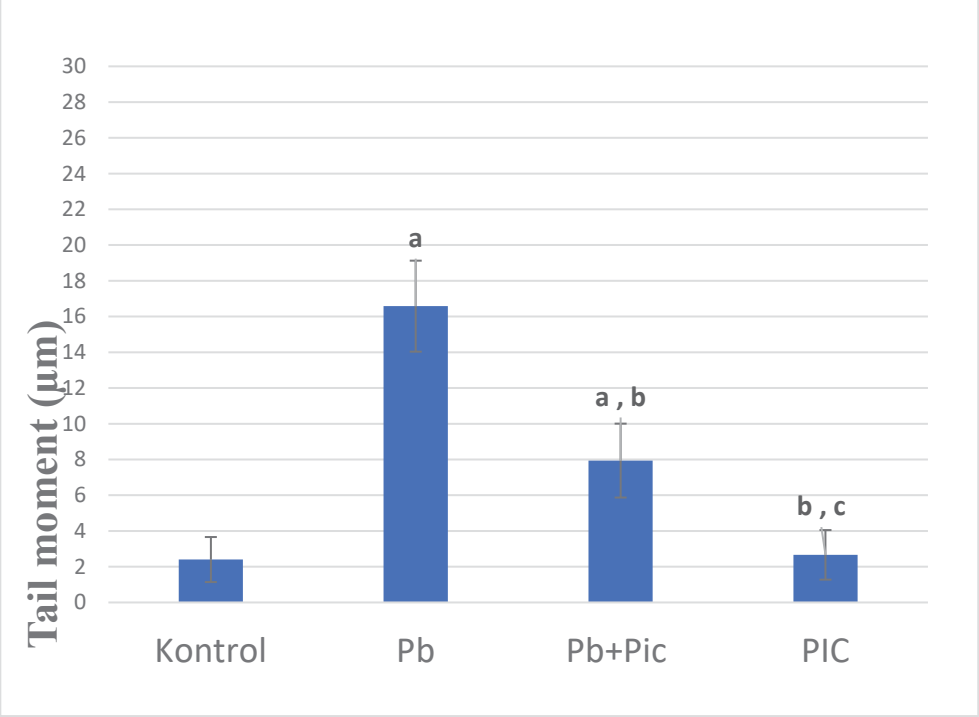

Figure 3.13. *Chemical formula of paracetamol*

Figure: Tail moment diagram. a significant difference compared to the control group (P< 0.05); b Significant differences compared to the Pb group (P< 0.05); c Significant differences compared to Pb+Pic group (P< 0.05). All data were expressed as mean ± SD. Statistical analysis of groups was performed with the SPSS package program and using one-way ANOVA followed by Dunnett T3 test as post hoc test.

3.9. Microscopic Findings

3.9.1. Sperm morphology

A healthy rat sperm consists of a hook-shaped head, a neck, and a long, motile tail. Morphologically healthy sperm are observed in the control group. Morphological deformities, such as fragmented heads and tails, are observed in the sperm visualized in the Pb-injected group. Additionally, morphological deformities such as breakage and bending in the tails are frequently encountered. These sperm are not functional for fertilization. In the Pb+PIC group, a significant improvement in sperm morphology is observed, with sperm resembling those in the control group. In the PIC group, sperm with morphologically undamaged parts similar to those in the control group are observed.

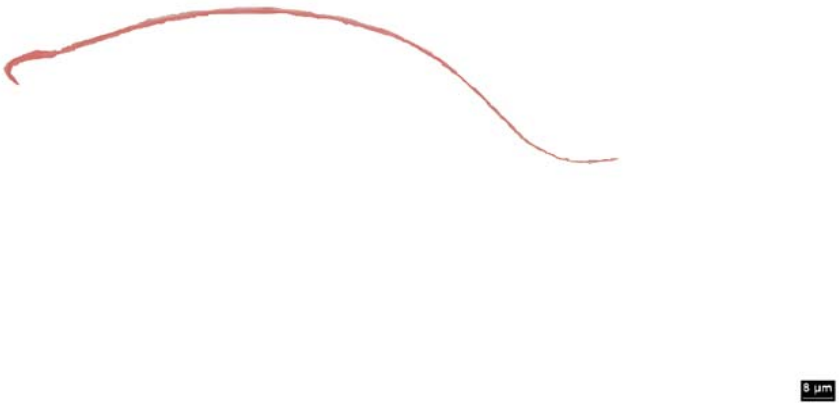

Figure 3.14. *Image of healthy sperm. It exhibits a hook-shaped head and a morphologically regular tail. Scale bar: 8µm.*

Figure 3.15. *Abnormal sperm, Banana-shaped head abnormality. Scale bar: 8µm.*

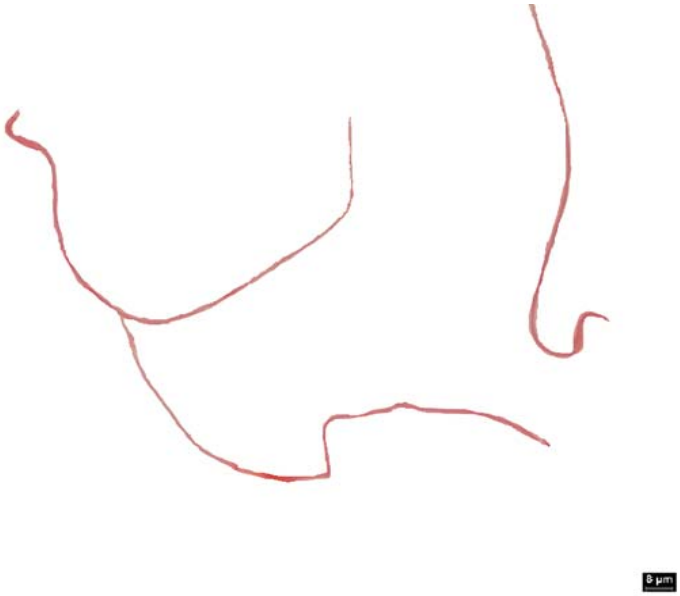

Figure 3.16. *Abnormal sperm Separated head and tail with deformities at multiple points in the tail. Bar: 8µm*

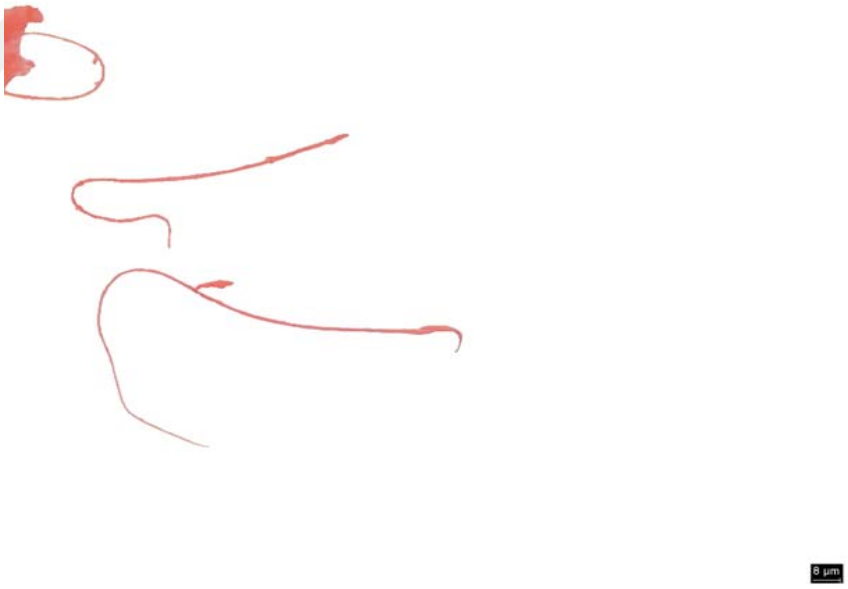

Figure 3.17. *Abnormal sperm Separated head and tail with deformities in the tail. Bar: 8µm*

Figure 3.18. *Abnormal sperm. Twisted neck. Bar: 8µm*

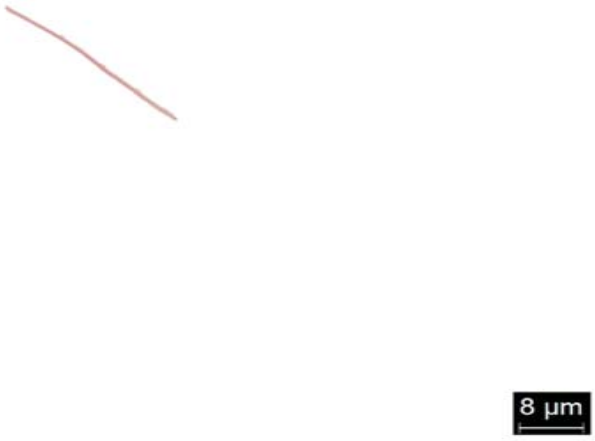

Figure 3.19. *Abnormal sperm. Headless semen and Broken observed in the tail. Bar: 8µm*

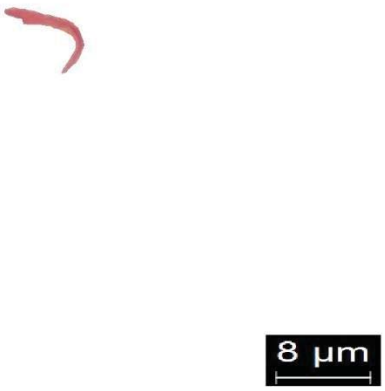

Figure 3.20. *Abnormal sperm. Detached and tailless. Scale bar: 8µm.*

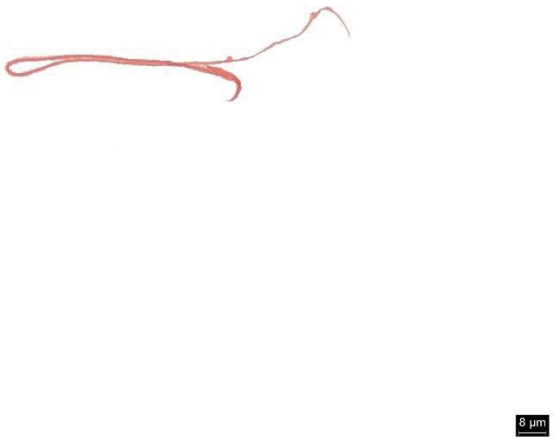

Figure 3.21. *Abnormal sperm. The twisting and broken observed in the tail. Scale bar: 8µm.*

Table 3.2. *Relative testis and relative epididymis weights*

Groups	Relative Testicular Weight	Relative Epididymal Weight
Control	0,53 ± 0,01	0,31 ± 0,004
Lead	0,44 ± 0,01 a	0,24 ± 0,014 a
Lead + PIC	0,50 ± 0,016 b	0,265 ± 0,002 b
PIC	0,54 ± 0,005 b	0,308 ± 0,005 b

Table 3.2. Relative testicular and epididymal weight values in tissues according to experimental groups, a: control, b: lead, c: lead + PIC, d: PIC; $P \leq 0.005$ significance.

3.10. Findings of Histopathological Examination

Microscopic photos of seminiferous tubules from different rat groups are shown in Figures. The histological study of testes stained with H&E revealed a significant difference across all groups. The control group appears normal, with completely compact seminiferous tubules, a narrow seminiferous tubule (TS) lumen, and a wide seminiferous epithelium (ES) with complete spermatogenesis. The same results were observed in the PIC group. The (Pb) group showed changes in the later stages of spermatogenesis, marked by abnormal basal membrane (MB), reduced thickness of ES, and empty spaces between germ cells, indicating a disappearance of Sertoli cells, disruption of spermiogenesis with a decrease in spermatozoa in the lumen. Additionally, there was proliferation of Leydig cells and vascular congestion. These results varied from one rat to another, and the intensive changes were observed approximately near the blood vessels (VS). Finally, the (Pb+PIC) group showed partial improvement with a wider diameter of ES, presence of spermatozoa, normal MB of TS, larger diameter of ES, reduced lumen of TS, and spermatogonia closely adjacent to the MB. Indeed, vascular congestion persists.

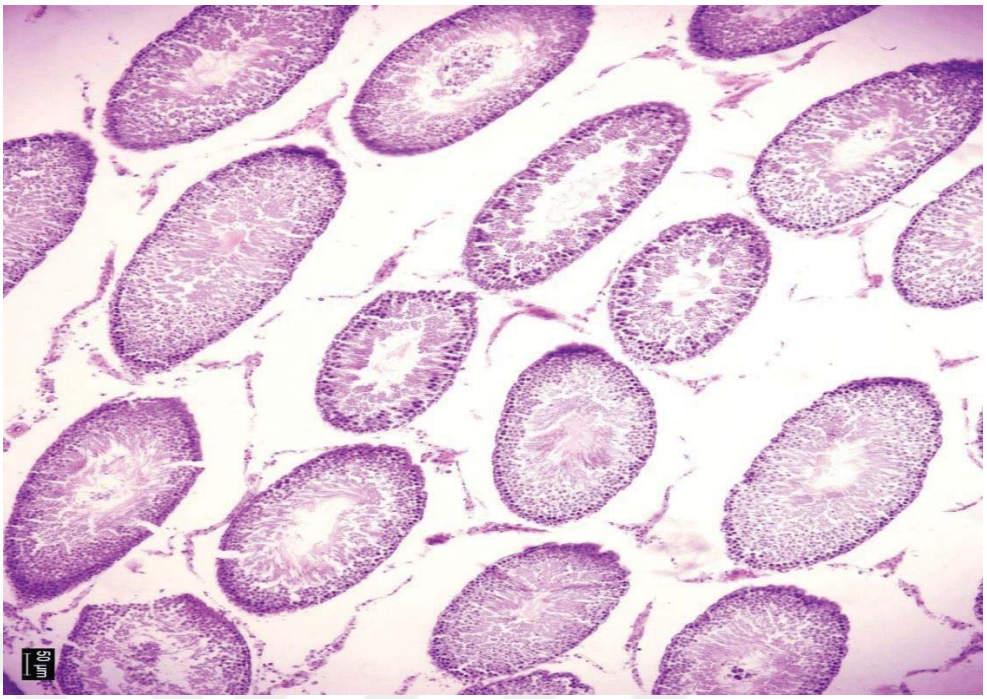

Figure 3.22. *Figure. Control: Regular organization and normal appearance of seminiferous tubules and interstitial cells. Scale bar: 50µm*

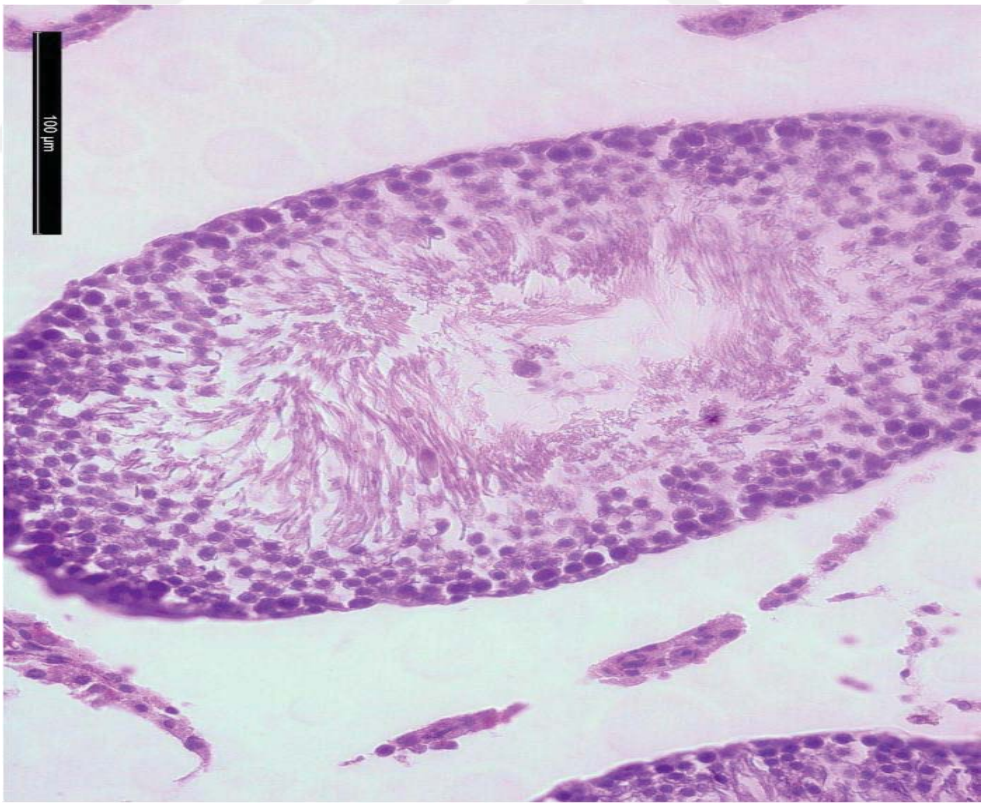

Figure 3.23. *Control: Regular appearance of spermatogenic cells and dense sperm formation with normal appearance. Leydig cells show a normal appearance. Scale bar: 100µm*

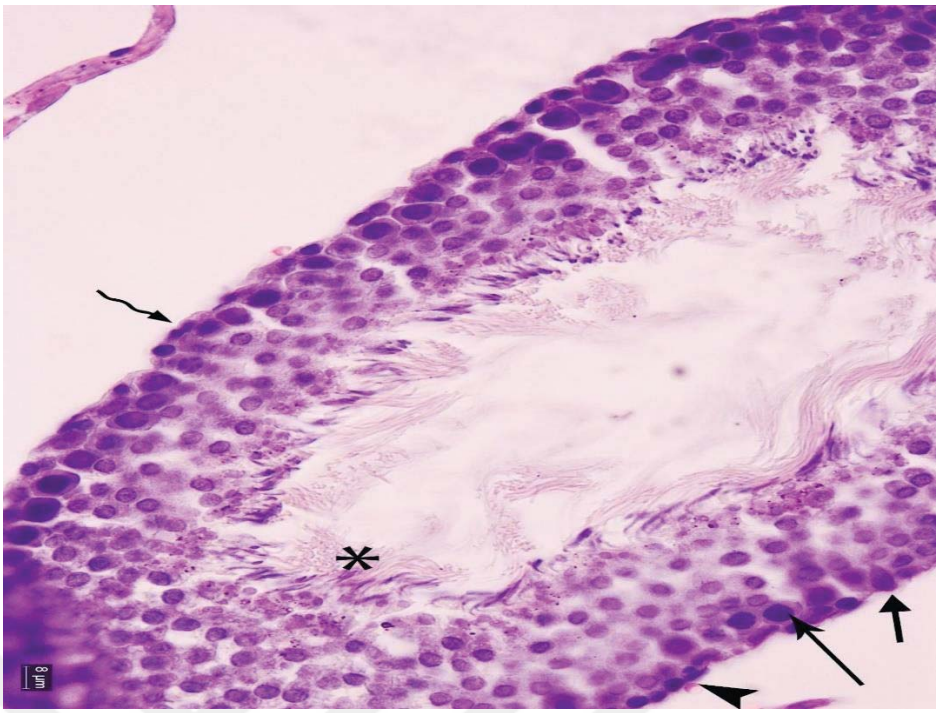

Figure 3.24. *Control: Regularly arranged and normal-looking spermatogenic cells. Basal lamina, myoid cells (broken arrow), spermatogonia cells (arrowhead), spermatocytes (long arrow), and normal appearance of Sertoli cells (short arrow). Numerous sperm formations (star). Scale bar: 8µm.*

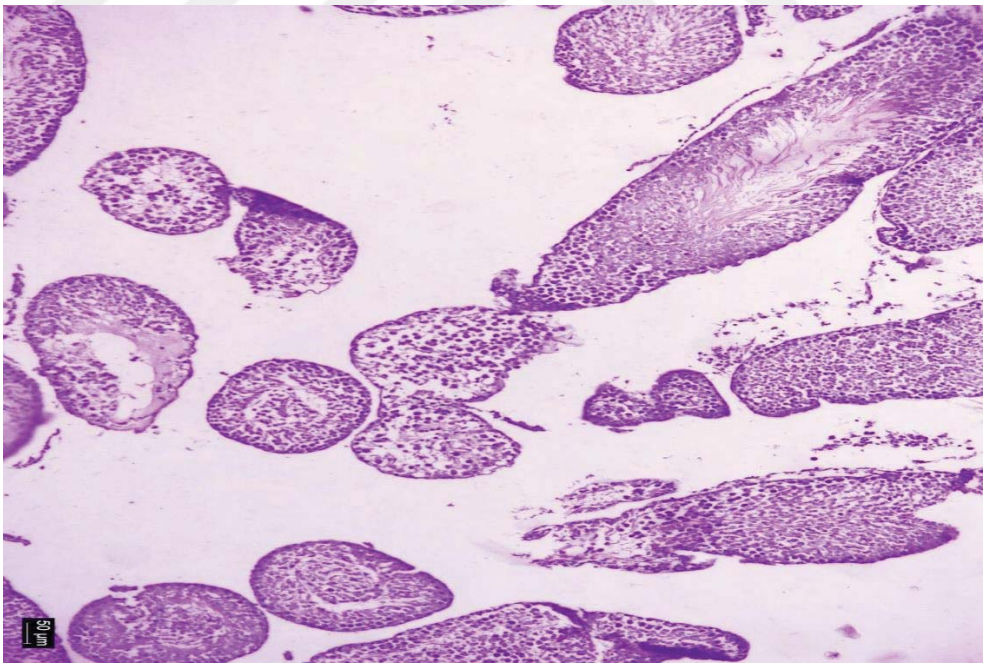

Figure 3.25. *Pb Group: Deformation of seminiferous tubules and organizational disruptions. Shape irregularities, fragmentation, and loss of interstitial cells in the tubules. Decrease in sperm formation. Scale bar: 50µm*

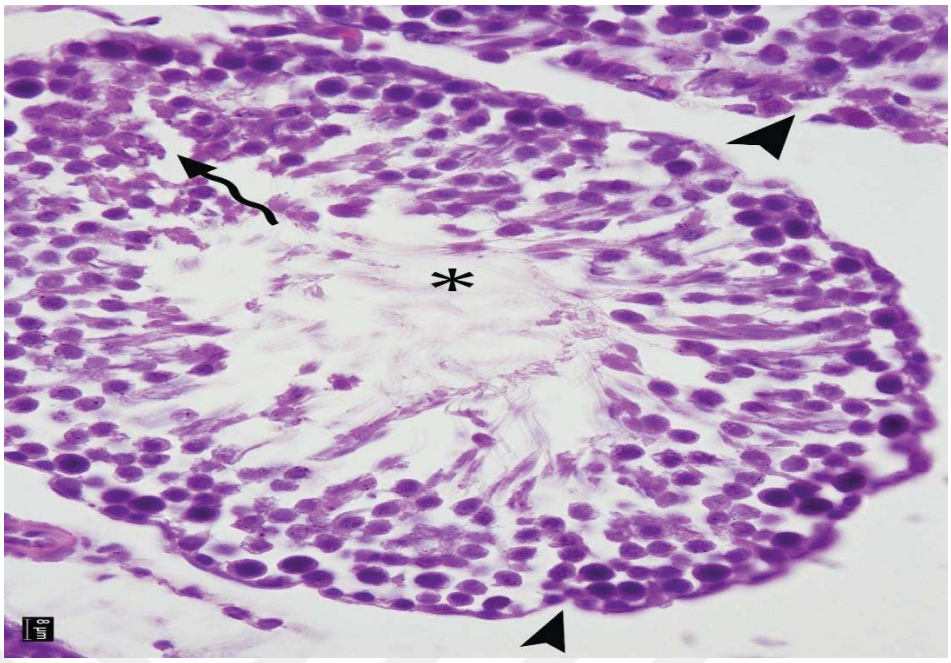

Figure 3.26. *Pb Group: Deformation and fragmentation in the basal lamina (arrowhead). Atrophy in spermatogenic cells, expansions in intercellular spaces (broken arrow). Decreased appearance of sperm formations (star). Scale bar: 8µm*

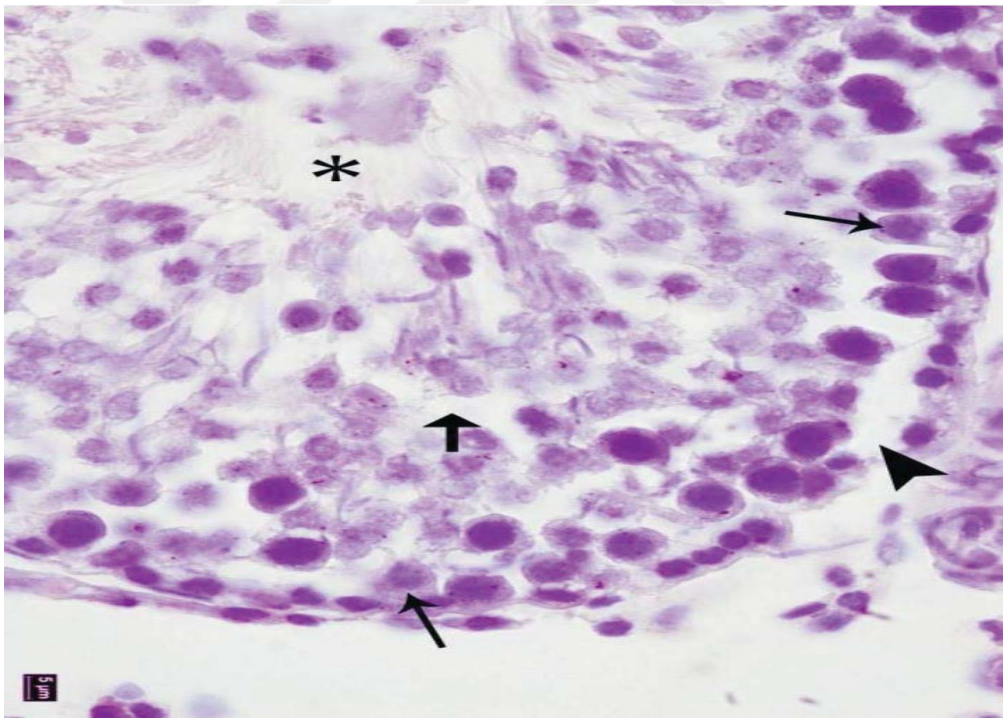

Figure 3.27. *Pb Group: Deformation in the basal lamina, myeloid cells, and spermatogonium cells (arrowhead). Organizational disruptions in the spermatogenic cell series, cellular atrophy, and increased intercellular spaces (short arrow). Damage to Sertoli cells (long arrow). Scale bar: 5µm*

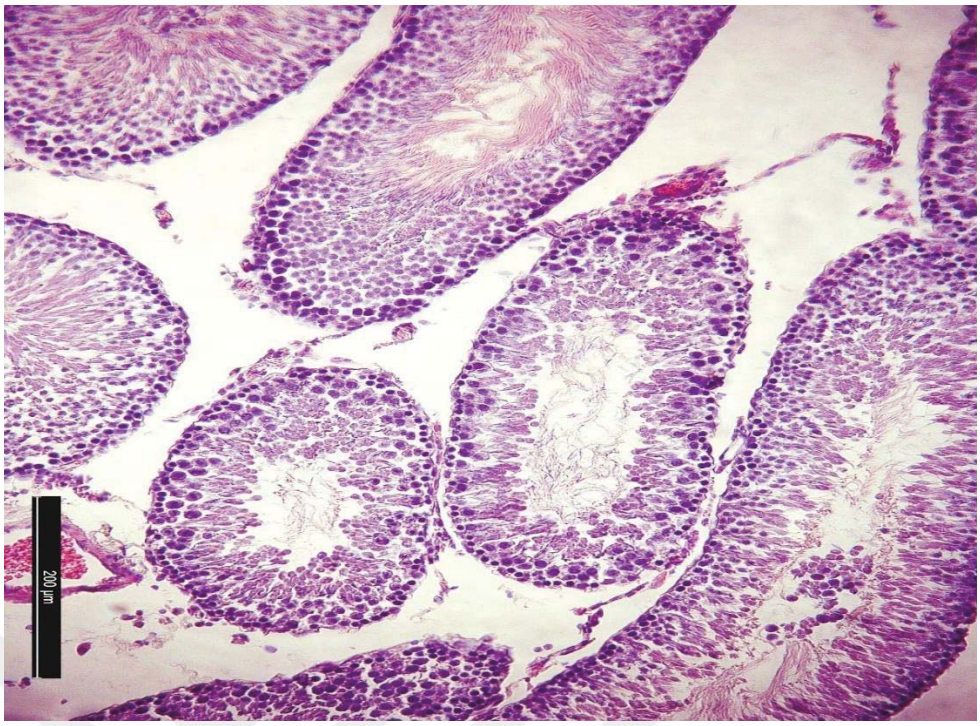

Figure 3.28. *Pb+PIC Group: Seminiferous tubules with partially preserved organizational structure and interstitial cells. Intense sperm formation is observed in many seminiferous tubules. Bar: 200µm*

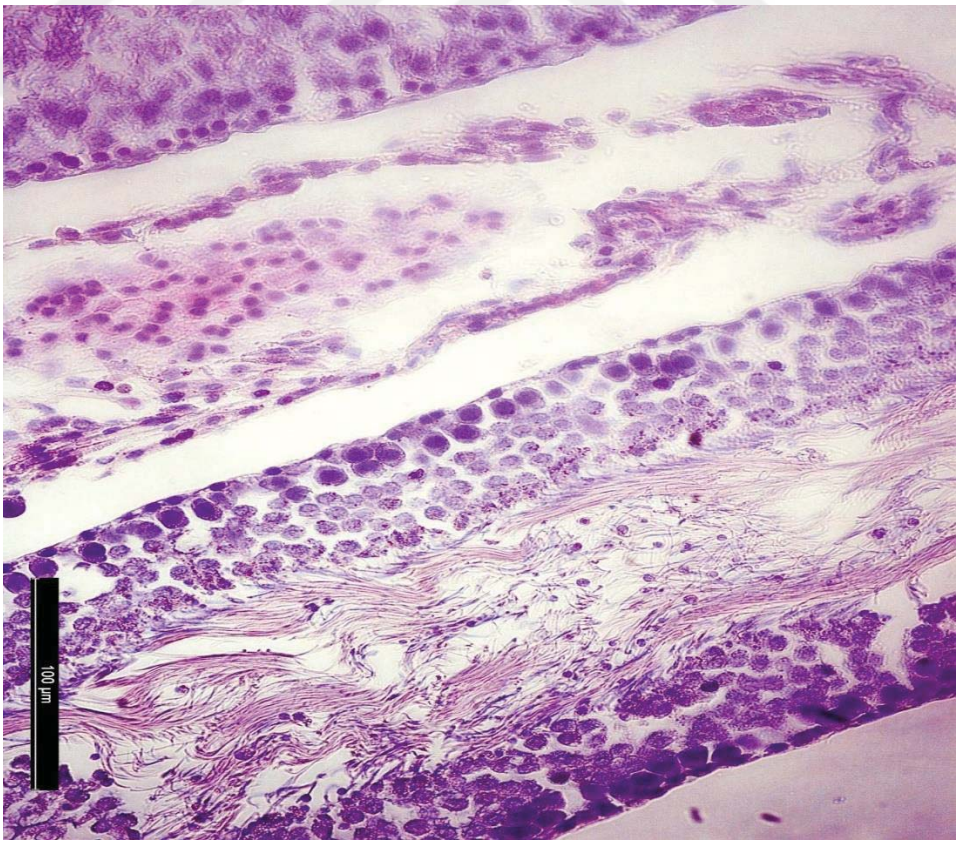

Figure 3.29. *Pb+PIC Group: Spermatogenic cells with a close-to-normal appearance, mildly expanded interstitial spaces, and numerous observed sperm formations. Leydig cells with a normal appearance. Bar: 100µm*

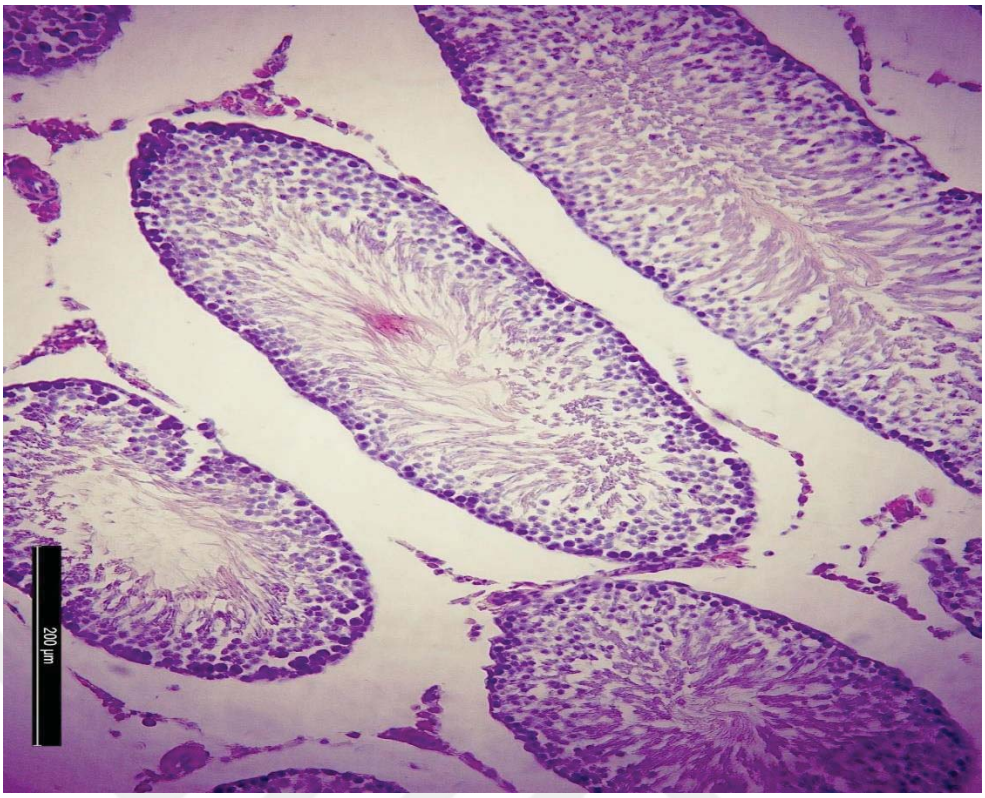

Figure 3.30. *PIC Group: Normally appearing spermatogenic cells and interstitial cells. Bar: 200µm*

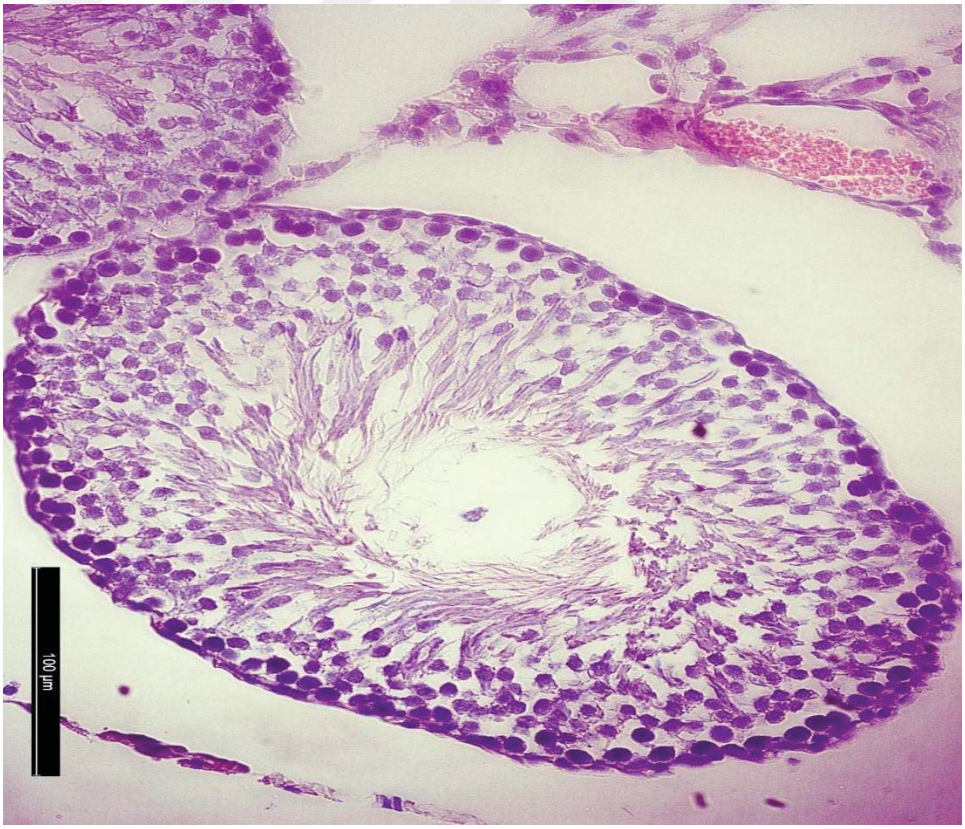

Figure 3.31. *PIC Group: Normal appearance of basal lamina, myoid cells, spermatogonia. Numerous observed sperm formations. Normally appearing Leydig cells. Bar: 200µm*

3.11. Findings of Proliferating Cell Nuclear Antigen (PCNA) Immunohistochemical Labeling

the level of PCNA protein expression was significantly ($P < 0.001$) increased in lead treated rats compared to the expression in other groups. The intensity of activated PCNA (deep brown) is predominant on spermatogonia and seminiferous tubules of lead treated rats.

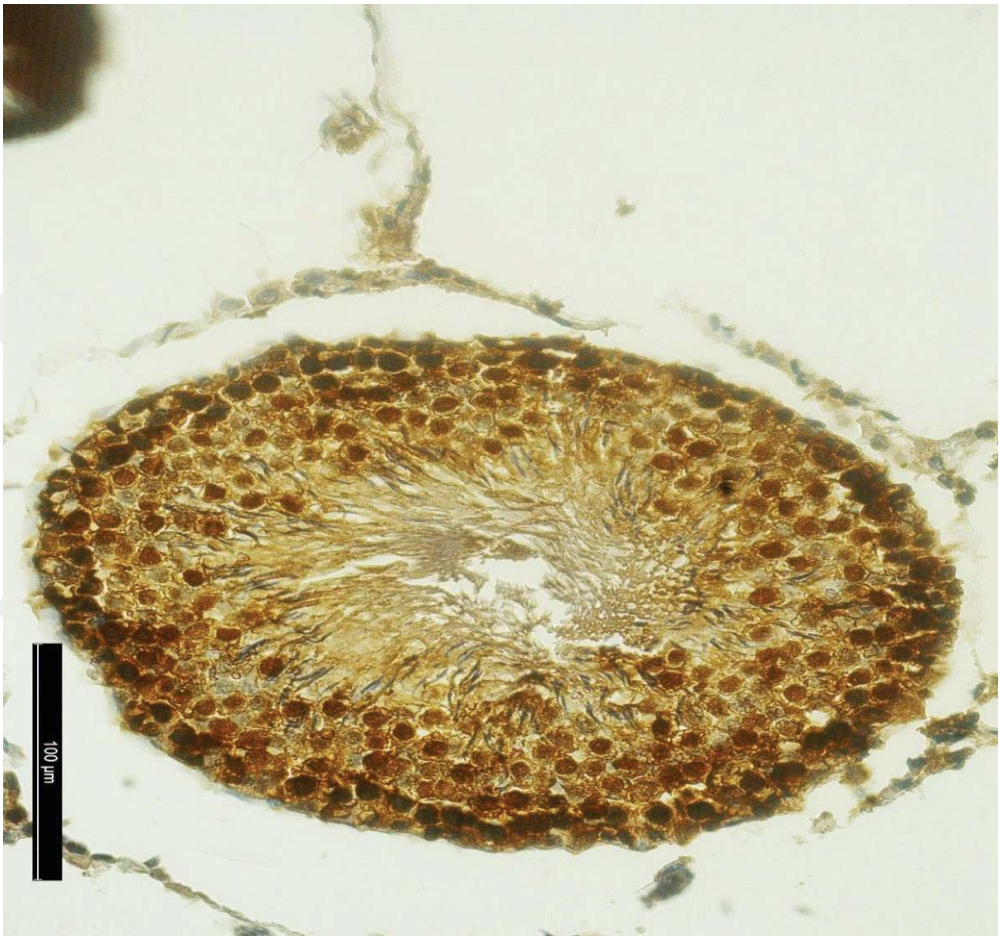

Figure 3.32. *Control Group: Intensely labeled PCNA markings due to cellular proliferation in the seminiferous tubule. Leydig cells without intense labeling were observed. Scale bar: 100µm*

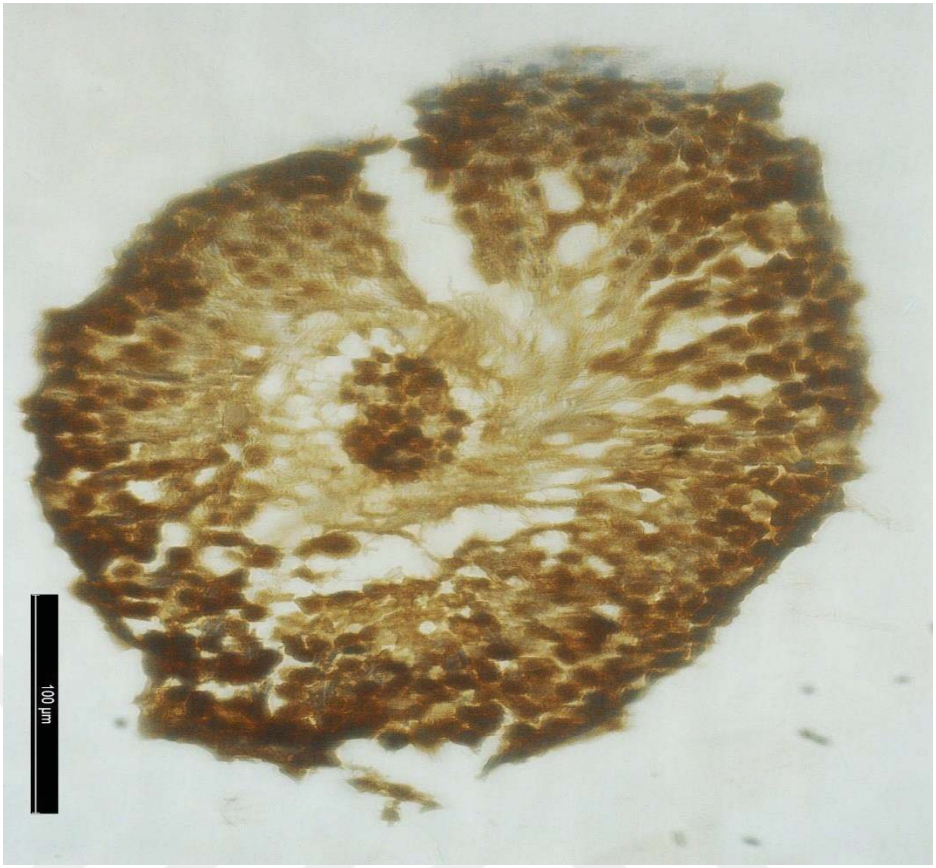

Figure 3.33. *Pb Group: Intensely labeled PCNA markings due to cellular proliferation in the seminiferous tubules. Scale bar: 100µm*

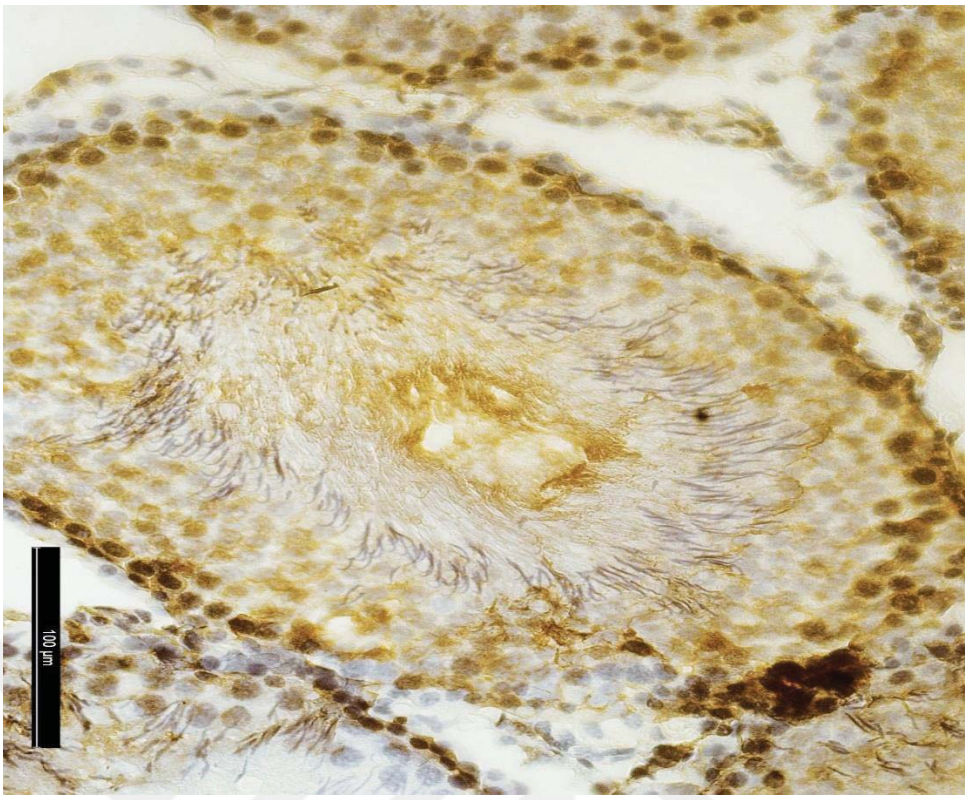

Figure 3.34. *Pb+PIC Group: Decreased cellular proliferation in the seminiferous tubules. Intensified PCNA labeling in spermatogonia. Scale bar: 100µm.*

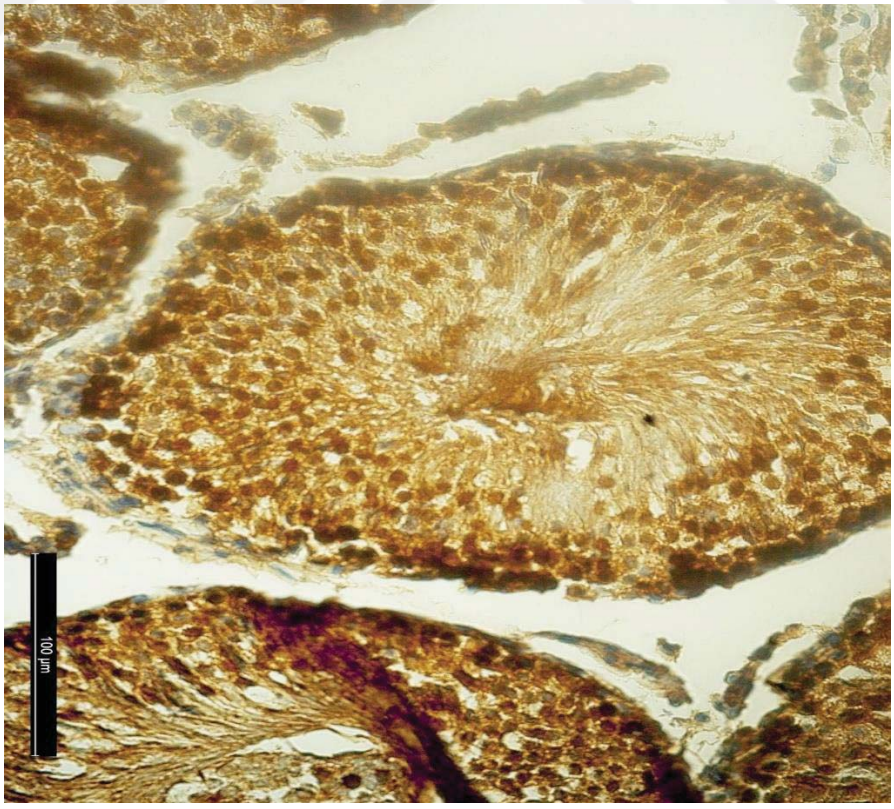

Figure 3.35. *PIC Group: Intense PCNA labeling associated with cellular proliferation in the seminiferous tubules. Leydig cells show no intense labeling. Scale bar: 100µm*

4. DISCUSSION

Heavy metals are widely acknowledged as major environmental pollutants, with lead being frequently identified as a prominent contaminant in both environmental and biological contexts. The persistent exposure to lead in animals and humans has been associated with various health risks (Juberg et al., 1997). Research has confirmed that lead causes multiple biochemical and physiological disorders in both humans and laboratory animals (Courtois et al., 2003). Further studies indicate that several metals accelerate oxidative damage to biological macromolecules, potentially contributing to their toxicity through the generation of free radicals (S.J. Stohs and D. Bagchi; 1995).

Polyphenols are great micronutrients that synergistically enhance the antioxidant functions of vitamins, enzymes, and other antioxidants, thereby defending against oxidative stress induced by reactive oxygen species. These compounds feature phenolic rings in their structure and occur in various natural forms (Tsao R, 2010). Recently, there has been increasing interest in exploring the role and application of natural antioxidants such as fruits and vegetables in fighting against oxidative damage associated with various health disorders characterized by oxidative stress (Khaki, A. et al., 2013). Phytotherapists have shown a growing interest in utilizing medicinal plants possessing antioxidant properties to mitigate the effects of heavy metal toxicity (Singh, M.K. et al., 2014). Piceatannol (3,3',4',5-tetrahydroxystilbene) is a naturally occurring hydroxylated derivative of resveratrol, characterized by two phenol rings connected via a styrene double bond (Kukreja A et al., 2013). ChatGPT Both compounds exhibit comparable biological activities, including antioxidant, antiproliferative, and anti-inflammatory properties (Piotrowska H et al., 2012).

Piceatannol demonstrates significantly enhanced biological activity and bioavailability compared to resveratrol. Consequently, piceatannol is increasingly garnering scientific interest for its potential to improve health-related conditions. However, further research is necessary to substantiate its efficacy in disease prevention and treatment (Ko YJ, et al., 2013). Lead is recognized as one of the most hazardous and persistent environmental pollutants, impacting all biological systems through exposure to air, water, and food sources (Patra, R.C., et al., 2011). Current data on lead-induced tissue pathologies indicates that the metal increases the production of several pro-inflammatory molecules and elevates oxidative stress markers (Huang, Y.S., et al., 2017). In this context, numerous human and experimental studies have shown that lead induces lipid

peroxidation and accumulates reactive oxygen species (ROS) in affected tissues, such as the kidney and testis (Zhang, Z., et al., 2017). Lead's capacity to produce reactive oxygen species (ROS) results in DNA strand breaks and the displacement of zinc in DNA-binding proteins (Kumar, A., et al., 2013). The toxic effects of lead have been confirmed by a study conducted by Elgawish and Abdelrazek (2014), which suggested that superoxide dismutase (SOD) levels were reduced in lead acetate-treated rats compared to other groups. Lead is reported to cause oxidative stress by generating reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals, and lipid peroxides. The heme synthesis pathway is also a primary target for lead toxicity, with delta aminolevulinic acid dehydratase (ALAD) being highly sensitive to lead.

Lead (Pb) is unevenly distributed in the body, accumulating in soft tissues, blood, and bones. Additionally, when the concentration of Pb in plasma reaches 40-80 µg/dl, it can inhibit ALAD-mediated protoporphyrin metabolism and disrupt oxidation-reduction reactions. Therefore, a blood lead concentration exceeding 40 µg/dl can pose a significant health risk (Dündar et al., 2005). Accumulation of delta aminolevulinic acid (ALA) restricts ALAD activity, which is noticeable in the urine and plasma even at lead levels of less than 10 µg/dl in blood. Although ALAD inhibition is evident at blood lead levels of 10-20 µg/dl, the biosynthesis of heme does not decrease until ALAD activity is restricted by 80-90%, which occurs at higher blood lead concentrations of approximately 55 µg/dl (Ahamed, M., et al., 2005).

In our study, ALAD enzyme activity was significantly reduced in the Pb group compared to the control group (~39%). While ALAD activity in the Pb+PIC group also showed a significant decrease compared to the control (~13%), it was notably higher (~30%) than in the Pb group. Lead can inhibit enzyme activities by reacting with the sulfhydryl groups of proteins and by displacing the zinc ion at the enzyme's three cysteine binding sites (Gonick, 2011).

PIC is believed to enhance the body's antioxidant capacity and also exhibit a chelating effect on lead (Pb). Previous studies have demonstrated that PIC, similar to other polyphenols, possesses chelating properties. PIC has the ability to bind to toxic metal ions, forming chelating agents that facilitate the removal of these ions from both intracellular and extracellular spaces. This process involves the formation of complex structures that are easily excreted from the body. Recent studies have highlighted piceatannol's role as an antioxidant and cytoprotector (Piotrowska H., et al., 2012),

attributing this to its strong capacity as a chelator and sequestrant of reactive oxygen species (Li B., Pratt D.A., 2015). Lipid peroxidation is recognized as a key marker of oxidative damage and has been implicated as a major factor in the toxicity of various xenobiotics. Furthermore, it disrupts the physiological and biochemical properties of biological systems (R. Anane and E.E. Creppy, 2001). Valverde et al. (M. Valverde, et al., 2001) reported the induction of lipid peroxidation and increased levels of reactive oxygen species (ROS) in mouse tissues after exposure to lead for 1 hour.

These findings are consistent with those of Marchlewicz et al. (M. Marchlewicz, et al., 2007), suggesting lead-induced cytotoxicity through indirect mechanisms. Our recent study found a notably increased concentration of malondialdehyde (MDA) in the serum of rats exposed to lead. Additionally, our results demonstrated significant elevations in the lipid peroxidation marker MDA, along with significant reductions in antioxidant markers such as glutathione (GSH) and catalase (CAT) in the tissues under investigation. In the Pb group, where elevated lipid peroxidation levels were anticipated, there was a significant increase, more than doubling that of the control group. In contrast, the Pb+PIC group showed a significant increase (~30%) in MDA levels compared to the control, yet this increase was lower compared to the Pb group. These results regarding lipid peroxidation align with the observations on antioxidant enzymes and total glutathione (GSH) levels. The significant reduction in malondialdehyde (MDA) observed in the group treated with PIC following lead exposure underscores the antioxidant properties and metal chelation abilities of this phenolic compound. Cervello et al. (I. Cervello, A. et al., 1992) suggested that the GST enzyme catalyzes reactions using the thiol (–SH) group of glutathione, thereby detoxifying substances and rendering them more water-soluble. GST (glutathione S-transferase) and GSH (reduced glutathione) are closely interconnected in the redox system. The decrease in GSH levels can partly be attributed to the reduction in GST activity (A.S. Newairy and H.M. Abdou, 2009). The decline in GST activity observed after lead exposure may be due to lead-induced changes in the enzyme's structure and the insufficient availability of GSH, which acts as a substrate for GST (R. Neal et al., 1999). Lead also inhibits glutathione reductase, the enzyme responsible for converting oxidized glutathione (glutathione disulfide; GSSG) back to its reduced form (reduced glutathione; GSH) (Ahmed E et al., 2011). Several studies have demonstrated that lead alters the activity of antioxidant enzymes such as SOD, CAT, GPx, GST, and G6PD, as well as the levels of glutathione (GSH) in both animals and humans.

Prolonged exposure to lead via intraperitoneal (IP) or oral routes in experimental studies has been shown to significantly decrease the activity of these antioxidant enzymes (Wang, J., et al., 2012; and Moneim, A.E.A., et al., 2011).

The mechanisms by which lead affects these enzymes can be complex. Lead can competitively inhibit the absorption of essential bio-elements and/or replace them in the active sites of enzymes. Additionally, lead can bind to thiol (–SH) groups of proteins, altering their function (Lakshmi, B.V.S. et al., 2013). In human sperm, the generation of reactive oxygen species (ROS) initiates lipid peroxidation by triggering a chain reaction within the membrane, which then spreads damage throughout the cell (Aitken and Fisher, 1994).

Studies have demonstrated that rat sperm exposed to lead produce significantly higher levels of reactive oxygen species (ROS) in a dose-dependent manner compared to control groups (Hsu et al., 1997). The effects of lead-induced reactive oxygen species (ROS) generation in sperm may be attributed to the indirect impact of lead on free radical scavenging enzymes and glutathione. Marchlewicz et al. (1993) further supported this by observing abnormal responses of antioxidant enzymes in the midpiece of sperm from lead-exposed rats.

An epidemiological study on the male reproductive system has shown positive correlations between lead levels in seminal plasma and levels of reactive oxygen species (ROS) in spermatozoa (Kiziler, A.R., et al., 2007). Increased activity of superoxide dismutase (SOD) has been observed in individuals with prolonged lead exposure, suggesting a mechanism for the increased production of reactive oxygen species (ROS) caused by lead exposure (Kasperczyk, S., et al., 2004). This oxidative stress in reproductive tissues due to lead exposure is closely linked to the production of reactive oxygen species (ROS). However, lead-induced oxidative stress elicits varying responses across different target sites, including sperm, depending on the dosage from low to high levels (Hsu, P.C. and Guo, Y.L., 2002).

Further evidence from animals chronically exposed to lead shows an increase in lipid peroxide concentration in reproductive organs (Marchlewicz, M. et al., 2007). Therefore, some studies have suggested that increased production of reactive oxygen species (ROS) induced by lead is a significant molecular mechanism contributing to reproductive disorders in males, affecting spermatogenesis and hormonal stages.

The mechanism behind lead-induced oxidative stress involves its impact on the antioxidative defense system within cells. Lead exhibits a strong affinity for thiol (–SH) groups present in enzymes of the antioxidative defense system, including glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), thereby inhibiting their activity (Nair, A.R., et al., 2013).

The oxidative status of the testes in adult male rats exposed to lead acetate was investigated over a period of 15 days using intraperitoneal administration adjusted according to body weight. Lead exposure resulted in increased testicular malondialdehyde (MDA) levels, indicating elevated reactive oxygen species (ROS) production, and led to decreased activities of testicular antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione-S-transferase (Pandya et al., 2011).

Another study measured the enzyme levels of SOD, CAT, and MDA in mice chronically exposed to lead, showing that SOD levels were reduced by approximately 1.5 times, CAT levels by 2 times, and MDA levels increased more than twofold compared to the control group (Shalan et al., 2005). Mense and Zhang (2006) discovered that lead poisoning can induce anemia by inhibiting ferrochelatase and delta-aminolevulinic acid dehydratase (ALAD), enzymes crucial for heme biosynthesis. Lead has been reported to affect the reproductive system in both experimental animals and humans exposed to this metal (Silbergeld, 1983). High levels of lead in animals have been shown to lead to reproductive failure (Ahmed, W.M., et al., 2008). The theoretical mechanisms of lead toxicity on the male reproductive system have not yet been fully elucidated. Several pathways are proposed to explain how lead exposure could reduce male fertility. For example, various potassium and calcium channel isoforms in human spermatozoa and testes may contribute to the initial stages of the acrosome reaction (Benoff, S., et al., 2000). Moreover, in the reproductive organs of lead-exposed rats, activities of certain enzymes such as sodium potassium ATPase and alkaline phosphatase (ALP) were found to be decreased (Batra, N., et al., 2001). Another significant aspect of lead's reproductive toxicity may be its association with excessive generation of reactive oxygen species (ROS), a concern that has recently garnered considerable attention. Accumulation of lead in tissues leads to cellular damage through oxidative stress, characterized by increased production of ROS and a reduction in the activities of the cellular antioxidant system (Gandhi, J. et al., 2017).

In both women and men, lead exerts adverse effects on the reproductive system. Common effects observed in men include abnormal spermatogenesis (reduced number and motility of sperm), decreased libido, abnormal prostatic function, chromosomal damage, alterations in serum testosterone levels, and infertility.The presence of excessive reactive oxygen species (ROS) in semen has been strongly associated with poor sperm concentration, motility, and morphology (Agarwal et al., 1994). Lead (Pb) exposure has been linked to decreased sperm count, reduced motility, and increased morphological abnormalities in both animals (Sokol and Berman, 1991; Chowdhury, 2009) and humans (Alexander et al., 1996). Lead exposure has been linked to tissue oxidative damage in chicks (Donaldson and Leeming, 1984) and rats (Sandhir et al., 1994). Studies have also shown that Pb exposure induces reactive oxygen species (ROS) generation in rat spermatozoa, which is negatively associated with fertility (Hsu et al., 1997).

In our study, while all essential aspects of healthy rat sperm were observed to be intact and functional in the control group, the Pb-exposed group exhibited morphological deformities, immobile sperm, and frequent detachment of heads and tails. Although sperm integrity was preserved, structural abnormalities in the head or tail rendered the sperm dysfunctional. The oxidative damage caused by Pb was evident in the sperm. However, in the group treated with PIC after Pb exposure, structural anomalies in the sperm were less frequent, indicating the beneficial effects of PIC in mitigating stress-induced damage.

Lead has adverse effects on sperm count and impairs the activity of viable sperm. Furthermore, reduced motility and prolonged latency of sperm liquefaction have been observed in both exposed individuals and experimental animals following lead (Pb) exposure (Chowdhury et al., 1986).

In the present study, a significant increase in sperm deformities was found in lead-treated rats compared to the control group. In another study where rats were injected with different concentrations of lead, sperm anomalies and hormone levels were examined. Rats exposed to 0.05%, 0.1%, and 0.3% PbAc showed a decrease in sperm motility compared to the control group, although this decrease was not statistically significant.

However, sperm velocity decreased in all groups. There was a significant increase of over 100% in the percentage of total abnormal sperm in the groups injected with 0.1% ($p < 0.01$) and 0.3% PbAc ($p < 0.05$). Specifically, in the 0.1% PbAc group, there were

significant increases in the frequency of sperm with misshapen heads ($p < 0.01$) and abnormal tails ($p < 0.01$), while the high lead exposure group showed an increased frequency of neck abnormalities ($p < 0.01$). Following lead exposure, LH (luteinizing hormone) and FSH (follicle-stimulating hormone) levels were not significantly affected, but a notable increase in serum testosterone levels was observed only in animals treated with 0.05% PbAc ($p < 0.01$) (Allouche et al., 2009).

The motility of sperm is highly dependent on the integrity of the mitochondrial sheath, where phospholipids play a crucial role as major components. Oxidation of fatty acids in these phospholipids by reactive oxygen species (ROS) can lead to sperm damage (Jones and Mann, 1977), impairing their motility (DeLamirande and Gagnon, 1992).Griveau et al. (1995) demonstrated that reactive oxygen species (ROS) cause a decrease in sperm motility, which correlates with increased lipid peroxidation and loss of membrane polyunsaturated fatty acids. In this study, in vivo lead (Pb) exposure was associated with sperm ROS generation, which was mitigated by administration of PIC (piceatannol). Prolonged exposure to Pb has also been reported to cause abnormal levels of sex hormones and significantly lower sperm counts, which exhibit morphological abnormalities and immotility (Telisman, S., et al., 2007). The effect of lead on the final body weight of intoxicated rats was significantly lower compared to that of the healthy normal group.

Additionally, the relative weights of the testis and epididymis were measured and statistically compared to the control group. The Pb-exposed group showed significantly lower weights for both the testis and epididymis compared to the control group. In contrast, the weights in the Pb+PIC group were similar to those of the control group and not statistically different. Histological examinations corroborated these findings, revealing increased intercellular spaces and reduced cell numbers in the testicular tissues of the Pb-exposed group compared to the control. These histological changes likely contributed to the lower weights observed in the Pb group compared to the control.

These results clearly indicate that lead caused a significant decrease in the gain of body weight. Nabil et al. (2012) also found that lead caused a decrease in the growth rate of rats when fed lead-containing diets. The decrease in body weight gain observed due to exposure to toxic ions such as lead could be associated with several factors, including an

imbalance in metabolism caused by impaired zinc status. Zinc-dependent enzymes play a crucial role in numerous metabolic processes, and their impairment due to lead exposure can contribute to metabolic disturbances affecting body weight regulation. Along with the decrease in body weight, a significant reduction in testicular weight was also observed in animals treated with lead acetate. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells. Hence, a reduction in testicular weight might be attributed to a decreased number of germ cells and elongated spermatids (Chapin et al., 1997). The weight of accessory sex organs was also decreased in animals treated with lead acetate. This weight loss in accessory sex organs corresponds with the decrease in serum testosterone concentration observed in this study. It has been reported that testosterone plays a major role in maintaining the structural integrity and functional activities of the accessory sex organs (Moor et al., 1930a).

Lead acetate administered orally to adult male rats at exposures of 273 or 819 mg/L caused significant decreases in the weight of the reproductive organs, reduction in epididymal sperm count, motile sperm, and viable sperm, indicating a significant decrease in sperm production and deteriorated sperm quality. Additionally, serum testosterone levels in treated rats were decreased, indicating impaired steroidogenesis (Anjum, M.R., et al., 2011).

Lead acetate administered orally to rats at a dose of 8 mg/kg body weight showed a significant decrease ($p < 0.05$) in the weight of the reproductive organs and some accessory glands. It also affected the levels of LH and testosterone hormones and sperm count, accompanied by an increase ($p < 0.05$) in the mitotic index and percentage of abnormal sperm (Al-Sa'ady, M.S.M., et al., 2011). Hammed et al. (2012) reported that oral exposure of rats to lead acetate at a dose of 10 mg/kg body weight showed significant decreases in the levels of LH and FSH compared to the control group.

For normal testicular functions to occur, anterior pituitary hormones such as FSH (follicle-stimulating hormone) and LH (luteinizing hormone) must be functional. LH stimulates Leydig cells in the testes to produce and secrete testosterone, which is essential for various aspects of male reproductive health and function.

FSH indirectly supports the functions of LH by inducing the formation of LH receptors in Leydig cells, thereby facilitating the action of LH on testosterone production. In our study, although no significant change was observed in LH hormone levels between the groups, the levels of FSH and testosterone were found to be decreased in the Pb-

exposed group. This decrease suggests that despite the normal secretion of LH in the Pb-exposed group, FSH and testosterone could not be adequately produced due to damage and reduced numbers of Leydig cells. This finding is consistent with the histological observations of the current study and with the documented effects of Pb on these hormones in the literature (Sokol et al., 1985). In the Pb+PIC group, the levels of FSH and testosterone were found to be consistent with those of the control group.

The presence of atrophic Leydig cells in the testes of lead-treated animals further supports the potential of lead to interfere with the process of steroidogenesis. Experimental findings revealed that animals exposed to lead exhibited lower levels of plasma luteinizing hormone (LH) after stimulation with gonadotropin-releasing hormone compared to controls, as well as a reduction in the inhibin/follicle-stimulating hormone (FSH) ratio (Foster, W.G., et al., 1993). In this study, serum testosterone levels, LH, and FSH were reduced in lead acetate-treated groups of rats compared to their respective controls. Significant changes in testosterone, LH, and FSH levels have been observed following exposure to certain heavy metals (Chattopadhyay et al., 2005; Atef Al Attar, 2011).

LH and FSH activity is influenced by both the amount of these hormones and the number of their specific receptors in the testis. Studies have demonstrated that exposure to environmental contaminants negatively impacts testicular function by decreasing pituitary LH secretion and reducing Leydig cell steroidogenesis (Murugesan et al., 2007).

Alongside gonadotrophins, testosterone is a crucial hormone that regulates spermatogenesis. The Leydig cells' secretion of testosterone is reliant on LH secretion by the pituitary gland. This dependency may be attributed to lead causing pathological changes in the Leydig cells within the interstitial tissues. In our study, rats treated with lead acetate showed significantly lower levels of FSH and LH compared to the control rats.

To better observe the general appearance and cell defects in the tubules of testis tissue, hematoxylin-eosin staining was performed, yielding significant images. Examination of the testis morphology in the control group revealed a well-defined basal lamina surrounded by myoid cells and spermatogonia cells located just below the basal lamina. Among the spermatogonia, Sertoli cells with abundant cytoplasm and large nuclei were observed, showing an orderly arrangement of cells towards the center of the tubule and a high amount of sperm formation. Additionally, normal-looking Leydig cells,

responsible for testosterone production, were frequently observed among the interstitial cells. In the Pb group, deformation and fragmentation of the basal lamina, atrophy of spermatogenic cells, expansion of intercellular spaces, a reduced number of Leydig cells, and a corresponding decrease in sperm formation were observed. In the Pb+PIC group, spermatogenic cells appeared near normal, with slightly expanded intercellular spaces, numerous observed sperm formations, and normal-looking Leydig cells. The overall histological structure was more consistent with the control group.

Madhavi et al. (2007) demonstrated that lead induced cytogenetic damage in the germ cells of mice. This testicular damage was further confirmed by histopathological lesions (Muthu and Krishnamoorthy, 2012).These results indicate that in male rats, lead specifically targets testicular spermatogenesis and sperm within the epididymis, leading to reproductive toxicity. These findings corroborate previous reports indicating that lead acetate significantly affects the testes and reproductive tract in male rats treated with lead (El-Shafai et al., 2011).

Different methodologies are employed to assess oxidative stress damage, including the selection of suitable markers capable of detecting such damage and conducting immunolabeling techniques. In our study, we chose proliferating cell nuclear antigen (PCNA) as one of the markers. Given the highly proliferative nature of testis tissue in sperm production and the maintenance of generational continuity, PCNA is well-suited for evaluating cell proliferation in this context. The main objective of selecting this marker is to assess the impact of lead (Pb) on cellular proliferation capacity and to compare differences in the group administered PIC following Pb exposure. PCNA is crucially involved in DNA repair, synthesis, cell cycle regulation, chromatin remodeling, and various other essential cellular processes (Mjelle et al., 2015).

The level of PCNA in the cell significantly increases during the S phase preceding DNA synthesis and decreases in the G2 and M phases (Cardano et al., 2020). In the control group of our study, intense PCNA staining was observed, corresponding to normal cellular proliferation within the seminiferous tubules. Similarly, the Pb group also showed intense PCNA staining. However, it is believed that the cellular proliferation observed in the Pb group is not due to normal physiological proliferation but is instead a response to DNA damage caused by Pb exposure. Due to DNA damage, there is a loss of cell cycle control, leading to cells continuing to proliferate with damaged DNA. This interpretation is supported by the SCGE findings of our study. According to the SCGE results, DNA

damage in the Pb group increased more than sevenfold compared to the control. In the Pb+PIC group, the damage was reduced by more than half (~56%) compared to the Pb group. Additionally, PCNA staining in the Pb+PIC group was significantly reduced compared to both the control and Pb groups. This clearly demonstrates the regulatory effect of PIC against Pb toxicity. Furthermore, the findings of PCNA and SCGE are supported by the BRCA1 results. The increased BRCA1 labeling observed in the Pb and Pb+PIC groups is significantly higher than the mild background fluorescence seen in the control and PIC groups. The increase in the Pb group is thought to be associated with BRCA1's role in cellular survival mechanisms and apoptosis.

The reason for the increased levels of the multifunctional protein BRCA1 in the Pb+PIC group is likely related to its role in DNA damage repair. The significant reduction in DNA damage observed in the Pb+PIC group, as seen in the SCGE findings, further supports the activation of DNA repair mechanisms controlled by BRCA1 in this group. The findings from this thesis indicate that PIC is significantly effective in mitigating Pb toxicity in the male reproductive system. PIC primarily reduces Pb levels in tissues through its chelating effect. Additionally, PIC's high antioxidant capacity effectively prevents radicals and radical-induced damage in testis tissue. Due to PIC's impact, Pb-induced DNA damage and uncontrolled cell proliferation decreased, while DNA repair increased, preventing genotoxicity in the testis tissue. Furthermore, hormone levels and sperm production were maintained at near-normal levels under PIC's influence, preserving sperm quality, quantity, and testis tissue histology. This study is anticipated to contribute to the literature by emphasizing the potential utility of antioxidant therapies in mitigating the impacts of prolonged lead exposure on the male reproductive system, which may arise from occupational and environmental sources.

5. CONCLUSION

Lead contamination and its detrimental effects on human and animal health have long been a significant concern. Chronic exposure to lead, even at low levels, has been associated with a range of health issues, including neurodevelopmental deficits, cardiovascular diseases, renal dysfunction, and significant reproductive impairments.

Our research primarily focused on assessing the reproductive toxic effects of lead exposure in male rats and evaluating whether piceatannol could mitigate these effects, utilizing a combination of biochemical assays, hormonal analyses, histological examinations, and DNA analyses.

Key findings from our study indicate that lead exposure resulted in significant hormonal disruptions, oxidative stress, and structural changes in reproductive tissues. Lead-exposed animals exhibited increased oxidative stress markers, disrupted endocrine function, and notable histological abnormalities. Our findings demonstrated that piceatannol administration significantly mitigated many lead-induced alterations. Piceatannol-treated animals showed reduced oxidative stress markers, less hormonal disruption, and fewer histological and sperm abnormalities compared to those exposed to the control group.

The importance of these results cannot be overstated, as they not only confirm the reproductive toxic potential of lead but also highlight the significant protective effects of piceatannol. The ability of piceatannol to counteract lead-induced oxidative stress and hormonal imbalances suggests that it could be a potent therapeutic agent for mitigating lead toxicity. This information is crucial for developing targeted interventions to protect vulnerable populations, particularly in areas with high environmental lead contamination, and offers a promising avenue for future research and therapeutic development.

By explaining the biological impacts of lead exposure in animals and the protective effects of piceatannol, we can extrapolate these findings to potential human outcomes, aiding in the formulation of public health policies and medical guidelines. Understanding the full scope of lead's impact and the potential benefits of protective agents like piceatannol is vital for designing effective prevention and intervention strategies. Additionally, this study highlights the importance of reducing environmental lead exposure through stringent regulations and public awareness campaigns.

This research primarily investigated the reproductive toxic effects of lead and the protective effects of piceatannol. Future studies should consider the systemic impacts of

lead exposure and piceatannol treatment, including effects on other organs and systems. Interdisciplinary approaches combining toxicology, reproductive biology, and epidemiology will be essential to provide a comprehensive understanding of lead toxicity and the potential benefits of protective agents, particularly those incorporating human subjects and diverse exposure scenarios, are essential to fully comprehend and combat the multifaceted challenges posed by lead toxicity and to explore the full potential of protective compounds like piceatannol.

REFERENCES

Abadin, H., Ashizawa, A., Stevens, Y., Llados, F., Diamond, G., Sage, G., . . . Swarts, S. (2007). *Toxicological Profile for Lead.* Agency for Toxic Substances and Disease Registry.

Abdel Moneim, A. E., Dkhil, M., & Al-Quraishy, S. (2011). The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats. *Journal of Hazardous Materials*, 250-255. doi:10.1016/j.jhazmat.2011.07.097

Agarwal, A., Ikemoto, I., & Loughlin, K. (1994). Relationship of sperm parameter with levels of reactive oxygen species in semen specimens. *J. Urol., 152*, 107-110.

Ahamed, M., Verma, S., Kumar, A., & Siddiqui, M. (2005). Environmental exposure to lead and its correlation with biochemical indices in children. *Sci. Total Environ., 346*(1), 48-55.

Ahmed, W., Abdel-Hameed, A., & Moghazy, F. (2008). Some reproductive and health aspects of female buffaloes in relation to blood lead concentration. *Int. J. Dairy Sci., 3*(2), 63-70.

Aitken, R., & Fisher, H. (1994). Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *Bioessays, 16*, 259-267.

Aitken, R., Clarkson, J., & Fishel, S. (1989). Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biol. Reprod., 40*, 183-197.

Al-Attar, A. (2011). Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. *Saudi J. of Biol. Sci., 18*, 63-72.

Alberts, B., Johnson, A., Lewis, J., & al., e. (2002). *Molecular Biology of the Cell* (Cilt 4th edition). New York: Garland Science.

Alexander, B., Checkoway, H., Van Netten, C., & et al. (1996). Semen quality of men employed at a lead smelter. *Occup. Environ. Med., 53*, 411-416.

Al-Sa'ady, M., Kamil, H., & Jasim, S. (2011). Effect of lead acetate in some physiological genetic parameters in white male rat rattus rattus. *Fac. Educ. Karbala Univ., 3*(9), 295-301.

Anane, R., & Creppy, E. (2001). Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase + catalase and vitamins E and C. *Hum. Exp. Toxicol., 20*(2001), 477-481.

Anjum, M., Sainath, S., Suneetha, Y., & Reddy, P. (2011). Lead acetate induced reproductive and paternal mediated developmental toxicity in rats. *Ecotoxicol. Environ. Saf., 74*(4), 793-799.

Batool, Z., Yousafzai, N., Murad, M., Shahid, S., & Iqbal, A. (2017). Lead toxicity and evaluation of oxidative stress in humans. *PSM Biol. Res., 2*(2), 79-82.

Batra, N., Nehru, B., & Bansal, M. (2001). Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels'. *J. Appl. Toxicol., 21*(6), 507-512.

Beier, E., Inzana, J., Sheu, T., Shu, L., Puzas, J., & Mooney, R. (2015). Effects of combined exposure to lead and high-fat diet on bone quality in juvenile male mice. *Environ. Health Perspect., 123*(10), 935-943.

Benoff, S., Jacob, A., & Hurley, I. (2000). Male infertility and environmental exposure to lead and cadmium. *Hum. Reprod. Update, 6*(2), 107-121.

Buiarelli, F., Coccioli, F., Jasionowska, R., Merolle, M., & Terracciano, A. (2007). Analysis of some stilbenes in Italian wines by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom., 21*, 2955e2964.

Cardano, M., Tribioli, C., & Prosperi, E. (2020). Targeting proliferating cell nuclear antigen (PCNA) as an effective strategy to inhibit tumor cell proliferation. *Current Cancer Drug Targets, 20*, 240–252.

Cervello, A., Lafuente, M., Giralt, J., & Mallol. (1992). Enhanced glutathione S-transferase (GST) activity in pregnant rats treated with benzo(a)pyrene. *Placenta, 13*, 273-280.

Chang, J., Hsu, Y., Teng, I., & Kuo, P. (2006). Piceatannol stimulates osteoblast differentiation that may be mediated by increased bone morphogenetic protein-2 production. *Eur. J. Pharmacol., 551*, 1-9.

Chapin, R., Harris Davis, M., Ward, B., Wilson, S., Mauney, R., Lockhart, M., . . . Collins, B. (1997). The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. *National Toxicology Program, NIEHS, Fundam. Appl. Toxicol., 40*(1), 138-57.

Chattopadhyay, R., Sarkar, M., & Biswas, N. (2005). Dose-dependent effect of copper chloride on male reproductive function in immature rats. *Kathmandu Univ Med J, 3*, 392-400.

Chiva-Blanch, G., & Visioli, F. (2012). Polyphenols and health: Moving beyond antioxidants. *Journal of Berry Research, 2*(2), 63-71.

Chojnacka, K., Zarzycka, M., & Mruk, D. (2016). Biology of the Sertoli Cell in the Fetal, Pubertal, and Adult Mammalian Testis. *Results Probl. Cell. Differ., 58*, 225-51.

Chowdhury, A., Rao, R., & Gautam, A. (1986). Histochemical changes in the testes of lead induced experimental rats. *Folia. Histochem. Cytobiol., 24*, 233-238.

Chowdhury, R. (2009). Recent advances in heavy metals induced effect on male reproductive function-A retrospective. *Al Ameen J. Med. Sci., 2*, 37-42.

Clarkson, T., Nordbekg, G., & Sager, P. (1983). Reproduction and developmental toxicity of metals. *Plenum Press*, 343-368.

Courtois, E., Marques, M., Barrientos, A., Casado, S., & López-Farré, A. (2003). Lead induced down regulation of soluble guanylate cyclase in isolated rat aortic segments mediated by reactive oxygen species and cyclooxygenase-2. *J. Am. Soc. Nephrol., 14*, 1464-1470.

Dart, R., Hurlbut, K., & Boyer-Hassen, L. (2004). *Lead. In: Medical Toxicology* (Cilt 3rd ed.). (D. RC, Dü.) Lippincot Williams and Wilkins.

DeLamirande, E., & Gagnon, C. (1992). Reactive oxygen species and human spermatozoa. I. effect on the motility of intact spermatozoa and on sperm axonemes. *J. Androl., 13*, 367-378.

Donaldson, W., & Leeming, T. (1984). Dietary lead: effects on hepatic fatty acid composition in chicks. *Toxicol. Appl. Pharmacol., 73*, 119-123.

Duan, P., Hu, C., Quan, C., Yu, T., Huang, W., Chen, W., . . . Yang, K. (2017). 4-Nonylphenol induces autophagy and attenuates mTOR-p70S6K/4EBP1 signaling by modulating AMPK activation in Sertoli cells. *Toxicol. Lett., 267*, 21-31.

Dündar, Y., & Aslan, R. (The Medical Journal of Kocatepe). Effects of Lead as a Life Surrounding Heavy Metal. *2005, 6*, 1-5.

Dustin, M., & Chan, A. (2000). Signaling takes shape in the immune system. *Cell., 103*, 283-294.

Elgawish, R., & Abdelrazek, H. (2014). Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. *Toxicol. Rep., 1*, 795-801.

El-Shafai, A., Zohdy, N., El-Mulla, K., Hassan, M., & Morad, N. (2011). Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of ascorbic acid. *Food and Chemical. Toxicol., 49*, 734-743.

Fang, J., Wang, P., Huang, C., & Hung, Y. P. (2014). Evaluation of the hepatotoxic risk caused by lead acetate via skin exposure using a proteomic approach. *Proteomics, 14*, 2588-2599.

Flora, S. (2011). Arsenic-induced oxidative stress and its reversibility. *Free Radical Biol. Med., 51*(2), 257-281.

Foster, W., McMahon, A., YoungLai, E., Hughes, E., & Rice, D. (1993). Reproductive endocrine effects of chronic lead exposure in the male cynomolgus monkey. *J. Reprod. Toxicol., 7*(3), 203-209.

Fujita, H., Nishitani, C., & Ogawa, K. (2002). Lead, chemical porphyria, and heme as a biological mediator. *Tohoku. J. Exp. Med., 196*(2), 53-64.

Gall, J., Boyd, R., & Rajakaruna, N. (2015). Transfer of heavy metals through terrestrial food webs: a review. *Environ. Monit. Assess., 187*(4), 1–21. doi:10.1007/s10661-015-4436-3

Gandhi, J., & et al. (2017). Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. *Zygote, 25*, 103-110.

Gonick, H. (2011). Lead-Binding Proteins: A Review. *Journal of Toxicology, 2011*, ID 686050. doi:10.1155/2011/686050

Grant, L. (2009). *Lead and compounds* (Cilt 3rg ed). (Lippmann M, Dü.) Wiley-Interscience.

Griswold, M. (2018). 50 years of spermatogenesis: Sertoli cells and their interactions with germ cells. *Biol. Reprod., 99*(1), 87-100.

Griveau, J., Dumont, E., Renard, P., Callegari, J., & Le Lannou, D. (1995). Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. *J. Reprod. Fertil., 103*, 17-26.

Gupta, N., Khan, D., & Santra, S. (2008). An assessment of heavy metal contamination in vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. *Bull. Environ. Contam. Toxicol., 80*(2), 115-118. doi:10.1007/s00128-007-9327-z

Han, X., Shen, T., & Lou, H. (2007). Dietary Polyphenols and Their Biological Significance. *Int J Mol Sci., 8*, 950-88.

Hassaan, Y., Handoussa, H., El-Khatib, A., Linscheid, M., El Sayed, N., & et al. (2014). Evaluation of plant phenolic metabolites as a source of Alzheimer's drug leads. *Biomed. Res. Int., 2014*, 843263.

He, S., & Yan, X. (2013). from resveratrol to its derivatives: new sources of natural antioxidant. *Curr. Med. Chem., 20*, 1005e1017.

Hijona, E., Aguirre, L., Pérez-Matute, P., Villanueva-Millan, M., MosquedaSolis, A., Hasnaoui, M., . . . C, C. (2016). Limited beneficial effects of piceatannol supplementation on obesity complications in the obese Zucker rat: gut microbiota, metabolic, endocrine and cardiac aspects. *J. Physiol. Biochem., 72*, 567e582. doi:10.1007/s13105-015-0464-2

Hsu, P., & Guo, Y. (2002). Antioxidant nutrients and lead toxicity. *Toxicology, 180*(1), 33-44.

Hsu, P., Liu, M., Hsu, C., Chen, L., & Guo, Y. (1997). Lead exposure causes generation of reactive oxygen species and functional impairment in rat sperm. *Toxicology, 122*, 133-143.

Huang, Y., & et al. (2017). Accumulation of methylglyoxal and d-lactate in Pb-induced nephrotoxicity in rats. *Biomed. Chromatogr., 31*, e3869.

Jones, R., & Mann, T. (1977). Damage to ram spermatozoa by peroxidation of endogeneous phospholipids. *J. Reprod. Fertil.*, 261-268.

Jones, R., Mann, T., & Sherins, R. (1979). Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. *Fertil. Steril., 31*, 531-537.

Juberg, D., Kleiman, C., & Kwon, S. (1997). Position paper of the American Council on Science and Health: lead and human health. *Ecotoxicol. Environ. Saf., 38*, 162-180.

Karri, S., Saper, R., & Kales, S. (2008). Lead Encephalopathy Due to Traditional Medicines. *Curr. Drug. Saf., 3*, 54–9.

Kasperczyk, S., Birkner, E., Kasperczyk, A., & ZalejskaFiolka, J. (2004). Activity of superoxide dismutase and catalase in people protractedly exposed to lead compounds. *Ann. Agric. Environ. Med., 11*(2), 291-296.

Khaki, A., Bayatmakoo, R., & Nouri, M. (2013). Remedial effect of Cinnamon zeylanicumon serum anti-oxidants levels in male diabetic Rat. *Life Sci. J., 10*(4s), 103-107.

Kim, Y., Park, C., Lee, J., Kim, G., Lee, W., & et al. (2008). Induction of apoptosis by piceatannol in human leukemic U937 cells through down-regulation of Bcl-2 and activation of caspases. *Oncol. Rep., 19*, 961-967.

Kiziler, A., Aydemir, B., Onaran, I., Alici, B., Ozkara, H., Gulyasar, T., & Akyolcu, M. (2007). High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. *Biol. Trace Elem. Res., 120*(1-3), 82-91.

Ko, Y., Kim, H., Kim, E., Katakura, Y., Lee, W., & et al. (2013). Piceatannol inhibits mast cell-mediated allergic inflammation. *Int. J. Mol. Med.*, 951-958.

Kosnett, M. (2006). *Lead. In: Poisoning and Drug Overdose* (Cilt 5th ed.). (Olson K.R, Dü.) McGraw Hill Professional.

Kucinska, M., Piotrowska, H., Luczak, M., Mikula-Pietrasik, J., Ksiazek, M. W., Wierzchowski, M., . . . Murias, M. (2014). Effects of hydroxylated resveratrol analogs on oxidative stress and cancer cells death in human acute T cell leukemia cell line: prooxidative potential of hydroxylated resveratrol analogs. *Chem. Biol. Interact., 209*, 96e110.

Kukreja, A., Mishra, A., & Tiwari, A. (2013). Source, production and biological activities of piceatannol: a review. *IJPSR, 4*, 1000-1007.

Kumar, A., Prasad, M., Achary, V., & Panda, B. (2013). Elucidation of lead-induced oxidative stress in Talinum triangulareroots by analysis of antioxidant responses and DNA damage at cellular level. *Environ. Sci. Pollut. Res., 20*(7), 4551-4561.

Kuo, P., & Hus, Y. (2008). The grape and wine constituent piceatannol inhibits proliferation of human bladder cancer cells via blocking cell cycle progression and inducing as/membrane-bound Fas ligand mediated apoptotic pathway. *Mol. Nutr. Food. Res.*, 408-418.

Kwon, J., Seo, S., Heo, Y., Yue, S., Cheng, J., & et al. (2012). Piceatannol, natural polyphenolic stilbene, inhibits adipogenesis via modulation of mitotic clonal expansion and insulin receptor-dependent insulin signaling in early phase of differentiation. *J. Biol. Chem., 287*, 11566-78.

Lakshmi, B., Sudhakar, M., & Aparna, M. (2013). Protective potential of Black grapes against lead induced oxidative stress in rats. *Environ. Toxicol. Pharmacol., 35*(3), 361-368.

Li, B., & Pratt, D. (2015). Methods for determining the efficacy of radical-trapping antioxidants. *Free Radic. Biol. Med., 82*, 187-202. doi:10.1016/j.freeradbiomed.2015.01.020

Liu, W., & Chang, L. (2010). Piceatannol induces Fas and FasL up regulation in human leukemia U937 cells via Ca2+/p38alpha MAPK-mediated activation of c-Jun and ATF-2 pathways. *Int J Biochem Cell Biol, 42*, 1498-1506.

Madhavi, D., Rudrama, K., Devi, K., Kesava, R., & Reddy, P. (2007). Modulating effect of Phyllanthus fruit extract against lead genotoxicity in germ cells of mice. *J. Enviro. Biol., 28*, 115-117.

Marchlewicz, M., Protasowicki, M., Piasecka, M., & Laszczynska, M. (1993). Effect of long-term exposure to lead on testis and epididymis in rats. *Folia. Histochem. Cyto.*, 55-62.

Marchlewicz, M., Wiszniewska, B., Gonet, B., Baranowska-Bosiacka, I., Safranow, K., Kolasa, A., . . . Rac, M. (2007). Increased lipid peroxidation and ascorbic acid

utilization in testis and epididymis of rats chronically exposed to lead. *Biometals, 20*, 13-19.

Matsui, Y., Sugiyama, K., Kamei, M., Takahashi, T., Suzuki, T., Katagata, Y., & Ito, T. (2010). Extract of passion fruit (Passiflora edulis) seed containing high amounts of piceatannol inhibits melanogenesis and promotes collagen synthesis. *J. Agric. Food Chem., 58*, 11112-11118.

Mense, S., & Zhang, L. (2006). Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases. *Cell Res., 16*(8), 681-692. doi:10.1038/sj.cr.7310086

Merill, J., Morton, J., & Soileau, S. (2007). *Principles and Methods of Toxicology* (Cilt 5th ed). (A. Hayes, Dü.) CRC Press.

Minakawa, M., Miura, Y., & Yagasaki, K. (2012). Piceatannol, a resveratrol derivative, promotes glucose uptake through glucose transporter 4 translocation to plasma membrane in L6 myocytes and suppresses blood glucose levels in type 2 diabetic model db/db mice,. *Biochem. Biophys. Res. Commun., 422*, 469e475.

Mjelle, R., Hegre, S., Aas, P., Slupphaug, G., Drablos, F., Saetrom, P., & Krokan, H. (2015). Cell cycle regulation of human DNA repair and chromatin remodeling genes. *DNA Repair (Amst), 30*, 53-67.

Moore, C., Price, D., & Gallagher, T. (1930a). Rat – Prostate cytology as a testis - hormone indicator and the prevention of castration changes by testis-extract injections. *America Journal of Anatomy, 45*, 71-107.

Mukherjee, A., Giri, A., Sharma, A., & Talukder, G. (1988). Relative efficacy of short-term tests in detecting genotoxic effects of cadmium chloride in mice in vivo. *Mutation Res.*, 206-285.

Murias, M., J€ager, W., Handler, N., Erker, T., Horvath, Z., Szekeres, T., . . . Gille, L. (2005). Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure-activity relationship. *Biochem. Pharmacol., 69*, 903e912.

Murugesan, P., Muthusamy, T., Balasubramanian, K., & Arunakaran, J. (2007). Effects of vitamins C and E on steroidogenic enzymes mRNA expression in polychlorinated biphenyl (Aroclor 1254) exposed adult rat Leydig cells. *Toxicology, 232*, 170-182.

Muthu, K., & Krishnamoorthy, P. (2012). Effect of Vitamin C and Vitamin E on Mercuric Chloride-Induced Reproductive Toxicity in Male Rats. *Muthu and Krishnamoorthy Biochem. Pharmacol., 1*, 7.

Nabil, M., Esam, A., Hossam, S., & Yasmine, E. (2012). Effect of lead acetate toxicity on experimental male rat. . *Asian Pacific J. Tropical Biomedecine*, 41-46.

Nair, A., DeGheselle, O., Smeets, K., Van Kerkhove, E., & Cuypers, A. (2013). Cadmium-induced pathologies: Where is the oxidative balance lost (or not)? *Int. J. Mol. Sci., 14*(3), 6116-6143.

Neal, R., Cooper, K., Kellogg, G., Gurer, H., & Ercal, N. (1999). Effects of some sulfurcontaining antioxidants on lead-exposed lenses. *Free Radic. Biol. Med., 26*, 239–243.

Needleman, H. (2004). Lead poisoning. *Annu. Rev. Med., 55*, 209–222.

Newairy, A., & Abdou, H. (2009). Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. *Food Chem. Toxicol.*, 813-818.

Patra, R., Rautray, A., & Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *J. Vet. Intern. Med.*, 457327.

Patrick, L. (2006). Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. *Altern. Med. Rev., 11*, 2-22.

Piotrowska, H., & Kucinska, M. M. (2012). Biological activity of piceatannol: leaving the shadow of resveratrol. *Mutat. Res., 750*, 60-82.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., & Pinelli, E. (2011). Lead uptake, toxicity, and detoxification in plants. *Reviews of Environmental Contamination and Toxicology, 213*, 113-136. doi:10.1007/978-1-4419-9860-6_4

Quinlan, G., Halliwell, B., Moorhouse, C., & Gutteridge, J. (1988). Action of lead and aluminum ions on iron stimulated lipid peroxidation in liposomes, erythrocytes and rat liver microsome fractions. *Biochem. Biophys. Acta., 962*, 196-200.

Ravel, C., & Jaillard, S. (2011). The Sertoli cell. *Morphologie, 95*(311), 151-8.

Rimando, A., Kalt, W., Magee, J., Dewey, J., & Ballington, J. (2004). Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J. Agric. Food Chem., 52*, 4713e4719.

Rubin, R., & Strayer, D. (2008). *pathology; Clinicopathologic Foundations of Medicine. Environmental and Nutritional pathology* (Cilt 5th ed). (Rubins, Dü.) Lippincot Williams & Wilkins.

Ruweler, M., Gulden, M. M., Murias, M., & Seibert, H. (2009). Cytotoxic, cytoprotective and antioxidant activities of resveratrol and analogues in C6 astroglioma cells in vitro. *Chem. Biol. Interact., 182*, 128e135.

Sandhir, R., Julka, D., & Gill, K. (1994). Lipoperoxidative damage on lead exposure in rat brain and its implications on membrane bound enzymes. *Pharmacol. Toxicol., 74*, 66-71.

Sano, S., Sugiyama, K., Ito, T., Katano, Y., & Ishihata, A. (2011). Identification of the strong vasorelaxing substance scirpusin B, a dimer of piceatannol, from passion fruit (Passiflora edulis) seeds. *J. Agric. Food Chem., 59*, 6209e6213.

Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M., & Tauqeer, H. (2013). Heavy metals contamination and what are the impacts on living organisms. *Greener J. Environ. Manag. Public Saf., 2*(4), 172-179. S.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical reviews in food science and nutrition, 45*(4), 287-306.

Seow, C., Chue, S., & Wong, W. (2002). Piceatannol, a Syk-selective tyrosine kinase inhibitor, attenuated antigen challenge of guinea pig airways in vitro. *Eur. J. Pharmacol., 443*, 189-196.

Setoguchi, Y., Oritani, Y., Ito, R., Inagaki, H., Maruki-Uchida, H., Ichiyanagi, T., & Ito, T. (2014). Absorption and metabolism of piceatannol in rats. *J. Agric. Food Chem., 62*, 2541e2548.

Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., & Dumat, C. (2015). Heavy metal stress and crop productivity. *In: Crop Production and Global Environmental Issues. Springer, Cham*, 1-25. doi:10.1007/978-3-319-23162-4_1

Shalan, M., Mostafa, M., Hassouna, M., Hassab El-Nabi, S., & El-Refaie, A. (2005). Amelioration of lead toxicity on rat liver with Vitamin C and silymarin supplements. *journal of toxicology, 206*(1), 1-15. doi:10.1016/j.tox.2004.07.006.

Silbergeld, E. (1983). *The effect of lead on reproduction - review of experimental studies, in: Lead versus health: Sourees and effects of low-level lead exposure.* (M. Rutter, & R. Jones, Dü) New York: John Wiley and Sons.

Singh, B., Singh, N., Thakur, S., & Kaur, A. (2017). Ultrasound assisted extraction of polyphenols and their distribution in whole mung bean, hull and cotyledon. *Journal of food science and technology, 54*(4), 921-932.

Singh, M., Dwivedi, S., Yadav, S., Sharma, P., & Khattri, S. (2014). Arsenic-induced hepatic toxicity and its attenuation by fruit extract of Emblica officinalis(amla) in mice. *Indian J. Clin. Biochem.,, 29*(1), 29-37.

Sokol, R. (1985). Lead toxicity and the hypothalamic-pituitary testicular axis. *Biol. Reprod., 33*, 722-728.

Sokol, R., & Berman, N. (1991). The effect of age of exposure on lead-induced testicular toxicity. *Toxicology, 69*, 269-278.

Stohs, S., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med., 18*, 321-336. doi:10.1016/0891-5849(94)00159-h

Tang, Y., & Chan, S. (2014). A review of the pharmacological effects of piceatannol on cardiovascular diseases. *Phytother. Res., 28*, 1581e1588.

Telisman, S., Colak, B., Pizent, A., Jurasovic, J., & Cvitkovic, P. (2007). Reproductive toxicity of low-level lead exposure in men. 105. *Environ. Res., 108*, 256–66.

Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients, 2*(12), 1231-1246.

Uchida-Maruki, H., Inagaki, H., Ito, R., Kurita, I., Sai, M., & Ito, T. (2015). Piceatannol lowers the blood glucose level in diabetic mice. *Biol. Pharm. Bull., 38*, 629e633.

Valverde, M., Trejo, C., & Rojas, E. (2001). Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA–metal interaction? *Mutagenesis, 16*, 265-270.

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A., & Pigna, M. (2010). Mobility and bioavailability of heavy metals and metalloids in soil environments. *J. Soil Sci. Plant Nutr., 10*(3), 268-292. doi:10.4067/S0718-95162010000100005

Wang, B., Lien, Y., & Yu, Z. (2004). Supercritical fluid extractive fractionation: study of the antioxidant activities of propolis. *Food. Chem., 86*, 237-243.

Wang, J., Yang, Z., Lin, L., Zhao, Z., Liu, Z., & Liu, X. (2012). Protective effect of naringenin against lead-induced oxidative stress in rats. *Biol. Trace Elem. Res., 146*(3), 354-359.

Weydert, C., & Cullen, J. (2009). Measurement of superoxide dismutase, catalase, and glutathione peroxidase in cultured cells and tissue. *National Institutes of Health, Nat Protoc., 5*(1), 51-66. doi:10.1038/nprot.2009.197

White, L., Cory-Slechta, D., Gilbert, M., Tiffany-Castiglioni, E., Zawia, N., Virgolini, M., . . . Basha, M. (2007). New and evolving concepts in the neurotoxicology of lead. *Toxicol. App. Pharmacol., 225*, 1-27.

Wiebe, J., Barr, K., & Buckingham, K. (1982). Lead administration during pregnancy and lactation affects steroidogenesis and hormone receptors in the testis of offsprings. *Toxicol. Environ. Health, 10*, 653-6GG.

Wittgen, H., & van Kempen, L. (2007). Reactive oxygen species in melanoma and its therapeutic implications. *Melanoma Res., 17*, 400-409.

Wolter, F., Clausnitzer, A., Akoglu, B., & Stein, J. (2002). Piceatannol, a natural analog of resveratrol, inhibits progression through the S phase of the cell cycle in colorectal cancer cell lines. *J. Nutr., 132*, 298-302.

Yokozawa, T., & Kim, Y. (2007). Piceatannol inhibits melanogenesis by its antioxidative actions. *Biol. Pharm. Bull., 30*, 2007-2011.

Yürekli, A. (2019). Lisansüstü Eğitim Enstitüsü Tez Yazım Kılavuzu. *Eskişehir Teknik Üniversitesi Lisansüstü Eğitim Enstitüsü*, 1-12.

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science, 8*, 33-42.

Zhang, Z., & et al. (2017). The Protective Efect of Baicalin Against Lead-Induced Renal Oxidative Damage in Mice. *Biol. Trace. Elem. Res., 175*, 129–135.

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