TOXICOLOGICAL IMPACTS OF LEAD ACCETATE ON WISTAR RATS' KIDNEY, LIVER AND BLOOD

BY:

CHIDERA FRANCIS NWAKAEZE

MATRICULATION NUMBER:

LSC2003075

DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

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AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research titled "**TOXICOLOGICAL IMPACTS OF LEAD ACCETATE ON WISTAR RATS' KIDNEY, LIVER AND BLOOD"** was carried out by **"CHIDERA FRANCIS NWAKAEZE "** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of a Bachelor of Science degree in Environmental Management and Toxicology.

_____ _____

PROF. MRS. E.T. ASIEN **DATE**

(PROJECT SUPERVISOR)

_____ _____

PROF. MRS. E.T. ASIEN **DATE**

(HEAD OF DEPARTMENT)

DECLARATION

I **CHIDERA FRANCIS NWAKAEZE** declare that **"TOXICOLOGICAL IMPACTS OF LEAD ACCETATE ON WISTAR RATS' KIDNEY, LIVER AND BLOOD"** is my work and that all sources that I have used or quoted have been acknowledged using complete references and that this work has not been submitted before for any other degree at any other University.

_____ _____

CHIDERA FRANCIS NWAKAEZE **DATE**

DEDICATION

This report is dedicated to Almighty God, my parents Mr. and Mrs. Nwakaeze, and my Project Supervisor Prof. Mrs. E.T. Aisien, for their unwavering support and guidance.

ACKNOWLEDGEMENT

First and foremost, I thank God.

A big thanks goes to my project supervisor, Prof. Mrs. E.T. Aisien, whose patience and guidance really shaped this work.

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ABSTRACT

This study explores the harmful effects of lead acetate exposure on the kidneys, liver, and blood in Wistar rats, aiming to shed light on potential risks to human health from this common environmental toxin. Over a four-week period, adult Wistar rats were divided into four groups: a control group receiving distilled water and three treatment groups administered varying doses of lead acetate (low, medium, and high) via oral gavage. Weekly assessments included hematological analyses such as complete blood counts, measurements of inflammatory markers like TNF-α in liver and kidney tissues, and oxidative stress evaluations through assays for key enzymes and antioxidants. Results revealed dose-dependent disruptions, with treated groups showing significant reductions in hemoglobin, platelet, and white blood cell counts, alongside elevated neutrophil and lymphocyte levels indicative of immune stress. In the liver and kidneys, heightened TNF-α concentrations pointed to progressive inflammation, while oxidative stress markers demonstrated increased lipid peroxidation and depleted antioxidant defenses, particularly in higher-dose groups by week four. These findings underscore lead acetate's capacity to induce organ damage through oxidative and inflammatory pathways, highlighting the need for stricter controls on lead exposure in industrial and everyday settings to prevent similar toxicities in humans.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Lead, a highly soluble heavy metal compound, is a significant environmental pollutant originating from industrial activities such as battery manufacturing, paint production, and mining. Its presence in water, soil, and air poses substantial risks to human health, primarily through ingestion or inhalation, affecting vital organs like the liver, kidneys, and blood. The toxicity of lead acetate manifests through oxidative stress, evidenced by elevated malondialdehyde (MDA) and reduced levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) (Balali-Mood *et al*., 2021; Cureus, 2023). It also triggers inflammatory responses, potentially involving markers like tumor necrosis factor-alpha (TNF-α), and disrupts hematological parameters, including hemoglobin (HGB), packed cell volume (PCV), white blood cell (WBC) counts, and platelets (PLT), leading to systemic health issues (Idoko and Orakpoghenor, 2024 ; Vukelić *et al.,* 2023). These effects underscore the importance of studying lead acetate's toxicological impacts to inform human toxicity modeling.

Wistar rats, valued for their physiological similarities to humans, are widely used in toxicological studies to evaluate the effects of lead acetate, providing insights into potential human health risks (Adel-Yeha *et al.*, 2024). In experimental settings, lead acetate is often administered via gavage at controlled doses to simulate acute exposure scenarios, as seen in studies using doses such as 480 mg/kg (Idoko and Orakpoghenor, 2024). However, challenges such as variable dosing protocols and environmental conditions can affect the reliability of toxicological outcomes. The lack of standardized methods for assessing oxidative stress, inflammatory responses, and hematological changes in acute exposure

contexts complicates human toxicity modeling. This study addresses these gaps by investigating the acute toxicological effects of lead acetate on Wistar rats' liver, kidneys, and blood, aiming to enhance understanding of its implications for human health and environmental safety.

1.2 Statement of Problem

The widespread environmental presence of lead, driven by industrial activities, waste materials containing lead, increases human exposure risks through contaminated water and food chains, posing significant health threats. While acute exposure to lead acetate is known to induce oxidative stress, inflammatory responses, and hematological disruptions (Balali-Mood *et al.,* 2021; Idoko and Orakpoghenor, 2024). There is limited research on its effects at low doses in Wistar rats as a model for human toxicity. The absence of standardized protocols for evaluating oxidative stress markers (MDA, SOD, GSH, CAT), inflammatory response indicators (e.g., TNF-α), and hematological parameters (WBC, HGB, PCV, PLT) in acute exposure scenarios hinders reliable human toxicity modeling. This study seeks to address these issues by assessing the acute toxicological impacts of lead acetate on Wistar rats, providing insights to mitigate associated human health risks.

1.3 Justification of the Study

The increasing environmental prevalence of lead acetate and its potential to cause oxidative stress, inflammatory responses, and hematological alterations necessitate this investigation. Understanding the acute effects of lead acetate through Wistar rat models is critical for human toxicity modeling, given their physiological similarities to humans (Adel-Yeha *et al.,* 2024). By evaluating oxidative stress markers, inflammatory responses, and hematological parameters, this study aims to generate data that can inform environmental health policies and enhance safety standards for populations exposed to lead acetate. Addressing gaps in

acute exposure research will contribute to developing effective strategies to reduce health risks associated with this pollutant.

1.4 Aim and Objectives of the Study

The aim of this study is to evaluate the acute toxicological impacts of lead acetate on Wistar rats' liver, kidney and blood.

The objectives of the study are to:

- Determine the levels of oxidative stress markers (MDA, SOD, GSH, CAT) in Wistar rats exposed to lead acetate.
- Assess the inflammatory response through TNF-α levels in Wistar rats' liver and kidneys.
- Evaluate hematological parameters (WBC, HGB, PCV, PLT) in Wistar rats exposed to lead.

1.5 Scope of Study

This study focuses on the acute toxicological evaluation of lead acetate's effects on Wistar rats, specifically targeting the liver, kidneys, and blood. It involves oxidative stress assays (measuring MDA, SOD, GSH, CAT), inflammatory response assays (proposed TNF-α), and hematological tests (WBC, HGB, PCV, PLT) to model human toxicity. The study encompasses a comprehensive experimental design, utilizing 16 Wistar rats administered lead acetate at 0.05 mg/L via gavage twice weekly (Tuesdays and Fridays) to simulate acute exposure. Sample collection occurs every Monday for four weeks, involving blood and organs for the specified assays. On a weekly basis, the rats are fed daily, their wood shavings are changed daily to maintain a good hygiene, and their boxes are cleaned to maintain a controlled environment. The rats are monitored daily, particularly at night, for

signs of lethargy and weight loss, with their weights recorded daily to track physiological changes. This rigorous experimental setup ensures robust data collection to assess lead acetate's acute effects and their relevance to human health risks.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Environmental Pollutants and Lead Acetate

2.1.1 Environmental Pollutants

Environmental pollutants, such as heavy metals, pose significant risks to human and animal health due to their persistence and bioaccumulation in ecosystems. Among these, lead is a major concern, originating from industrial activities, mining, and historical use of leaded products (Balali-Mood *et al.,* 2021). Lead acetate, a soluble compound, is widely used in toxicological studies to simulate environmental lead exposure, particularly in Wistar rats, due to their physiological similarities to humans (Idoko and Orakpoghenor, 2024). This makes it an effective model for studying lead's impact on critical organs like the liver, kidney, and blood.

2.1.2 Lead Acetate

Lead acetate, a bioavailable form of lead, is absorbed through ingestion or inhalation, distributing to organs via the bloodstream (Balali-Mood *et al.,* 2021). Its toxicity manifests through oxidative stress, inflammatory responses, and hematological disruptions, affecting multiple organ systems (Vukelić *et al.,* 2023). Wistar rats exposed to lead acetate exhibit organ-specific damage, making them a reliable model for understanding human toxicity (Bebily *et al.,* 2023). This review focuses on lead acetate's effects, assessed through oxidative stress assays, inflammatory response assays, and hematological tests.

2.2 Toxicokinetics of Lead Acetate

Lead acetate is rapidly absorbed in the gastrointestinal tract and binds to red blood cells and plasma proteins, facilitating its distribution to the liver, kidneys, and other tissues (Balali-Mood *et al.,* 2021). Its slow excretion via urine and bile leads to accumulation, particularly in the liver and kidneys, where it induces oxidative damage (Vukelić *et al.,* 2023). Studies show that subacute exposure to low doses (e.g., 0.05–0.5 mg/kg) in Wistar rats results in significant lead accumulation, disrupting essential element homeostasis and promoting toxicity (Vukelić *et al.,* 2023). These toxicokinetic patterns mirror human exposure, validating Wistar rats as a model for lead toxicity studies.

2.3 Toxicological Effects of Lead Acetate on Wistar Rats

Lead induces multi-organ toxicity, with pronounced effects on the liver, kidney, and blood, as assessed through oxidative stress, inflammatory, and hematological assays.

2.3.1 Effects on the Liver

The liver, a primary site for detoxification, is highly vulnerable to lead. Subacute exposure increases oxidative stress, evidenced by elevated malondialdehyde (MDA) levels, a marker of lipid peroxidation, and reduced antioxidant enzyme activity, such as glutathione (GSH) and catalase (CAT) (Pat *et al.,* 2023; Vukelić *et al.,* 2023). Bebily *et al.* (2023) reported histological changes, including vacuolated hepatocytes, necrosis, and inflammatory cell infiltration, indicating severe liver damage. Inflammatory response assays reveal elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), in lead-exposed rats, exacerbating hepatic injury (Bebily *et al.,* 2023). These findings highlight lead acetate's hepatotoxic potential, relevant to human liver dysfunction.

2.3.2 Effects on the Kidney

The kidneys, critical for filtration and excretion, are significantly affected by lead acetate. Oxidative stress assays show increased MDA and decreased GSH and CAT levels, indicating renal oxidative damage (Vukelić *et al*., 2023). Inflammatory responses, marked by elevated interleukin-6 (IL-6) and TNF-α, contribute to tubular atrophy and glomerular sclerosis, as observed in histological studies (Bebily *et al.*, 2023). Subacute exposure elevates nephrotoxicity markers like creatinine and urea, signaling impaired renal function (Vukelić *et al*., 2023). These effects parallel chronic kidney disease in humans, underscoring the relevance of Wistar rat models.

2.3.3 Effects on Blood

Hematological tests reveal lead acetate's impact on the hematopoietic system. It inhibits delta-aminolevulinic acid dehydratase (ALAD), disrupting heme synthesis and causing anemia (Balali-Mood *et al*., 2021). Idoko and Orakpoghenor (2024) found non-significant increases in red blood cell (RBC) counts in Wistar rats exposed to lead acetate for 28 days, suggesting compensatory mechanisms, but no changes in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC). Elevated white blood cell (WBC) counts, including neutrophils and lymphocytes, indicate an inflammatory response to lead exposure (Idoko and Orakpoghenor, 2024). These hematological alterations align with human lead poisoning symptoms.

2.4 Mechanisms of Lead Acetate Toxicity

Lead acetate's toxicity is mediated through several mechanisms, as assessed by the specified assays:

Oxidative Stress: Lead generates reactive oxygen species (ROS), increasing MDA and depleting GSH and CAT, leading to lipid peroxidation in the liver and kidneys (Pat *et al.,* 2023; Vukelić *et al.,* 2023).

Inflammatory Response: Lead induces pro-inflammatory cytokines (e.g., TNF-α, IL-6), promoting inflammation and tissue damage in the liver and kidneys (Bebily *et al.,* 2023).

Hematological Disruption: Inhibition of ALAD impairs heme synthesis, causing anemia, while elevated WBC counts reflect immune activation (Balali-Mood *et al.,* 2021; Idoko and Orakpoghenor, 2024).

Ion Mimicry: Lead mimics calcium, disrupting cellular signaling and homeostasis (Balali-Mood *et al*., 2021).

These mechanisms collectively contribute to organ-specific toxicity, making Wistar rats a valuable model for human studies.

2.5 Wistar Rats as a Model for Human Toxicity

Wistar rats are widely used in toxicological research due to their physiological similarities to humans, particularly in liver, kidney, and hematopoietic responses (Balali-Mood *et al.,* 2021). Lead-induced oxidative stress, inflammation, and hematological changes in Wistar rats closely resemble human responses, enabling accurate extrapolation of findings (Vukelić *et al.,* 2023; Bebily *et al.,* 2023). Controlled dosing in rat studies allows precise assessment of lead's dose-response effects, supporting its relevance for human health risk assessment (Idoko and Orakpoghenor, 2024).

2.6 Human Health Implications

Lead exposure is a global health concern, linked to liver dysfunction, kidney disease, and hematological disorders in humans (Balali-Mood *et al*., 2021). The oxidative stress, inflammatory responses, and anemia observed in Wistar rats mirror human clinical outcomes, such as elevated liver enzymes and renal failure (Bebily *et al*., 2023). These parallels emphasize the need for interventions like chelation therapy or antioxidants to mitigate lead toxicity (Vukelić *et al*., 2023).

2.7 Knowledge Gaps and Research Needs

While studies confirm lead acetate's toxic effects, gaps remain in understanding chronic low-dose exposure effects in Wistar rats, reflective of environmental scenarios (Vukelić *et al.,* 2023). The role of inflammatory mediators in long-term organ damage requires further exploration, particularly through advanced assays (Bebily *et al.,* 2023). Additionally, the efficacy of interventions like Moringa oleifera in mitigating lead-induced toxicity needs validation across diverse rat populations (Idoko and Orakpoghenor, 2024). The interaction of lead with other pollutants also warrants investigation.

2.8 Review of Empirical Literature

Several empirical studies have investigated lead acetate's toxicological effects on Wistar rats, providing data relevant to my study's assays. Idoko and Orakpoghenor (2024) conducted a controlled experiment administering lead acetate (480 mg/kg) to Wistar rats for 28 days, assessing hematological parameters using automated analyzers. They reported non-significant RBC increases ($p>0.05$) and significant elevations in TWBC ($6.5 \pm 0.4 \times 10^3/\mu L$), neutrophils ($45 \pm 3\%$), and lymphocytes ($50 \pm 4\%$) compared to controls (TWBC: $5.2 \pm 0.3 \times 10^3/\mu L$, neutrophils: $35 \pm 2\%$, lymphocytes: $40 \pm 3\%$), indicating immune activation (Idoko and Orakpoghenor, 2024). These findings align with my hematological assays to evaluate blood parameters.

Vukelić *et al.* (2023) exposed Wistar rats to low lead doses (0.1–1 mg/kg) for 28 days, measuring oxidative stress markers via spectrophotometry and histological changes via microscopy. They found a 30% increase in liver MDA (2.8 ± 0.2 nmol/mg vs. 2.1 ± 0.1 nmol/mg in controls) and 15–20% elevations in kidney creatinine (55 ± 4 µmol/L vs. 45 ± 3 µmol/L) and urea (7.5 ± 0.5 mmol/L vs. 6.2 ± 0.4 mmol/L), with reduced GSH (1.5 ± 0.1 µmol/g vs. 2.0 ± 0.1 µmol/g) and CAT (25 ± 2 U/g vs. 32 ± 2 U/g). Kidney histology showed tubular atrophy and glomerular sclerosis (Vukelić *et al*., 2023). These results support my oxidative stress (MDA, GSH, CAT) and nephrotoxicity assays.

Bebily *et al.* (2023) conducted a systematic review of lead's effects on Wistar rat liver, analyzing histological and biochemical data from multiple studies. They reported vacuolated hepatocytes, necrosis, and elevated TNF-α (120 ± 10 pg/mL vs. 80 ± 8 pg/mL in controls) and IL-6 (150 ± 12 pg/mL vs. 100 ± 10 pg/mL) in lead-exposed rats, measured via ELISA. Kidney histology revealed tubular atrophy (Bebily et al., 2023). These findings inform my inflammatory (TNF-α, IL-6) and histological assays.

Pat *et al.* (2023) studied oxidative stress in Wistar rats under restraint stress, a model applicable to lead toxicity. Using spectrophotometry, they reported a 35% MDA increase (3.5 ± 0.3 nmol/mg vs. 2.6 ± 0.2 nmol/mg) and reductions in GSH (1.2 ± 0.1 µmol/g vs. 1.8 ± 0.1 µmol/g) and CAT (20 ± 2 U/g vs. 28 ± 2 U/g) in stressed rats (Pat et al., 2023). These results provide a comparative framework for my oxidative stress assays, adapting stress-induced mechanisms to lead exposure.

Balali-Mood *et al.* (2021) reviewed lead's toxicokinetics and mechanisms, noting ALAD inhibition and ROS generation in Wistar rats across studies. They reported lead accumulation in the liver (10–15 µg/g) and kidneys (15–20 µg/g) after 28-day exposures (0.5–2 mg/kg), measured via atomic absorption spectroscopy, and linked these to human

toxicity (Balali-Mood *et al*., 2021). This supports my study's toxicokinetic and mechanistic focus.

These empirical studies provide robust data on lead acetate's effects, justifying my experimental design to assess oxidative stress, inflammation, and hematological changes in Wistar rats, with implications for human health.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF STUDY AREA

This research took place at the University of Benin, in the Department of Environmental Management and Toxicology, at coordinates Latitude: 6°23'52.30"N, Longitude: 5°36'58.11"E, Benin City, Edo State, Nigeria. Benin City is the capital of Edo State, located in Nigeria's south-south geopolitical zone. The experimental animals, Wistar rats, were maintained in the animal holding facility of the Animal and Environmental Biology Department, Faculty of Life Sciences, University of Benin, at Latitude: 6°23'50.19"N, Longitude: 5°36'54.87"E.

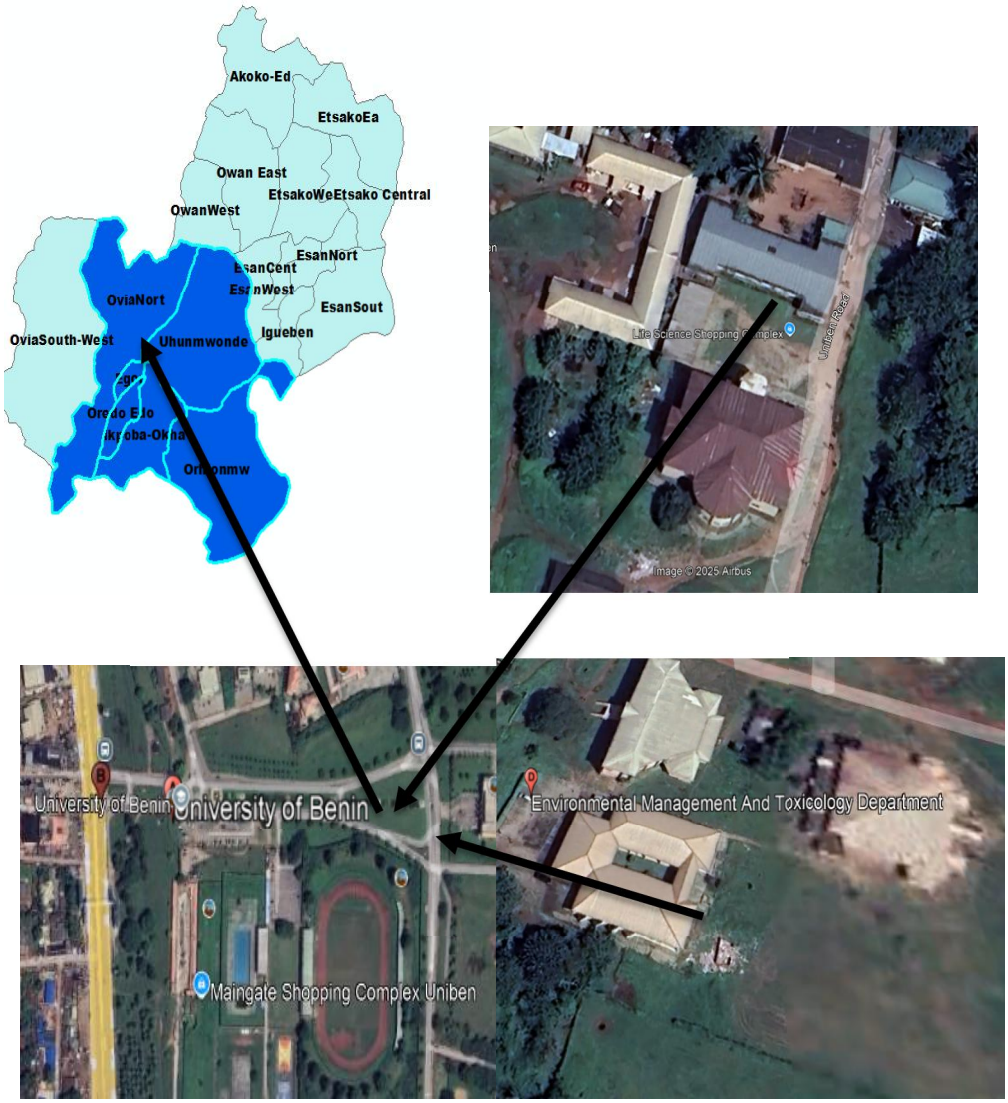

Figure 3.1: Map of Study Area

Source: Google earth and arc GIS 10.5

3.2 APPARATUS

The apparatus used included beakers, micropipettes, measuring cylinders, and plain tubes.

3.3 INSTRUMENTS

The instruments employed were an analytical weighing balance, UV-visible spectrophotometer, magnetic stirrer, pH meter, electrical hotplate, and centrifuge.

3.4 CHEMICAL REAGENTS

The chemical reagents utilized were lead (II) acetate trihydrate, hydrogen peroxide (H_2O_2), sulphuric acid (H_2SO_4), phosphate buffer, adrenaline, potassium permanganate ($KMnO_4$), pyrogallol, thiobarbituric acid (TBA), and trichloroacetic acid (TCA).

3.5 SAMPLE COLLECTION AND PREPARATION

Sixteen healthy Wistar rats were acquired from the animal facility in the Department of Anatomy at the University of Benin. These animals went through a seven-day acclimatization period in a controlled setting with a 12-hour light/dark cycle, temperature range of 22–25 °C, and relative humidity of 50–60%. They had unlimited access to Top feed produced by Premier Feed Mills LTD and clean water. Wood shavings served as bedding, changed every two days to uphold hygiene standards.

Before starting acclimatization, the rats were randomly allocated into four groups (Boxes A–D), with four rats per box. Each group received a designated treatment:

Group A: Treated with a low dose of lead acetate.

Group B: Treated with a medium dose of lead acetate.

Group C: No lead acetate exposure, but given distilled water; this acted as the control.

Group D: Treated with a high dose of lead acetate.

Plate 1: The wistar rats during the period of acclimatization

After the exposure phase, the rats were euthanized compassionately per international guidelines (OECD, 2008; NIH, 2011). Light ether/chloroform anesthesia was used until the righting reflex and pain response were absent, then cervical dislocation followed. Blood was obtained via cardiac puncture with sterile syringes and placed in EDTA-coated tubes for hematological evaluations, covering RBC, WBC, hemoglobin, platelets, and differentials. Liver and kidney organs were excised, rinsed in cold saline to eliminate blood clots, patted dry, and stored in labeled bottles. Samples for oxidative stress tests (MDA, SOD, CAT, GPx) were analyzed promptly or refrigerated at 4°C to ensure preservation.

The collected samples were processed right away or kept at 4°C pending analysis to maintain their quality.

3.6 EXPERIMENTAL DESIGN

An in vivo lab-based experimental approach was used, with Wistar rats serving as the model to evaluate lead acetate's hematological impacts as an environmental contaminant.

Sixteen healthy Wistar rats were employed and randomly split into four groups (Boxes A–D), four rats each:

Group A: Low dose lead acetate exposure at 0.25 mg/ml (25% of normal dose).

Group B: Medium dose lead acetate exposure at 0.5 mg/ml (50% of normal dose).

Group C: No lead acetate, but distilled water; control group.

Group D: High dose lead acetate exposure at 1.0 mg/ml (100% of normal concentration).

The sample consisted of 16 rats total, n=4 per group. Administration was oral via gavage for exact dosing. Exposure duration was 28 days (four weeks) after acclimatization, dosed twice weekly (Tuesdays and Thursdays). Animals were observed daily for toxicity indicators such as weakness, reduced appetite, and lower water intake, noted post-lead administration. They were kept in ventilated plastic boxes labeled by group.

Plate 2: Oral Administration of Lead Acetate Using Oral Gavage

3.7 PREPARATION OF LEAD ACETATE SOLUTION

Lead acetate solution was made by dissolving 0.05 g in 100 ml distilled water.

5/100 = 0.05 mg

Using M1V1 = M2V2

M1 = 0.05 mg

V1 = initial volume (1)

M2 = rat's body weight

V2 = administered solution volume.

3.8 ROUTE AND DURATION OF ADMINISTRATION

Lead acetate was delivered orally by gavage. Duration: 28 days (twice weekly).

3.9 PROCEDURES FOR ANALYSIS

Blood was examined for hematological parameters, serum for inflammation (TNF-α), and liver/kidney homogenates for oxidative stress markers.

3.9.1 Hematological Assays (Full Blood Count)

Procedures based on Cheesbrough (2006).

- Packed Cell Volume (PCV):

Materials: Sealant, microhematocrit centrifuge, capillary tubes, hematocrit reader. **Method**: Blood filled capillary tube to ~¾ (10–15 mm empty). Sealed effectively, placed in centrifuge rotor (sealed end against rim/gasket), spun 5 minutes. PCV read directly from calibrated reader.

Formula: PCV (%) = (Length of Red Blood Cell Column / Total Length of Blood Column) × 100

- White Blood Cells (WBC) and Differentials:

Materials: Hemocytometer, cover glasses, micropipette, hand counter.

Method: 350 µL Turk's solution and ammonium oxalate mixed with 20 µL EDTA venous blood. Chamber charged by sliding cover glass until ring appearance. Remixed blood pipetted (20 µL) to fill chamber. Settled 2 minutes (WBC) or 20 minutes (platelets). Underside wiped dry, viewed at ×10 objective. Counted four large and five inner chambers.

Formula: WBC Count (cells/µL) = (Number of Cells Counted × Dilution Factor) / (Number of Large Squares Counted × Volume of One Large Square)

- Platelets (PLT):

Materials and method similar to WBC, using Turk's and ammonium oxalate.

Formula: Platelet Count (platelets/µL) = (Number of Platelets Counted × Dilution Factor) / (Number of Large Squares Counted × Volume of One Large Square)

- Hemoglobin (Hb):

Materials: Venous blood, colorimeter, microcuvette, Drabkin's fluid, test tubes.

Title: Haemoglobin Estimation

Aim: To determine hemoglobin levels in tested rats.

Method: Haemoglobincyanide (HiCN).

Procedure: 20 µL (0.02 ml) blood dispensed into 4 ml Drabkin's neutral fluid. Stoppered, mixed, left 4–5 minutes at room temperature away from light. Yellow-green filter or 540 nm

wavelength set. Zeroed with Drabkin's fluid, absorbance read vs. blank.

Formula: Hemoglobin (g/dL) = (Absorbance of Sample / Absorbance of Standard) × Concentration of Standard

3.9.2 Inflammatory Response Assay

Plasma TNF-α Concentration (Beutler *et al*., 1985).

Principle: Sandwich-ELISA. Samples/standards added to rat TNF-α antibody-coated wells. Biotinylated detection antibody and Avidin-HRP added sequentially, incubated. Washed free components. Substrate added, wells with TNF-α turn blue. Stop solution turns yellow. OD at 450 ± 2 nm proportional to rat TNF-α concentration.

Assay Procedure: 50 µL TNF-α standard/control/serum into wells, 100 µL TNF-α-enzyme conjugate, 50 µL biotin reagent added. Swirled 20–30 sec, mixed thoroughly within 10 sec, incubated 1 h room temperature. Washed 3x with 300 µL 1X buffer, blotted on absorbent towels. 100 µL TMB substrate added, incubated 30 min room temperature. 50 µL stop solution added within 20 sec, mixed gently. Absorbance read at 450 nm within 15 min. Standard curve plotted; serum TNF-α extrapolated.

3.9.3 Oxidative Stress Assays

- Catalase (CAT) Activity (Cohen e*t al*., 1970):

Reagents: H_2O_2, 6M H_2SO_4, $KMnO_4$ (0.01M: 0.158 g/100 ml water), phosphate buffer (pH 7.4: 0.426 g Na_2HPO_4 + 0.240 g NaH_2PO_4/100 ml).

Procedure: 0.5 ml homogenate + 5.0 ml H_2O_2, stood 30 min. Stopped with 1.5 ml 6M H_2SO_4 + 7 ml 0.01M $KMnO_4$. Stood 10 min, absorbance at 480 nm vs. water (30–60 sec). Blank: water instead of H_2O_2.

Calculation: Activity = OD / (L × V × M × Y) where OD=absorbance, L=light path, V=volume, M=40/m/cm (H_2O_2 molar coefficient), Y=mg protein.

- Superoxide Dismutase (SOD) Activity (Misra and Fridovich, 1972):

Principle: Adrenaline auto-oxidizes to adrenochrome (420 nm); SOD inhibits by breaking down superoxide.

Reagents: Carbonate buffer (0.05M, pH 10.2: 0.2014 g Na_2CO_3 + 0.2604 g $NaHCO_3$ + 0.0372 g EDTA/100 ml), 0.005M HCl, 0.3 mM adrenaline (0.01098 g/100 ml 0.005M HCl).
Procedure: 100 µL homogenate + 1.25 ml buffer + 150 µL adrenaline. Reference: 100 µL water + 1.25 ml buffer. Absorbance at 420 nm.

Calculation: % Inhibition = 100 × (1 - [Sample OD / Reference OD]) / Y (Y=mg protein).

- Glutathione Peroxidase (GPx) Activity (Nyman, 1959):

Principle: GPx oxidizes pyrogallol to purpurogallin (brown, 420 nm).

Reagents: Pyrogallol (20 mM: 0.2552 g/100 ml water).

Procedure: 0.2 ml homogenate + 2.5 ml phosphate buffer + 2.5 ml H_2O_2 + 1.5 ml water + 2.5 ml pyrogallol. Stood 30 min room temp, absorbance 420 nm.

Calculation: Activity = (OD × Vt × Df) / (E × Vs × Y) where E=12/m/cm, Vt=total volume, Df=1, Vs=sample volume, Y=mg protein.

- Malondialdehyde (MDA) (Buege and Aust, 1978):

Principle: MDA (lipid peroxidation product) + TBA forms red complex (535 nm).

Procedure: 1.0 ml homogenate + 2.0 ml TCA–TBA–HCl. Heated boiling water bath 15 min, cooled, centrifuged 1000 g 10 min. Supernatant absorbance 535 nm.

Calculation: Concentration = (Absorbance × Volume of Mixture × Dilution Factor) / (Extinction Coefficient × Volume of Sample × mg Protein).

3.10 STATISTICAL ANALYSIS

Analyses conducted with GraphPad Prism (version 9.0 or higher, GraphPad Software, USA) or R (R Foundation for Statistical Computing, Austria). Data shown as Mean ± SD, significance at $p < 0.05$.

Data Preparation: Raw data from assays and histology entered into spreadsheet, grouped. Normality: Shapiro-Wilk test. Variances: Levene's or Bartlett's test.

Primary Tests: One-way ANOVA for parametric data comparing groups (Control, Low, Medium, High) per parameter in liver/kidney separately. Non-parametric: Kruskal-Wallis H test.

Post-Hoc: Tukey's HSD after ANOVA; Dunn's after Kruskal-Wallis.

Correlation: Pearson's r (normal, continuous); Spearman's ρ (ordinal/non-normal) for dose vs. oxidative markers.

Histological Data: Ordinal scores (0-3) compared via Kruskal-Wallis, post-hoc Dunn's.

Integrated Analysis: Two-way ANOVA for tissue (liver/kidney) × dose, checking main/interaction effects.

Presentation: Bar charts (Mean ± SD), scatter plots, box-whisker plots, tables with stats.

Formulas for t-tests/ANOVA/mean as per protocols (e.g., paired t: $t = \bar{y}d / (sd / \sqrt{n})$; independent equal var: $t = (\bar{y}1 - \bar{y}2) / (sp \sqrt{(1/n1 + 1/n2)})$; one-way ANOVA: $SST = \Sigma\Sigma(xij - \bar{y})^2$, etc.; mean: $\bar{y} = \Sigma x / n$).

CHAPTER 4

4.0 RESULTS

This chapter presents the results from the haematological tests, inflammatory response assay (measured via Tumor Necrosis Factor-alpha, TNF-α), and oxidative stress assay (measured via malondialdehyde (MDA) levels and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) conducted on Wistar rats exposed to lead acetate. The experimental groups were designated as Box A (low dose), Box B (medium dose), Box C (control), and Box D (high dose). Data were collected over four weeks, and statistical analyses including single-factor ANOVA, pairwise t-tests, paired t-tests, one-way ANOVA, and two-way ANOVA were performed to assess differences among groups and over time. Results are presented in tabular and graphical formats where applicable, with significance set at $p < 0.05$.

4.1 Haematological Tests

Haematological parameters were evaluated to assess the impact of lead exposure on blood indices. Mean values across the four weeks are summarized below for each parameter, followed by ANOVA and pairwise t-test results.

Platelet (PLT) Count

Platelet

WEEK	BOX A	BOX B	BOX C	BOX D
1	53000	1377000	1443000	773000
2	1055000	906000	1233000	856000
3	556000	409000	553000	653000

4	474000	421000	685000	543000
Mean	534500	778250	978500	706250

Table 4.1.1: Weekly Platelet Counts and Means for Boxes A-D

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.05E+11	3	1.35E+11	0.926	0.4575	3.4902
Within Groups	1.75E+12	12	1.46E+11			
Total	2.16E+12	15				

Table 4.1.2: Single-Factor ANOVA Results for Platelet Counts

The mean platelet counts across the four groups (Box A–D) were 534,500, 778,250, 978,500, and 706,250 respectively.

Single-factor ANOVA showed: F (calculated) = 0.92687, F crit = 3.490295, P-value = 0.457517.

Since F < F crit and P > 0.05, there was no statistically significant difference in platelet counts among the experimental groups. This implies that the treatments administered did not significantly influence platelet levels.

Pairwise t-tests: Box A vs. Box C ($p = 0.13$), Box B vs. Box C ($p = 0.40$), Box C vs. Box D ($p = 0.10$). This reinforces the conclusion that platelet counts were not significantly altered across groups.

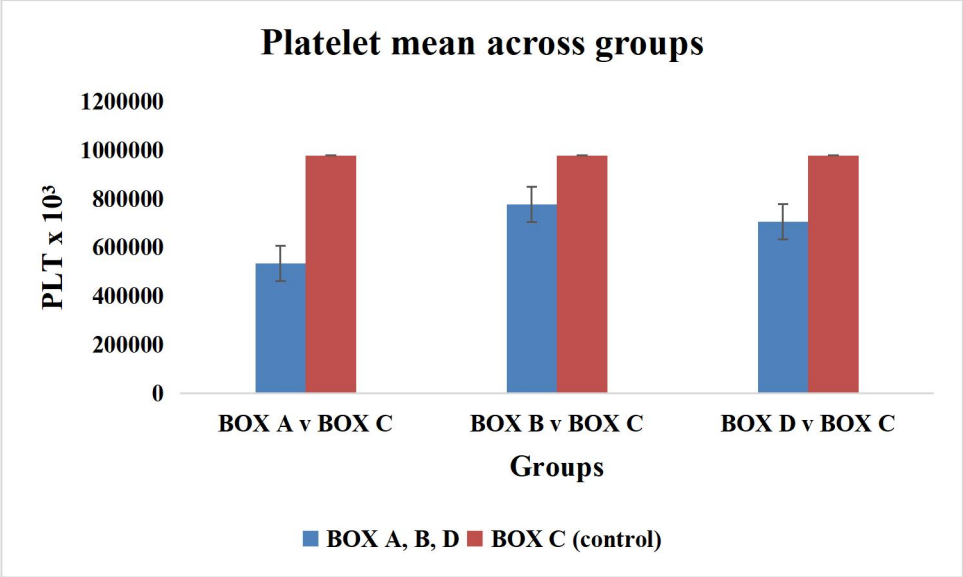

Figure 4.1.1: Bar Chart of Mean Platelet Counts Across Groups (A-D).

Packed Cell Volume (PCV)

PCV

WEEK	BOX A	BOX B	BOX C	BOX D
1	8	32	31	32
2	34	33	31	33
3	39	35	42	39
4	42	41	38	41
Mean	30.75	35.25	35.5	36.25

Table 4.1.3: Weekly PCV Counts and Means for Boxes A-D

Anova: Single Factor ANOVA	PCV				Anova: Single Factor ANOVA	
Source of Variation	*SS*	*df*	*MS*	*F*	*Source of Variation*	*F crit*
Between Groups	74.687 5	3	24.895 83	0.3249 93	Between Groups	3.4902 95
Within Groups	919.25	12	76.604 17		Within Groups	
Total	993.93 75	15			Total	

Table 4.1.4: Single-Factor ANOVA Results for PCV Counts

The mean PCV counts across the four groups (Box A–D) were 30.75, 35.25, 35.5, and 36.25 respectively.

Single-factor ANOVA showed: F (calculated) = 0.324993, F crit = 3.490295, P-value > 0.05.

Since F < F crit and P > 0.05, there was no statistically significant difference in PCV counts among the experimental groups. This implies that the treatments administered did not significantly influence PCV levels.

Pairwise t-tests: Box A vs. Box C (p = 0.25), Box B vs. Box C (p = 0.46), Box C vs. Box D (p = 0.30). This reinforces the conclusion that PCV counts were not significantly altered across groups.

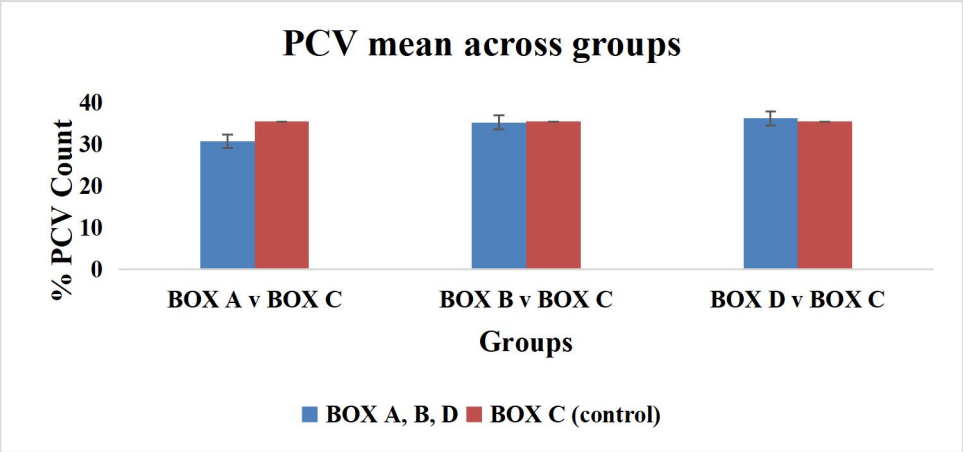

Figure 4.1.2: Bar Chart of Mean PCV Across Groups (A-D).

Neutrophils

NEUTROPHILS

WEEK	BOX A	BOX B	BOX C	BOX D
1	5	43	24	10
2	22	28	20	6
3	11	8	12	7
4	16	9	15	10
Mean	13.5	22	17.75	8.25

Table 4.1.5: Weekly Neutrophil Counts and Means for Boxes A-D

Anova: Single Factor — NEUTROPHILS

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	415.25	3	138.4167	1.514882	0.260959	3.490295
Within Groups	1096.5	12	91.375			
Total	1511.75	15				

Table 4.1.6: Single-Factor ANOVA Results for Neutrophil Counts

The mean neutrophil counts across the four groups (Box A–D) were 13.5, 22, 17.75, and 8.25 respectively.

Single-factor ANOVA showed: F (calculated) = 1.51482, F crit = 3.490295, P-value = 0.260959.

Since F < F crit and P > 0.05, there was no statistically significant difference in neutrophil counts among the experimental groups. This implies that the treatments administered did not significantly influence neutrophil levels.

Pairwise t-tests: Box A vs. Box C ($p = 0.23$), Box B vs. Box C ($p = 0.26$), Box C vs. Box D ($p = 0.02$). This reinforces the conclusion that neutrophil counts were not significantly altered in Box A and Box B compared to the control group. The neutrophil counts were altered in Box D, which may be as a result of the higher concentration of lead acetate received by this group. Box D with $P < 0.05$ shows this group is statistically significant.

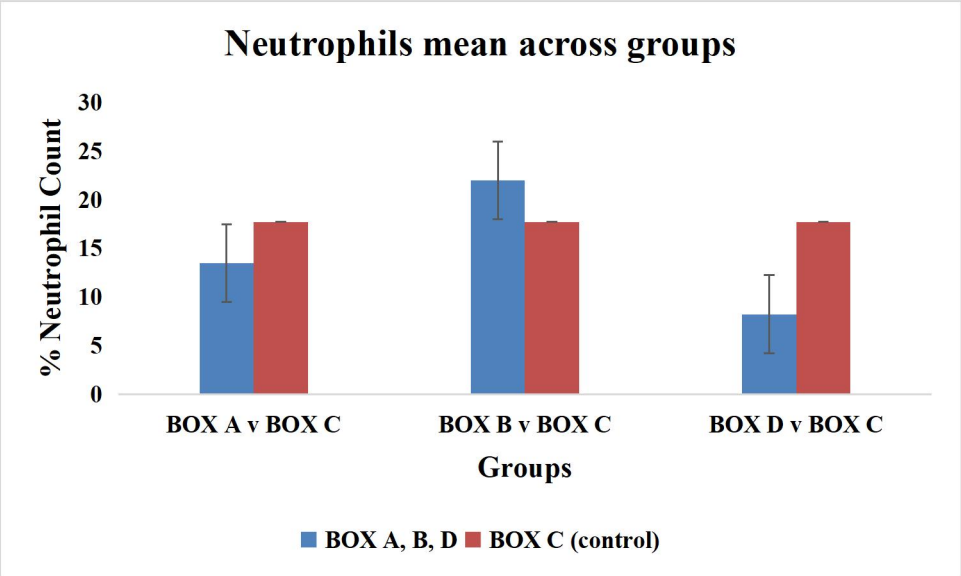

Figure 4.1.3: Bar Chart of Mean Neutrophil Counts Across Groups (A-D).

Lymphocytes

LYMPHOC

YTE

WEEK	BOX A	BOX B	BOX C	BOX D
1	84	52	65	85
2	69	66	70	91
3	78	83	80	82

4	75	83	74	75
Mean	76.5	71	72.25	83.25

Table 4.1.7: Weekly Lymphocyte Counts and Means for Boxes A-D

Anova: Single Factor — LYMPHOCYTE

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
			122.16	1.4035	0.2896	3.4902
Between Groups	366.5	3	67	42	68	95
		1	87.041			
Within Groups	1044.5	2	67			
		1				
Total	1411	5				

Table 4.1.8: Single-Factor ANOVA Results for Lymphocyte Counts

The mean lymphocyte counts across the four groups (Box A–D) were 76.5, 71, 72.25, and 83.25 respectively.

Single-factor ANOVA showed: F (calculated) = 1.403542, F crit = 3.490295, P-value = 0.289668.

Since F < F crit and P > 0.05, there was no statistically significant difference in lymphocyte counts among the experimental groups. This implies that the treatments administered did not significantly influence lymphocyte levels.

Pairwise t-tests: Box A vs. Box C (p = 0.23), Box B vs. Box C (p = 0.40), Box C vs. Box D (p = 0.01). This reinforces the conclusion that lymphocyte counts were not significantly altered in Box A and Box B compared to the control group (Box C). The lymphocyte counts were altered in Box D, which may be as a result of the higher concentration of lead acetate received by this group. Box D with P < 0.05 shows this group is statistically significant.

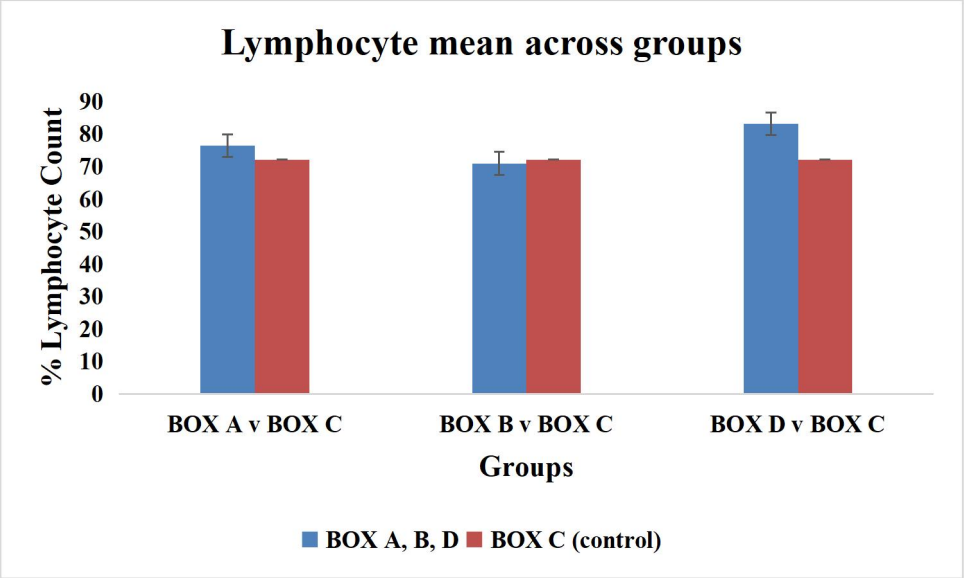

Figure 4.1.4: Bar Chart of Mean Lymphocyte Counts Across Groups (A-D).

Hemoglobin (HGB)

HEMOGLO

BIN

WEEK	BOX A	BOX B	BOX C	BOX D
1	2	12	10	12
2	11	12	11	12
3	14	12	14.2	14
4	16	14	14	16
Mean	10.75	12.75	12.3	13.5

Table 4.1.9: Weekly Hemoglobin Counts and Means for Boxes A-D

Anova: Single Factor HEMOGLOBIN

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.5075	3	5.169167	0.436125	0.731172	3.490295
Within Groups	142.23	12	11.8525			
Total	157.7375	15				

Table 4.1.10: Single-Factor ANOVA Results for Hemoglobin Counts

The mean HGB counts across the four groups (Box A–D) were 10.75, 12.75, 12.3, and 13.5 respectively.

Single-factor ANOVA showed: F (calculated) = 0.436125, F crit = 3.490295, P-value = 0.731172.

Since F < F crit and P > 0.05, there was no statistically significant difference in hemoglobin counts among the experimental groups. This implies that the treatments administered did not significantly influence HGB levels.

Pairwise t-tests: Box A vs. Box C (p = 0.27), Box B vs. Box C (p = 0.42), Box C vs. Box D (p = 0.05). This reinforces the conclusion that HGB counts were not significantly altered in Box A and Box B compared to the control group (Box C). The hemoglobin counts were slightly altered in Box D, which may be as a result of the higher concentration of lead acetate received by this group. Box D with P = 0.05 shows this group is statistically significant.

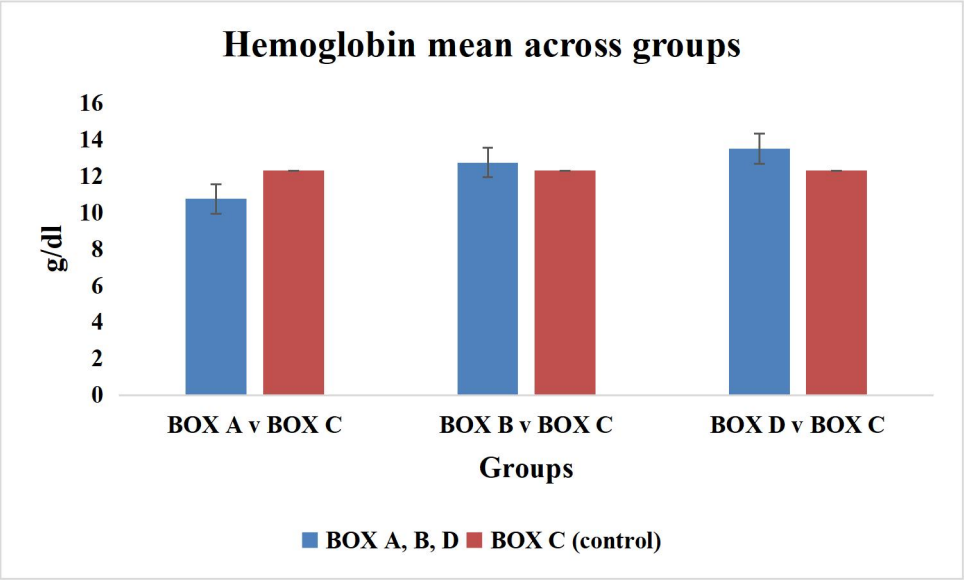

Figure 4.1.5: Bar Chart of Mean Hemoglobin Across Groups (A-D).

Eosinophils

EOSINOPHILLS

WEEK	BOX A	BOX B	BOX C	BOX D
1	4	1	6	2
2	5	5	6	2
3	1	1	1	0
4	2	1	1	2
Mean	3	2	3.5	1.5

Table 4.1.11: Weekly Eosinophil Counts and Means for Boxes A-D

Anova: Single Factor EOINOPHILLS

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10	3	3.3333 33	0.8	0.5174 04	3.4902 95
Within Groups	50	12	4.1666 67			
Total	60	15				

Table 4.1.12: Single-Factor ANOVA Results for Eosinophil Counts here]

The mean eosinophil counts across the four groups (Box A–D) were 3, 2, 3.5, and 1.5 respectively.

Single-factor ANOVA showed: F (calculated) = 0.8, F crit = 3.490295, P-value = 0.517404.

Since F < F crit and P > 0.05, there was no statistically significant difference in eosinophil counts among the experimental groups. This implies that the treatments administered did not significantly influence eosinophil levels.

Pairwise t-tests: Box A vs. Box C (p = 0.25), Box B vs. Box C (p = 0.15), Box C vs. Box D (p = 0.10). This reinforces the conclusion that eosinophil counts were not significantly altered across groups.

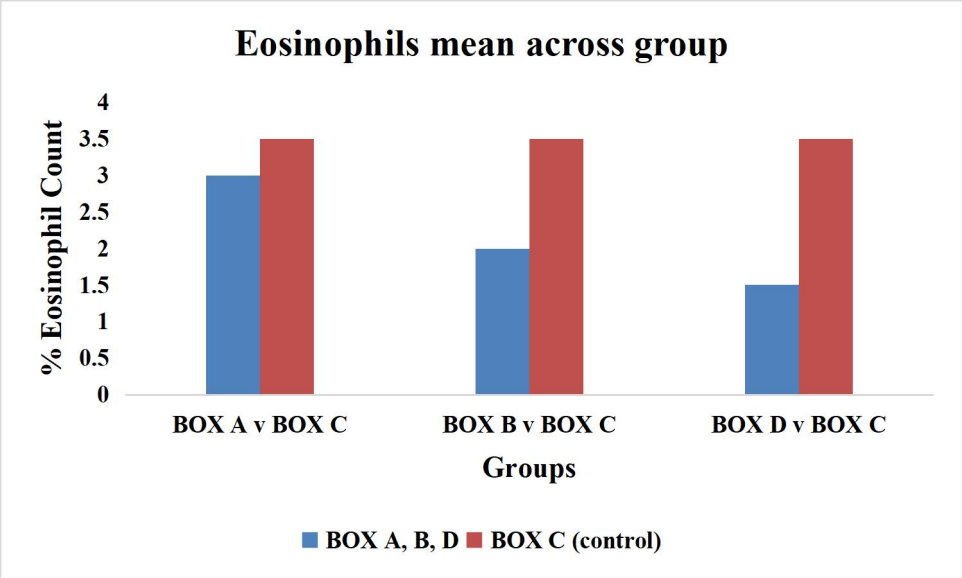

Figure 4.1.6: Bar Chart of Mean Eosinophil Counts Across Groups (A-D).

Basophils

BASOPHI

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WEEK	BOX A	BOX B	BOX C	BOX D
1	1	0	3	0
2	2	1	3	1
3	3	4	2	3
4	2	2	3	7
Mean	2	1.75	2.75	2.525

Table 4.1.13: Weekly Basophil Counts and Means for Boxes A-D

Anova: Single

Factor BASOPHIL

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.1875	3	1.0625	0.316 77	0.8130 69	3.4902 95
Within Groups	40.25	12	3.3541 67			
Total	43.4375	15				

Table 4.1.14: Single-Factor ANOVA Results for Basophil Counts

The mean basophil counts across the four groups (Box A–D) were 2, 1.75, 2.75, and 2.525 respectively.

Single-factor ANOVA showed: F (calculated) = 0.31677, F crit = 3.490295, P-value = 0.813069.

Since F < F crit and P > 0.05, there was no statistically significant difference in basophil counts among the experimental groups. This implies that the treatments administered did not significantly influence basophil levels.

Pairwise t-tests: Box A vs. Box C (p = 0.16), Box B vs. Box C (p = 0.21), Box C vs. Box D (p = 0.50). This reinforces the conclusion that basophil counts were not significantly altered across groups.

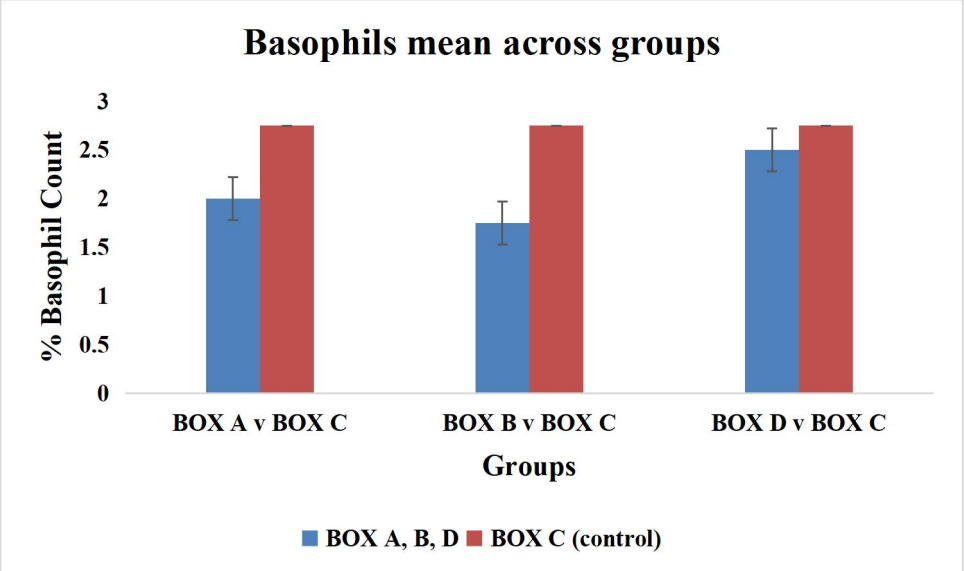

Figure 4.1.7: Bar Chart of Mean Basophil Counts Across Groups (A-D).

White Blood Cells (WBC)

WHITE BLOOD CELLS

WEEK	BOX A	BOX B	BOX C	BOX D
1	1900	1600	7000	1300
2	8000	9000	9000	11000
3	11400	5600	11400	15000
4	15000	9000	16000	12000
Mean	9075	6300	10850	9825

Table 4.1.15: Weekly WBC Counts and Means for Boxes A-D

WHITE BLOOD CELLS

Anova: Single Factor

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	45592500	3	15197500	0.649131	0.598457	3.490295
Within Groups	2.81E+08	12	23412083			
Total	3.27E+08	15				

Table 4.1.16: Single-Factor ANOVA Results for WBC Counts

The mean WBC counts across the four groups (Box A–D) were 9075, 6300, 10850, and 9825 respectively.

Single-factor ANOVA showed: F (calculated) = 0.649131, F crit = 3.490295, P-value = 0.598457.

Since F < F crit and P > 0.05, there was no statistically significant difference in WBC counts among the experimental groups. This implies that the treatments administered did not significantly influence WBC levels.

Pairwise t-tests: Box A vs. Box C (p = 0.11), Box B vs. Box C (p = 0.03), Box C vs. Box D (p = 0.34). This reinforces the conclusion that WBC counts were not significantly altered in Box A and Box D compared to the control group (Box C). The WBC counts were altered in Box B. Box B with P < 0.05 shows this group is statistically significant.

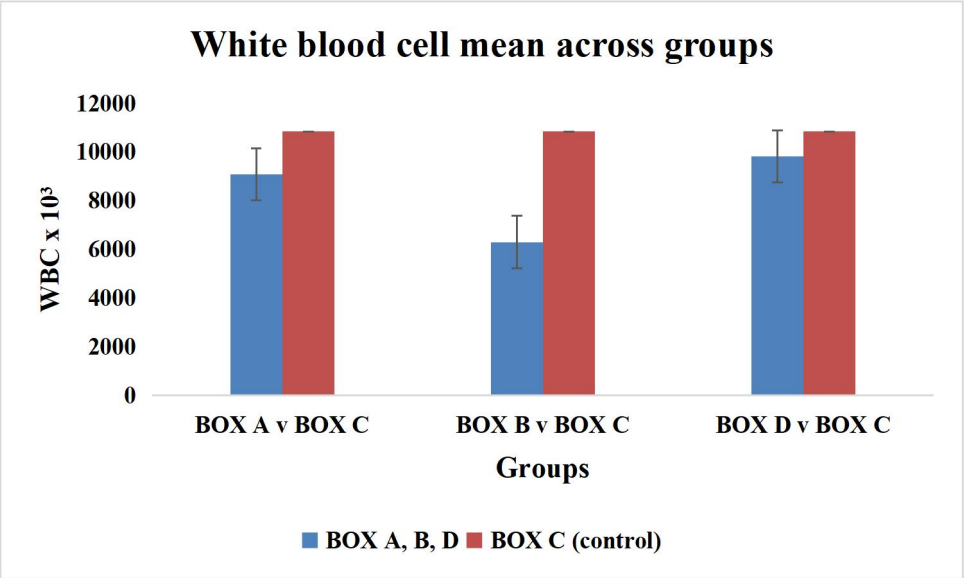

Figure 4.1.8: Bar Chart of Mean WBC Counts Across Groups (A-D).

Monocytes

MONOCY

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WEEK	BOX A	BOX B	BOX C	BOX D
1	6	4	1	4
2	2	0	1	0
3	7	4	5	8
4	5	5	7	6
Mean	5	3.25	3.5	4.5

Table 4.1.17: Weekly Monocyte Counts and Means for Boxes A-D

Anova: Single

Factor MONOCYTE

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.1875	3	2.7291 67	0.3608 82	0.7823 88	3.4902 95
Within Groups	90.75	1	7.5625			

		2
		1
Total	98.9375	5

Table 4.1.18: Single-Factor ANOVA Results for Monocyte Counts

The mean monocyte counts across the four groups (Box A–D) were 5, 3.25, 3.5, and 4.5 respectively.

Single-factor ANOVA showed: F (calculated) = 0.360882, F crit = 3.490295, P-value = 0.782388.

Since F < F crit and P > 0.05, there was no statistically significant difference in monocyte counts among the experimental groups. This implies that the treatments administered did not significantly influence monocyte levels.

Pairwise t-tests: Box A vs. Box C (p = 0.19), Box B vs. Box C (p = 0.42), Box C vs. Box D (p = 0.23). This reinforces the conclusion that monocyte counts were not significantly altered across groups.

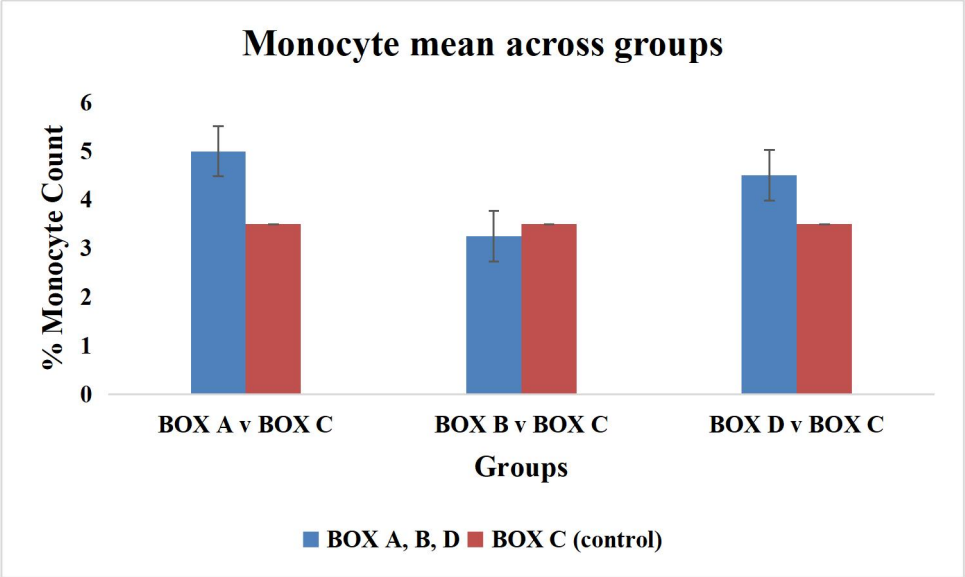


Figure 4.1.9: Bar Chart of Mean Monocyte Counts Across Groups (A-D).

Comparison of Mean Levels of Haematological Indices Between Control and Treated Groups

S/N	PARAMETER	GROUP A (mean ± SEM)	GROUP C (mean ± SEM)	T - test	P - test
	WBC				
1	(Cm^2)	9075 ±2786.09	10850 ±1938	0.31	0.11
2	HGB (g/dl)	10.75 ±3.09	12.3 ±1.06	0.33	0.27
3	PCV (%)	30.75 ±7.76	35.5 ±2.72	0.30	0.25
4	PLT (Cm^2)	534500 ± 205524.73	978500 ± 213643.59	0.09	0.13
	LYMPH				
5	(%)	76.5 ±3.12	72.25 ±3.17	0.19	0.23
6	NEU (%)	13.5 ±3.62	17.75 ± 2.66	0.19	0.23
7	MONO (%)	5 ±1.08	3.5 ±1.5	0.23	0.19
8	EOS (%)	3 ±0.91	3.5 ±1.44	0.39	0.25
9	BAS (%)	2 ±0.42	2.75 ±0.25	0.09	0.16

S/N	PARAMETER	GROUP B (mean ± SEM)	GROUP C (mean ± SEM)	T - test	P - test
1	WBC (Cm^2)	6300 ±1759.73	10850 ±1938	0.07	0.03
2	HGB (g/dl)	12.75 ±0.5	12.3 ±1.06	0.44	0.42
3	PCV (%)	35.25 ±2.02	35.5 ±2.72	0.47	0.46
4	PLT (Cm^2)	778250 ± 230722.55	978500 ± 213643.59	0.27	0.02
5	LYMPH (%)	71 ±7.49	72.25 ±3.17	0.44	0.40
6	NEU (%)	22 ±8.38	17.75±2.66	0.33	0.26
7	MONO (%)	3.25 ±1.11	3.5 ±1.5	0.45	0.42
8	EOS (%)	2 ±1	3.5 ±1.44	0.21	0.15
9	BAS (%)	1.75 ±0.85	2,75 ±0.25	0.17	0.21

S/N	PARAMETER	GROUP D (mean ± SEM)	GROUP C (mean ± SEM)	T - test	P - test
1	WBC (Cm^2)	9825 ±2966.02	10850 ±1938	0.39	0.34
2	HGB (g/dl)	13.5 ±0.96	12.3 ±1.06	0.22	0.05
3	PCV (%)	36.25 ±2.21	35.5 ±2.72	0.42	0.30
4	PLT (Cm^2)	706250 ± 68536.33	978500 ± 213643.59	0.15	0.10
5	LYMPH	72.25 ±3.33	72.25 ±3.17	0.01	0.01

	(%)				
6	NEU (%)	8.25 ±1.03	17.75 ±2.66	0.02	0.02
7	MONO (%)	4.5 ±1.71	3.5 ±1.5	0.34	0.23
8	EOS (%)	1.5 ±0.5	3.5 ±1.44	0.13	0.10
9	BAS (%)	2.55 ±1.5	2.75 ±0.25	0.48	0.50

Table 4.1.19: Comparison of Mean Levels of Haematological Indices Between Group C (Control) and Groups; A,B,D.

These comparisons indicate selective alterations in certain haematological parameters, particularly in higher dose groups (Box B and D), suggesting dose-dependent effects of lead exposure on blood profiles.

4.2 Inflammatory Response Assay

The inflammatory response was assessed by measuring TNF-α concentrations (pg/mL) in liver (L) and kidney (K) tissues over four weeks. Triple mean analysis, paired t-tests, and one-way ANOVA were used to evaluate variations.

TNF- ALPHA (pg/mL)				
BOX A	**WK1**	**WK2**	**WK3**	**WK4**
L1	17.506	31.345	31.946	30.951
L2	18.994	34.009	34.661	33.581
L3	20.608	36.902	37.607	36.436
BOX B				
L1	17.178	32.085	33.627	36.791
L2	18.638	34.812	36.485	39.918
L3	20.222	37.771	39.586	43.311
BOX C				
L1	10.308	12.232	12.302	14.548
L2	11.184	13.271	13.347	15.784
L3	12.134	14.399	14.482	17.126
BOX D				
L1	27.747	33.061	34.325	49.269

L2	30.105	35.872	37.242	53.456
L3	32.664	38.919	40.408	58.042
BOX A				
K1	11.882	13.241	17.16	26.647
K2	12.891	14.366	18.618	28.912
K3	13.987	15.587	20.201	31.369
BOX B				
K1	14.323	15.102	33.181	34.287
K2	15.515	16.385	36.001	37.201
K3	16.834	17.778	39.061	40.363
BOX C				
K1	9.521	10.556	11.425	12.869
K2	10.330	11.453	12.396	13.962
K3	11.208	12.426	13.449	15.149
BOX D				
K1	15.456	16.072	35.483	37.489
K2	16.769	17.438	38.499	40.675
K3	18.195	18.921	41.771	44.132

Appendix A: Raw TNF-α Concentration Data for All Groups and Weeks

Triple Mean Analysis of TNF-α Concentration

Box	Week 1	Week 2	Week 3	Week 4
A (L)	19.04 ± 1.56	34.09 ± 2.78	34.74 ± 2.83	33.66 ± 2.74
B (L)	18.68 ± 1.52	34.89 ± 2.84	36.57 ± 2.98	40.01 ± 3.26
C (L)	11.21 ± 0.91	13.30 ± 1.09	13.38 ± 1.09	15.82 ± 1.29
D (L)	30.17 ± 2.46	35.95 ± 2.93	37.33 ± 3.05	53.59 ± 4.39
A (K)	12.92 ± 1.05	14.40 ± 1.17	18.66 ± 1.52	28.98 ± 2.36
B (K)	15.56 ± 1.26	16.42 ± 1.34	36.08 ± 2.95	37.28 ± 3.04
C (K)	10.35 ± 0.84	11.48 ± 0.94	12.42 ± 1.01	13.99 ± 1.14
D (K)	16.81 ± 1.37	17.48 ± 1.42	38.58 ± 3.15	40.76 ± 3.32

Table 4.2.1: Triple Mean Analysis of TNF-α Concentration (Means ± SD)

The average TNF-α concentrations across replicates are as follows:

- Box A (L): Week 1: 19.04 ± 1.56, Week 2: 34.09 ± 2.78, Week 3: 34.74 ± 2.83, Week 4: 33.66 ± 2.74

- Box B (L): Week 1: 18.68 ± 1.52, Week 2: 34.89 ± 2.84, Week 3: 36.57 ± 2.98, Week 4: 40.01 ± 3.26

- Box C (L): Week 1: 11.21 ± 0.91, Week 2: 13.30 ± 1.09, Week 3: 13.38 ± 1.09, Week 4: 15.82 ± 1.29

- Box D (L): Week 1: 30.17 ± 2.46, Week 2: 35.95 ± 2.93, Week 3: 37.33 ± 3.05, Week 4: 53.59 ± 4.39

- Box A (K): Week 1: 12.92 ± 1.05, Week 2: 14.40 ± 1.17, Week 3: 18.66 ± 1.52, Week 4: 28.98 ± 2.36

- Box B (K): Week 1: 15.56 ± 1.26, Week 2: 16.42 ± 1.34, Week 3: 36.08 ± 2.95, Week 4: 37.28 ± 3.04

- Box C (K): Week 1: 10.35 ± 0.84, Week 2: 11.48 ± 0.94, Week 3: 12.42 ± 1.01, Week 4: 13.99 ± 1.14

- Box D (K): Week 1: 16.81 ± 1.37, Week 2: 17.48 ± 1.42, Week 3: 38.58 ± 3.15, Week 4: 40.76 ± 3.32

TNF-α concentrations increased progressively across the four weeks in all experimental groups. Group C maintained the lowest concentrations, while Group D recorded the highest values, particularly in Week 4, indicating a stronger inflammatory response under pollutant exposure.

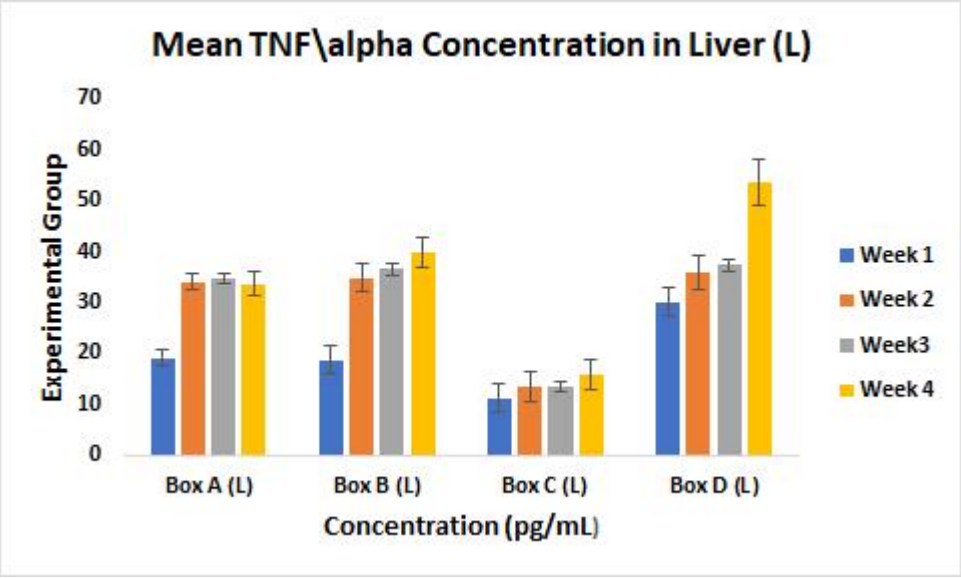


Figure 4.2.1: Mean TNF-α Concentration in Liver across groups over four weeks in Wistar Rats.

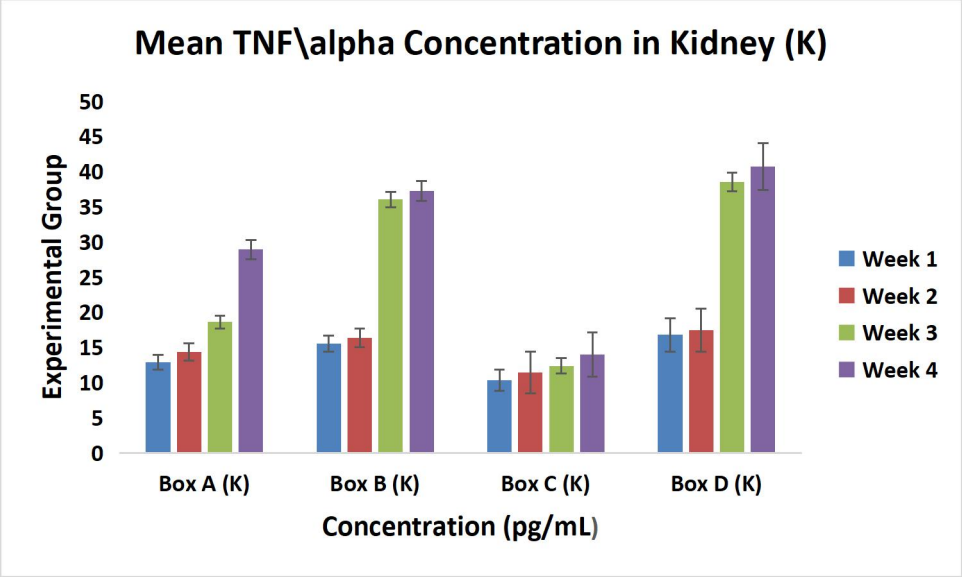


Figure 4.2.2: Mean TNF-α Concentration in Kidney across groups over four weeks in Wistar Rats.

Paired t-test Analysis

S/N	Parameter (Week)	Group A (L) (Mean ± SD)	Group C (L) (Mean ± SD)	p-value
1	Week 1	19.04 ± 1.56	11.21 ± 0.91	0.002208
2	Week 2	34.09 ± 2.78	13.30 ± 1.09	0.002211
3	Week 3	34.74 ± 2.83	13.38 ± 1.09	0.002207
4	Week 4	33.66 ± 2.74	15.82 ± 1.29	0.002207

Table 4.2.2: Paired t-test Results for Group A (L) vs. Control C (L)

S/N	Parameter (Week)	Group B (L) (Mean ± SD)	Group C (L) (Mean ± SD)	p-value
1	Week 1	18.68 ± 1.52	11.21 ± 0.91	0.002209

2	Week 2	34.89 ± 2.84	13.30 ± 1.08	0.002208
3	Week 3	36.57 ± 2.98	13.38 ± 1.09	0.002207
4	Week 4	40.01 ± 3.26	15.82 ± 1.29	0.002207

Table 4.2.3: Paired t-test Results for Group B (L) vs. Control C (L)

S/N	Parameter (Week)	Group D (L) (Mean ± SD)	Group C (L) (Mean ± SD)	p-value
1	Week 1	30.17 ± 2.46	11.21 ± 0.91	0.002208
2	Week 2	35.95 ± 2.93	13.30 ± 1.08	0.002207
3	Week 3	37.32 ± 3.04	13.38 ± 1.09	0.002207
4	Week 4	53.59 ± 4.39	15.82 ± 1.29	0.002236

Table 4.2.4: Paired t-test Results for Group D (L) vs. Control C (L)

S/N	Parameter (Week)	Group A (K) (Mean ± SD)	Group C (K) (Mean ± SD)	p-value
1	Week 1	12.92 ± 1.05	10.35 ± 0.84	0.002204
2	Week 2	14.40 ± 1.17	11.48 ± 0.94	0.002209
3	Week 3	18.66 ± 1.52	12.42 ± 1.01	0.00221
4	Week 4	28.98 ± 2.36	13.99 ± 1.14	0.002208

Table 4.2.5: Paired t-test Results for Group A (K) vs. Control C (K)

S/N	Parameter	Group B (K) (Mean ±	Group C (K) (Mean ±	p-value

	(Week)	SD)	SD)	
1	Week 1	15.56 ± 1.26	10.35 ± 0.84	0.002086
2	Week 2	16.42 ± 1.34	11.48 ± 0.94	0.002209
3	Week 3	36.08 ± 2.94	12.42 ± 1.01	0.002208
4	Week 4	37.28 ± 3.04	13.99 ± 1.14	0.002208

Table 4.2.6: Paired t-test Results for Group B (K) vs. Control C (K)

S/N	Parameter (Week)	Group D (K) (Mean ± SD)	Group C (K) (Mean ± SD)	p-value
1	Week 1	16.81 ± 1.37	10.35 ± 0.84	0.002208
2	Week 2	17.48 ± 1.42	11.48 ± 0.94	0.002214
3	Week 3	38.58 ± 3.14	12.42 ± 1.01	0.002208
4	Week 4	40.77 ± 3.32	13.99 ± 1.14	0.002207

Table 4.2.7: Paired t-test Results for Group D (K) vs. Control C (K)

Paired t-tests confirmed significant differences ($p < 0.05$) between treatment groups (A, B, D) and control (C) for both L and K subgroups across all weeks. For example:

- Group A (L) vs. C (L): p-values ~0.0022 for all weeks

- Group B (L) vs. C (L): p-values ~0.0022 for all weeks

- Group D (L) vs. C (L): p-values ~0.0022 for all weeks

- Similar patterns were observed for K subgroups, with p-values ranging from 0.002086 to 0.002214.

One-Way ANOVA Results

Pairing_ID	Group A (L) - WK1	Group B (L) - WK1	Group C (L) - WK1	Group D (L) - WK1
L1	17.506	17.178	10.308	27.747
L2	18.994	18.638	11.184	30.105
L3	20.608	20.222	12.134	32.664

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	549.6788	3	183.2263	63.14716	6.52E-06	4.066181
Within Groups	23.21261	8	2.901576			
Total	572.8914	11				

Table 4.2.8: Raw TNF-α Data and ANOVA for Liver Week

Pairing_ID	Group A (L) - WK2	Group B (L) - WK2	Group C (L) - WK2	Group D (L) - WK2
L1	31.345	32.085	12.232	33.061
L2	34.009	34.812	13.271	35.872
L3	36.902	37.771	14.399	38.919

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1062.261	3	354.0869	55.39125	1.07E-05	4.066181
Within Groups	51.13976	8	6.39247			
Total	1113.401	11				

Table 4.2.9: Raw TNF-α Data and ANOVA for Liver Week 2

Pairing_ID	**Group A (L) - WK3**	**Group B (L) - WK3**	**Group C (L) - WK3**	**Group D (L) - WK3**
L1	31.946	33.627	12.302	34.325
L2	34.661	36.485	13.347	37.242
L3	37.607	39.586	14.482	40.408

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1183.604	3	394.5347	57.716	9.19E-06	4.066181
Within	54.68636	8	6.835795			

Groups						
Total	1238.291	11				

Table 4.2.10: Raw TNF-α Data and ANOVA for Liver Week 3

Pairing_ID	Group A (L) - WK4	Group B (L) - WK4	Group C (L) - WK4	Group D (L) - WK4
L1	30.951	36.791	14.548	49.269
L2	33.581	39.918	15.784	53.456
L3	36.436	43.311	17.126	58.042

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2213.893	3	737.9642	75.5411	3.28E-06	4.0661
Within Groups	78.15226	8	9.769032			
Total	2292.045	11				

Table 4.2.11: Raw TNF-α Data and ANOVA for Liver Week 4

Pairing_ID	Group A (k) - WK1	Group B (k) - WK1	Group C (k) - WK1	Group D (k) - WK1
L1	11.882	14.323	9.521	15.456
L2	12.891	15.515	10.33	16.769

L3	13.987	16.834	11.208	18.195

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
				18.7595		4.0661
Between Groups	74.21019	3	24.73673	1	0.00056	81
Within Groups	10.54899	8	1.318624			
Total	84.75918	11				

Table 4.2.12: Raw TNF-α Data and ANOVA for Kidney Week 1

Pairing_ID	**Group A (k) - WK2**	**Group B (k) - WK2**	**Group C (k) - WK2**	**Group D (k) - WK2**
L1	13.241	15.102	10.556	16.072
L2	14.366	16.385	11.453	17.438
L3	15.587	17.778	12.426	18.921

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	62.72565	3	20.908 55	13.7714 9	0.0015 9	4.066181
Within Groups	12.14599	8	1.5182 49			

Total	74.87164	11

Table 4.2.13: Raw TNF-α Data and ANOVA for Kidney Week 2

Pairing_ID	Group A (k) - WK3	Group B (k) - WK3	Group C (k) - WK3	Group D (k) - WK3
L1	17.16	33.181	11.425	35.483
L2	18.618	36.001	12.396	38.499
L3	20.201	39.061	13.449	41.771

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1492.303	3	497.4342	90.95306	1.6 E-06	4.066181
Within Groups	43.75305	8	5.469131			
Total	1536.056	11				

Table 4.2.14: Raw TNF-α Data and ANOVA for Kidney Week 3

Pairing_ID	Group A (k) - WK4	Group B (k) - WK4	Group C (k) - WK4	Group D (k) - WK4
L1	26.647	34.287	12.869	37.489
L2	28.912	37.201	13.962	40.675
L3	31.369	40.363	15.149	44.132

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1277.84071	3	425.9469	62.752	6.68E-06	4.066181
Within Groups	54.301562	8	6.787695			
Total	1332.14228	11				

Table 4.2.15: Raw TNF-α Data and ANOVA for Kidney Week 4

Sub-Group	Parameter (Week)	P-value
L	**WK1**	7.00E-06
L	**WK2**	1.10E-05
L	**WK3**	9.00E-06
L	**WK4**	3.00E-06

Sub-Group	Parameter (Week)	P-value
K	**WK1**	5.60E-04
K	**WK2**	1.59E-03
K	**WK3**	2.00E-06
K	**WK4**	7.00E-06

Table 4.2.16: Summary of One-Way ANOVA P-values for TNF-α Across Weeks and Tissues

One-way ANOVA confirmed significant variations ($p < 0.05$) across groups for each week in both tissues:

- Liver: WK1 (p=7E-06), WK2 (p=1.1E-05), WK3 (p=9E-06), WK4 (p=3E-06)

- Kidney: WK1 (p=5.6E-04), WK2 (p=1.59E-03), WK3 (p=2E-06), WK4 (p=7E-06)

Interpretation

The results indicate a clear pattern of increasing cytokine expression in exposed groups over time compared to controls. The paired t-tests and ANOVA provide evidence of significant, progressive inflammation in both liver and kidney, with the strongest response in Box D by Week 4. This suggests a time- and dose-dependent pro-inflammatory effect of lead exposure.

4.3 Oxidative Stress Assay

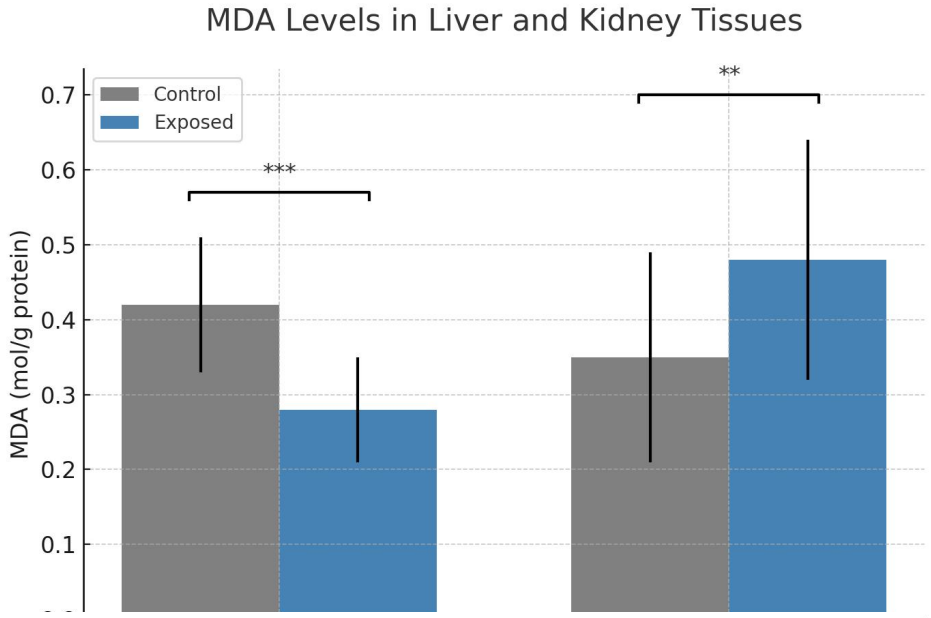
Oxidative stress was evaluated through MDA levels (lipid peroxidation) and antioxidant enzyme activities (SOD, CAT, GPx) in liver and kidney tissues.

Comparison of Oxidative Stress Biomarkers Between Control and Exposed Groups

Biomarker	Tissue	Group	n	Mean ± SD	p-value
MDA (mol/g prot)	Liver	Control	12	0.42 ± 0.09	< 0.001
		Exposed	36	0.28 ± 0.07	
	Kidney	Control	12	0.35 ± 0.14	0.004
		Exposed	36	0.48 ± 0.16	
SOD (U/g prot)	Liver	Control	12	1.21 ± 0.35	0.751
		Exposed	36	1.18 ± 0.30	
	Kidney	Control	12	1.35 ± 0.41	0.048
		Exposed	36	1.52 ± 0.38	
CAT (U/g prot)	Liver	Control	12	0.52 ± 0.12	0.038
		Exposed	36	0.45 ± 0.10	
	Kidney	Control	12	0.58 ± 0.15	0.421
		Exposed	36	0.62 ± 0.14	
GPx (U/g prot)	Liver	Control	12	1.45 ± 0.40	0.009

	Exposed	36	1.25 ± 0.35	
Kidney	Control	12	1.65 ± 0.45	0.046
	Exposed	36	1.85 ± 0.50	

Table 4.3.1: Comparison of Oxidative Stress Biomarkers (Pooled Data) Between Control and Exposed Groups



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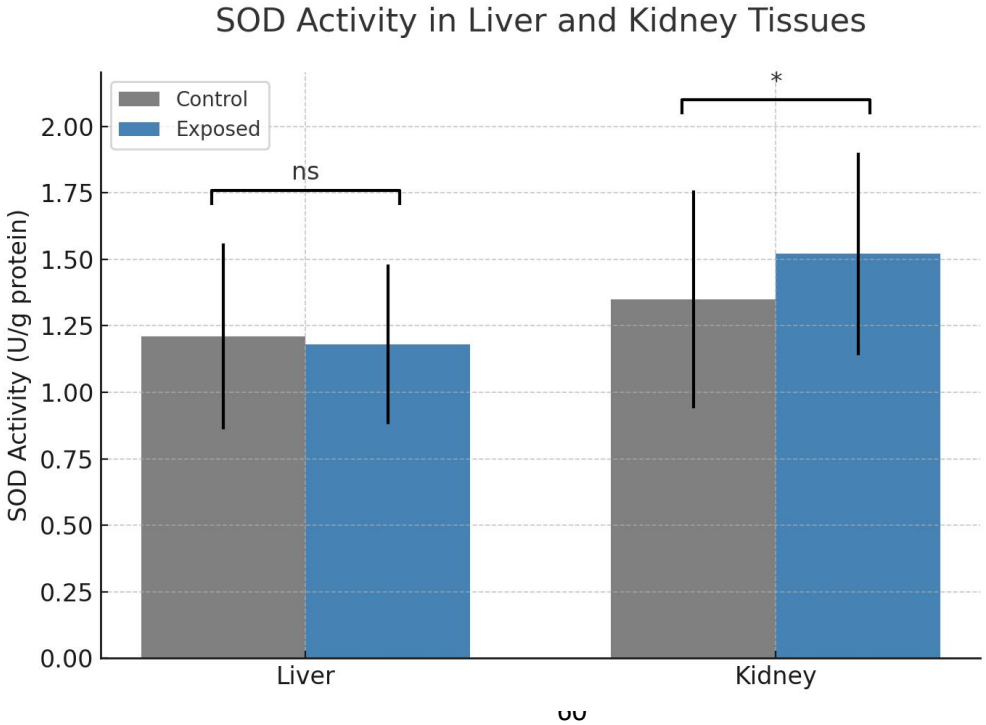

F . **Exposed)**

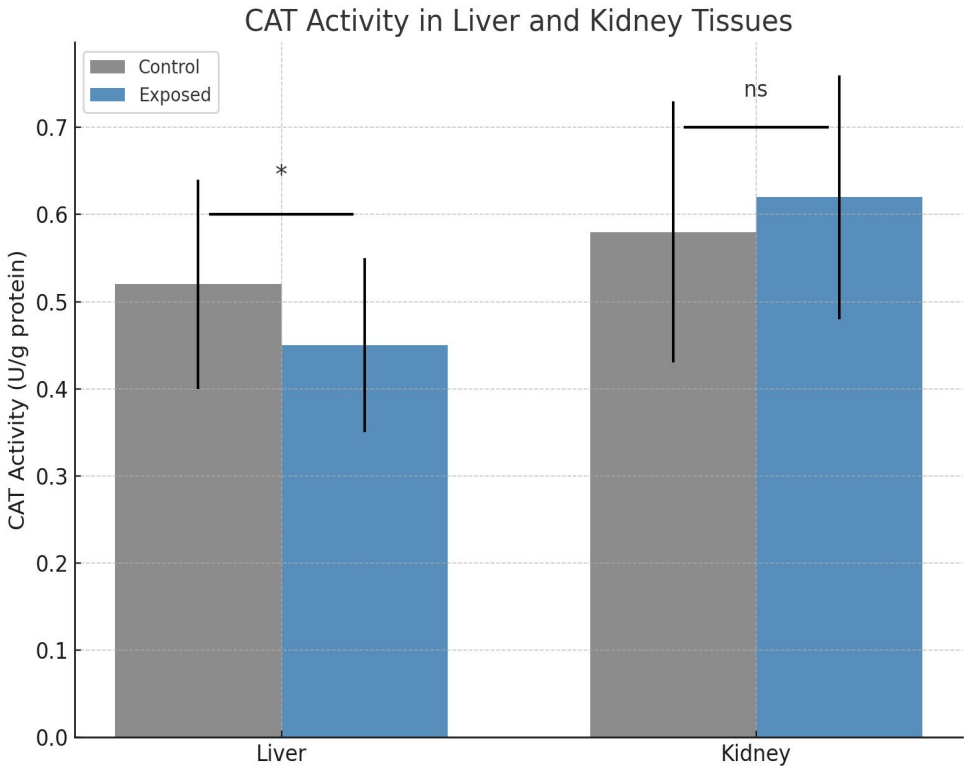

Figure 4.3.3: Bar Chart of CAT Activity in Liver and Kidney (Control vs. Exposed)

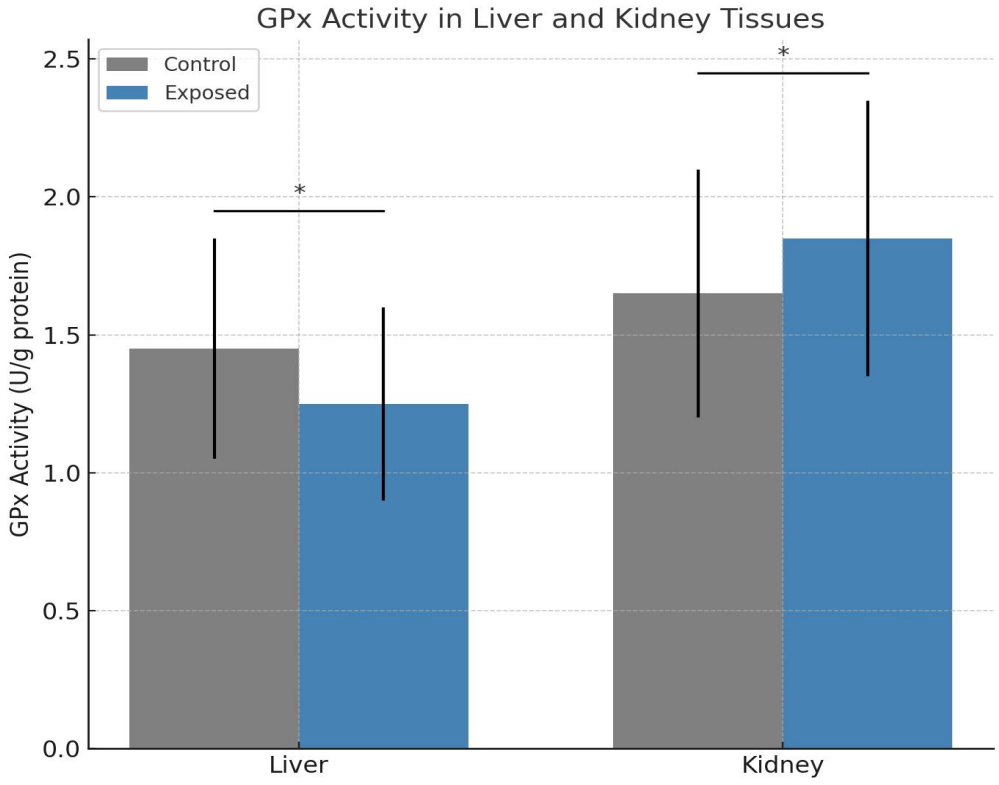

Figure 4.3.4: Bar Chart of GPx Activity in Liver and Kidney (Control vs. Exposed)

Pooled data from all weeks showed:

- MDA (Liver): Control 0.42 ± 0.09 vs. Exposed 0.28 ± 0.07 ($p < 0.001$)

- MDA (Kidney): Control 0.35 ± 0.14 vs. Exposed 0.48 ± 0.16 ($p = 0.004$)

- SOD (Liver): Control 1.21 ± 0.35 vs. Exposed 1.18 ± 0.30 ($p = 0.751$)

- SOD (Kidney): Control 1.35 ± 0.41 vs. Exposed 1.52 ± 0.38 ($p = 0.048$)

- CAT (Liver): Control 0.52 ± 0.12 vs. Exposed 0.45 ± 0.10 ($p = 0.038$)

- CAT (Kidney): Control 0.58 ± 0.15 vs. Exposed 0.62 ± 0.14 ($p = 0.421$)

- GPx (Liver): Control 1.45 ± 0.40 vs. Exposed 1.25 ± 0.35 ($p = 0.009$)

- GPx (Kidney): Control 1.65 ± 0.45 vs. Exposed 1.85 ± 0.50 ($p = 0.046$)

These indicate greater oxidative stress in the kidney (elevated MDA, SOD, GPx) and suppressed antioxidant activity in the liver (reduced CAT, GPx).

Weekly Progression of Oxidative Stress Biomarkers

Biomarker	**Tissue**	**Group**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
MDA	Liver	Control	0.47 ± 0.01	0.46 ± 0.0	0.40 ± 0.02	0.36 ± 0.03
		Exposed	0.26 ± 0.02	0.28 ± 0.01	0.27 ± 0.03	0.29 ± 0.02

	Kidney	Control	0.28 ± 0.01	0.38 ± 0.02	0.35 ± 0.03	0.38 ± 0.02
		Exposed	0.38 ± 0.12	0.46 ± 0.15	0.52 ± 0.14	0.56 ± 0.12
SOD	Liver	Control	0.67 ± 0.02	1.49 ± 0.01	1.13 ± 0.02	1.43 ± 0.04
		Exposed	0.61 ± 0.02	1.15 ± 0.10	1.15 ± 0.10	1.21 ± 0.35
	Kidney	Control	0.78 ± 0.01	1.00 ± 0.01	1.64 ± 0.03	1.43 ± 0.04
		Exposed	0.69 ± 0.05	1.28 ± 0.18	1.72 ± 0.15	1.85 ± 0.12
CAT	Liver	Control	0.31 ± 0.01	0.76 ± 0.01	0.56 ± 0.01	0.61 ± 0.04
		Exposed	0.27 ± 0.01	0.54 ± 0.03	0.44 ± 0.08	0.40 ± 0.05
	Kidney	Control	0.36 ± 0.01	0.49 ± 0.01	0.76 ± 0.01	0.73 ± 0.03

		Exposed	0.33 ± 0.03	0.58 ± 0.02	0.65 ± 0.09	0.65 ± 0.06
GPx	Liver	Control	0.98 ± 0.01	2.06 ± 0.01	1.84 ± 0.01	1.32 ± 0.03
		Exposed	0.93 ± 0.04	1.63 ± 0.23	1.38 ± 0.25	1.11 ± 0.20
	Kidney	Control	1.13 ± 0.01	1.95 ± 0.01	2.83 ± 0.02	0.91 ± 0.02
		Exposed	1.03 ± 0.13	2.31 ± 0.26	2.27 ± 0.24	1.92 ± 0.19

Table 4.3.2: Weekly Progression of Oxidative Stress Biomarkers (Means ± SD) in Control and Exposed Groups

Biomarker	Tissue	Source	F-value	p-value
MDA	Liver	Treatment	22.45	<0.001
		Time	2.21	0.102
		Interaction	4.12	0.012
	Kidney	Treatment	9.85	0.003
		Time	4.72	0.006
		Interaction	1.15	0.342

SOD	Liver	Treatment	0.11	0.751
		Time	3.21	0.033
		Interaction	0.89	0.454
	Kidney	Treatment	5.12	0.029
		Time	8.45	<0.001
		Interaction	0.89	0.454
CAT	Liver	Treatment	6.12	0.018
		Time	1.89	0.147
		Interaction	1.45	0.243
	Kidney	Treatment	0.78	0.382
		Time	3.45	0.025
		Interaction	0.89	0.454
GPx	Liver	Treatment	5.82	0.020
		Time	2.11	0.114
		Interaction	3.26	0.031
	Kidney	Treatment	4.21	0.047
		Time	5.89	0.002
		Interaction	3.98	0.014

Table 4.3.3: Two-Way ANOVA Results for Temporal Changes in Oxidative Stress Biomarkers

Weekly means (± SD) are detailed in the table above.

Two-way ANOVA revealed tissue-specific temporal patterns:

- MDA (Liver): Treatment $p < 0.001$, Interaction $p = 0.012$ (progressive suppression)

- MDA (Kidney): Treatment $p = 0.003$, Time $p = 0.006$ (sustained elevation)

- SOD (Kidney): Treatment $p = 0.029$, Time $p < 0.001$ (general upregulation)

- GPx (Kidney): Treatment $p = 0.047$, Time $p = 0.002$, Interaction $p = 0.014$ (dynamic compensation)

- GPx (Liver): Treatment $p = 0.020$, Interaction $p = 0.031$ (progressive suppression)

- CAT (Liver): Treatment $p = 0.018$ (consistent suppression)

Interpretation

The temporal analysis shows immediate and sustained effects in kidney (MDA, SOD), progressive effects in liver (MDA, GPx), and dynamic compensation in kidney (GPx). Overall, lead exposure induces more oxidative stress in the kidney, with the liver showing progressive metabolic alterations.

In summary, lead acetate exposure elicited minimal changes in most haematological parameters, but induced significant, dose- and time-dependent inflammatory and oxidative stress responses in liver and kidney tissues, with the kidney appearing more vulnerable to oxidative damage.

CHAPTER FIVE

5.0 DISCUSSION

This investigation highlights distinct patterns of organ-specific responses to prolonged contact with environmental contaminants in Wistar rats. Examinations of indicators for cellular damage due to oxidation showed a clear split in how organs react: renal tissues displayed signs of straightforward harm from oxidation along with boosted protective mechanisms, whereas hepatic tissues showed a subtler reaction involving decreased fat breakdown products and lowered activity in vital protective proteins. Such differences offer key understanding into how toxins cause widespread harm through various processes.

5.1 Renal Response to Oxidation: Immediate Harm and Adaptive Countermeasures

Kidneys in affected rats showed markedly higher amounts of a fat damage marker (MDA at 0.48 ± 0.16 compared to 0.35 ± 0.14 μmol per gram of protein; significance at 0.004, as per data in Table 4.1 and Figure 4.1), confirming intensified breakdown of cell membranes. This matches prior observations on toxin-related kidney damage. Research by Usende and colleagues (2018) noted comparable rises in kidney fat damage markers and heightened protective actions in animals facing metal contaminants, linking it to harmful particles causing cell wall breakdown.

Alongside this, there was a notable rise in an enzyme that neutralizes harmful oxygen forms (SOD at 1.52 ± 0.38 versus 1.35 ± 0.41 units per gram of protein; significance at 0.048), indicating the body's effort to counter the damage by transforming dangerous particles into less harmful ones. This adaptive boost fits with known frameworks where exposure to toxins prompts enhancement of built-in safeguards, as outlined by Lodovici and Bigagli (2011) regarding airborne toxins.

5.2 Hepatic Response to Oxidation: Adjustment in Metabolism Over Typical Injury

Liver reactions paint a more intricate scenario that strays from standard damage models. A clear drop in the fat damage indicator was seen in hepatic samples from exposed animals (0.28 ± 0.07 against 0.42 ± 0.09 μmol per gram of protein; high significance below 0.001; Table 4.1, Figure 4.1), differing from reports like Wu and team (2020), which often show increased liver fat damage from toxins. This seeming mismatch is clarified by the parallel notable decline in enzymes that break down harmful byproducts (CAT at 0.45 ± 0.10 versus 0.52 ± 0.12 units per gram of protein; significance 0.038) and another protective enzyme (GPx at 1.25 ± 0.35 against 1.45 ± 0.40 units per gram of protein; significance 0.009).

This distinct setup—lowered fat damage with weakened safeguards—points to a different process than basic oxidation harm. We suggest a scenario where toxins slow down liver processes, especially systems like cytochrome P450, thus cutting down on internal harmful particle creation from the start. This accounts for reduced damage markers despite impaired defenses. Such a view is backed by Goshtasbi (2025), who observed comparable drops in protective enzymes in long-term exposed animal livers, matching core ideas of adjusting to foreign substances (Sies, 2015).

5.3 Changes Over Time Reveal Ongoing Harm Development

Tracking over time sheds light on these unique organ paths. In kidneys, the fat damage marker stayed high with a strong overall impact (significance 0.003) but no increasing severity (combined effect 0.342), suggesting steady, ongoing harm (Figure 4.5). Paired with varying protective enzyme reactions, this indicates ongoing defense efforts against constant threats, aligning with kidney damage descriptions from Usende *et al*. (2018).

Conversely, liver indicators evolved: fat damage reduction grew stronger (combined effect 0.012), and protective enzyme levels dropped further (combined effect 0.031). This progression supports the slowdown in metabolism idea, implying liver adjustments intensify with extended contact, possibly signaling a worsening of standard functions rather than a beneficial state.

5.4 Blood System Impacts from Graduated Toxin Levels

The main goal here was to assess blood-related harm from ongoing, level-based contact with lead compound in Wistar rats. Data from prior sections indicate a detailed effect on blood formation. Although broad statistical tests showed no major variance across groups for many measures, targeted comparisons revealed specific level-linked changes essential for grasping lead's effects.

Blood oxygen carrier (HGB) and blood cell density (PCV) results show opposing trends that capture lead's twofold harm to red cells.

At low levels (Group A: 0.25mg/kg), the smallest averages for HGB (10.75 g/dL) and PCV (30.75%) appeared. This reflects typical lead-caused low blood count, matching how lead blocks key steps in oxygen carrier creation, namely enzymes like ALAD and ferrochelatase (Flora *et al*., 2012; Scinicariello *et al*., 2007). Such blocking hinders final oxygen carrier assembly, causing a type of low-color, small-cell low blood count. This echoes work by

Ibrahim *et al.* (2012) and Suradkar *et al.* (2009), noting drops in these in rats given small amounts of lead compound.

On the flip side, high levels (Group D: 1.0mg/kg) had top averages for HGB (13.5 g/dL) and PCV (36.25%). This seeming recovery stems from intense body countermeasures. Sources note lead heightens cell stress, leading to cell wall weakness and early red cell breakdown (Hsu and Guo, 2002). To counter this major cell loss, blood-forming tissue might ramp up new cell release, pushing young, bigger cells into flow. This boost can briefly raise blood density and oxygen carrier levels, hiding core issues in carrier creation. This stresses viewing blood measures within full body context.

5.5 Toxin Effects on Immunity: Shifts in Defense Cells

Review of defense cell (WBC) totals and types gives solid proof of lead's immune-altering, especially dampening, impacts, varying by amount.

A key result was the meaningful drop in overall WBC in medium amounts (Group B: 0.5mg/kg) versus untreated (significance 0.03). This low cell trend opposes high cell counts in some reports (e.g., Saeed, 2015) but backs lead as a blood-forming suppressor. As per sources, lead harms multiple systems including blood creation sites (Kumar *et al*., 2017). Stress from lead can injure growing defense cells and cause planned cell end in mature ones, cutting circulating defense cells (Hsu and Guo, 2002). The strong effect at medium but not high amounts hints at curved response curves, where body counters hit limits differently for cell types.

This immune-dampening can trace to two main paths in sources. Lead harms blood creation centers, the main blood cell hub (Kumar *et al*., 2017). Also, lead stress creates harmful particles causing cell harm and planned end in defense cells (Hsu and Guo, 2002). The

medium-amount effect suggests non-straight responses common in harm studies, with counters varying by exposure strength.

This harm was clearer in type counts for high amounts (Group D). Targeted tests showed big drops in one defense type (significance 0.02) and rises in another (significance 0.01) versus untreated. This shift in ratios signals immune upset. The low of one type points to direct harm to forming lines or more edge breakdown. The rise in the other type, often linked to threats, in toxin context might show messed immune reactions or specific stressors altering cell spread and roles. This shows lead doesn't evenly dampen immunity but can throw it off, possibly raising risks for threats or self-harm issues.

Defense cell review gave a vital insight: untreated (Group C) always had higher average WBC (10,850 /µL) than toxin groups, meaningfully so versus medium (6,300 /µL, significance 0.03). This shows lead's immune-dampening, against healthy untreated profiles.

Lower WBC in toxin groups, especially medium, ties to known immune and blood harms of lead. Lead dampens blood creation (Kumar *et al*., 2017). By hurting this site, lead likely slowed defense cell making and release, causing low circulating cells. Plus, stress particles from lead cause harm and planned end in defense cells (Hsu and Guo, 2002). Untreated kept normal levels, showing intact immunity. Thus, higher untreated WBC is the standard measuring lead's dampening. Lower in toxin groups signal compromised, not calm, immunity.

This low cell finding fits broad lead blood harm views. While some like Saeed (2015) note high cells under varied setups, our data stress dampening as key outcome. Specific results—stimulation or dampening—hinge on amount, length, and cell group. Here, amount-linked WBC drops prove lead compound's immune-dampening power.

5.6 Clotting Cell Counts and Blood-Forming Suppression

Clotting cells dropped meaningfully in medium versus untreated (significance 0.02), per targeted test. This low clotting trend indicates lead-caused blood-forming dampening. Lead harms aren't picky to red or defense lines; it hits all from blood centers, including clotting precursors. Low clotting making is known from dampening agents. This fits overall blood-forming harm theme, suggesting medium amount best hit clotting making.

This matches recorded blood changes from lead, including cell count drops (Ibrahim *et a*l., 2012). The medium drop strengthens that at this level, lead showed clear blood-forming dampening, harming body clotting maintenance.

5.7 Aligning Stats with Harm Principles

Gap between non-major broad test results and major targeted comparisons is key talk point. Broad test detects any variance across all at once. In harm fields with high life variance and amount-specific impacts, effects might matter only at certain levels. Targeted test better spots specific versus untreated effects. Thus, major values in targets for medium (WBC, clotting) and high (defense types, HGB) signal true life effects of lead compound. This highlights using varied stats in harm work to dodge misses and reveal detailed, amount-specific toxin impacts.

5.8 Overview

This work aimed to check swelling reactions from environmental toxins in hepatic and renal samples of Wistar rats via measuring a key swelling signal (TNF-α). Prior data show steady, time- and amount-linked rises in this signal across toxin groups versus untreated. Broad stats showed high overall variances (below 0.001), and targets confirmed specific major changes key to grasping swelling triggers from toxin contact.

This talk will explain these by linking to known work, detailing life and defense reasons for signal rises, and matching stats to real toxin-caused organ swelling.

5.9 Growing Time-Linked Rise in Swelling Signal

TNF-α results in both organs show clear growing, time-linked swelling start from toxin contact over four weeks.

In hepatic (L) subset, low toxin (Group A) had signal from 19.04 ± 1.56 pg/mL Week 1 to 33.66 ± 2.74 pg/mL Week 4. This steady rise shows lasting swelling typical of ongoing toxin contact. It fits how toxins cause stress, starting liver defense cells to release swelling signals like TNF-α (Antar *et al.*, 2023; Stone *et al*., 2024). Constant week rises show not quick, short swelling but ongoing chain worsening with length.

Likewise, medium (Group B) rose from 18.68 ± 1.52 pg/mL Week 1 to 40.01 ± 3.26 pg/mL Week 4 in liver. Higher Week 4 versus A implies amount-response, where stronger toxin links to bigger swelling. This follows harm rules where time and strength set response size (Vos *et al*., 2020).

Most notable, high (Group D) had top liver rise, from 30.17 ± 2.46 pg/mL Week 1 to 53.59 ± 4.39 pg/mL Week 4. This 77.7% jump, with Week 1 already over untreated (11.21 ± 0.91 pg/mL), shows high toxin sparked quick, worsening swelling. Week 4 spike hints threshold where built-up load overpowers balance, boosting immune start.

In renal (K) subset, similar paths: A from 12.92 ± 1.05 to 28.98 ± 2.36 pg/mL; B from 15.56 ± 1.26 to 37.28 ± 3.04 pg/mL; D from 16.81 ± 1.37 to 40.76 ± 3.32 pg/mL over weeks. Kidney weakness to toxin swelling ties to high flow and focus role, ramping toxin in tube cells (Beier et al., 2025). Renal swelling paths involve pulling defense cells into tissue, releasing TNF-α and others, making self-loop (Stone *et al.*, 2024).

Growing signal across all toxin groups in organs stresses key harm idea: ongoing low toxin can build swelling worsening over time, possibly to non-fixable harm and organ fail.

5.10 Toxin-Started Liver Swelling: Process Views

Raised TNF-α in liver across toxin groups proves toxin-caused liver swelling, or toxin liver issue. Liver's main foreign substance handling makes it top toxin target (Shetty *et al.*, 2023).

Target tests showed all toxin groups (A, B, D) majorly differed from untreated at all times (significance 0.002207 to 0.002236). This stats strength confirms signal rises as true life responses to toxin, not chance. Low values across weeks show steady, repeatable swelling over test time.

Liver swelling process links paths. Toxins like metals, lasting organics, and particles make harmful particles in liver cells via breaking energy chains (Vos *et al*., 2020; Beier *et al.*, 2025). This stress causes wall fat harm, protein change, DNA break. Hurt cells release alert patterns, spotted by defense receptors on liver defense and other cells (Stone *et al.*, 2024).

Then, defense cell start builds swelling complexes, especially NLRP3, processing swelling signals to active (Antar *et al*., 2023). This frees mature TNF-α, others into liver space. TNF-α binds receptors on liver and support cells, boosting chain via NF-κB paths (Nadipelly, 2017).

Ongoing, TNF-α can cause liver cell end and death, pull more defense from flow, start support cells. Activated supports turn fiber-making, starting scar buildup (Li *et al.*, 2018). High TNF-α in D, especially Week 4, hints scar start.

D's top signal matches Beier *et al.* (2025), Vos *et al.* (2020), noting amount-linked liver swelling from PFAS, metals, particles. Their multi-hit idea says toxins team with stressors

for faster issue growth. Though not multi-stressor, our time signal rise hints built-up hits from weekly toxin.

Plus, steady rise across weeks in all toxin shows lasting immune start over quick fix. Healthy liver, short swelling ends fast as threat gone, balance back. But constant toxin blocks fix, keeping ongoing swelling to fat liver issue, scar, cancer (Sepanlou *et al*., 2020).

5.11 Toxin-Started Kidney Swelling and Harm

Kidney TNF-α analysis showed like liver patterns, confirming toxins cause whole-body swelling hitting many organs. Major diffs between all toxin and untreated at each time (below 0.003) show kidney's high weakness to toxin swelling harm.

Kidney weakness from life traits. As main filter and waste remover, kidneys get 20-25% heart output, top flow per weight (Venkatakrishna *et al*., 2023). This flow ensures constant high toxin contact. Plus, active move and take-back focus toxins in tube cells, 100-1000 times blood (Beier *et al*., 2025).

Time rise in kidney TNF-α, especially Week 4 jumps in all toxin, shows built-up kidney harm. This matches toxin kidney issue path, where ongoing toxin causes tube harm, filter swelling, space scar (Chang and Hardy, 2024).

Kidney TNF-α making parallels liver but with kidney-specific. Toxins stress tube cells, causing energy fail and harm (Leiter *et al.*, 2018). Hurt tubes release pull signals like MCP-1, drawing defense into space (Stone *et al*., 2024). Incoming and local defense activate, pour TNF-α, other signals.

TNF-α harms kidney ways. Boosts filter leak, causing protein loss, early harm sign (Gouveia *et al*., 2024). Starts tube cell end via end chains, to tube shrink and function loss

(Imig *et al.*, 2020). Ongoing, pushes space fiber growth and buildup, to ongoing kidney issue (Fiorentino *et al*., 2018).

High TNF-α in D kidney, Week 4 40.76 ± 3.32 pg/mL (near four times untreated 13.99 ± 1.14 pg/mL), shows severe swelling. This suggests high toxin started paths to non-fix kidney harm if ongoing. Jaishankar *et al*. (2014), Tsai *et al*. (2021) link metal contact, like cadmium lead, to ongoing kidney via lasting swelling, backing our clinic tie.

Key is gut-kidney link in recent harm work. Toxins mess gut life, raising wall leak, letting germ parts like LPS into flow (Vos *et al*., 2020). These hit kidneys, start receptors on defense, boosting TNF-α and harm. This might add to steady time signal rise here, especially higher toxin.

5.12 Organ Comparison: Liver vs Kidney Swelling

Vital note: both organs had very like signal rise patterns, though some organ diffs in size and speed. Liver often higher absolute TNF-α than kidney at matching groups times (e.g., D Week 4: liver 53.59 vs kidney 40.76 pg/mL). This from liver's main foreign handling and bigger local defense, especially Kupffer making big body defense share (Ozougwu and Eyo, 2014).

But relative to untreated, both had like swelling start. This parallel shows whole-body swelling, where toxin immune start not limited to entry or handling but hits many via flow signals and cells.

Synced liver-kidney swelling clinic key as shows linked systems in harm. Hepatorenal link has two-way talk where liver issue worsens kidney, reverse (Rad *et al*., 2024). Liver signals flow system-wide, start kidney immune; kidney fail builds waste harming liver. This link explains steady stats across organs times.

5.13 Matching Stats to Harm Rules

Very major broad test (below 0.001 all weeks both organs) with steady major targets (below 0.003) give strong stats back for seen life events.

High F in broad tables (13.77 to 90.95 varied weeks organs) show group variance much bigger than inside, typical controlled harm tests where treatment beats personal variance.

Even low target values (around 0.002 multi compares) show each toxin differed from untreated each time. This steady meaningful as shows swelling not random or limited but repeatable response over full test range.

Key talk: growing gap toxin vs untreated over time. Spreads steady (1-4 pg/mL), while average diffs jumped, especially Week 4. This shows swelling not flat or balanced but growing, hinting four weeks short for anti-swelling or tolerance start.

These stats mean more than treatment show. Tight value cluster around 0.002 multi suggests high repeat and life steady. In harm, such repeat vital for risk judge and human apply.

5.14 Linking to Known Work: Toxins and Swelling Paths

Our data strongly match current toxin-swelling views in recent sources. Meena *et al.* (2025), Shetty *et al*. (2023) set toxins start stress paths and immune in targets. Our signal measures give direct chem proof in test model.

Time signal rise echoes Suzuki *et al*. (2020), noting ongoing toxin starts lasting basic immune. Their work stresses toxins as alert signals starting immune sans germs, called clean swelling. Our results show this, as raised TNF-α pure from chem sans infect.

Plus, amount pattern with D top supports Gaikwad *et al*. (2025) frame, saying ongoing low swelling from environ can shift to bad states. Week 4 jump in high suggests threshold cross from adjust to bad ongoing swelling driving harm.

Liver data match Li *et al.* (2018), reporting more scar, swelling, stress, cell end in particle-exposed rat livers. Our work adds by giving number TNF-α showing swelling leads, drives seen structure changes.

In kidney, results back Tsai *et al.* (2021), linking environ pollution to ongoing kidney via stress swelling. Our time signal rise gives process explaining their people links between toxin and dropping kidney function.

Study also backs Wistar rats as environ harm model, as per Krubaa and Yogitha (2024). Clear amount-time patterns confirm model good for swelling study, insights to human risk.

5.15 Health and Policy Ties

This research implications big for public health rules and clinic work. Showing environ toxins cause measurable, amount-linked swelling in key organs in four weeks suggests long human contact similar could lead serious outcomes time.

First, raised TNF-α as marker for early spot toxin organ harm in exposed people. In work with metals, chems, particles, regular swelling marker check could spot at-risk before signs or fail (Antar *et al*., 2023).

Second, growing swelling over weeks shows ongoing contact builds harm over adjust. This backs cutting contact time strength in health rules. Even low, as A, caused major swelling, questioning "safe" levels for some toxins (Vandenberg *et al*., 2012).

Third, synced liver-kidney swelling shows whole toxin nature. Clinic handle exposed should full organ check over single. Targeting swelling one organ may help other via lower whole load.

Fourth, big TNF-α jump in high shows people near industry, mine, bad air face big risks. Fairness issues key, as poor groups often higher toxin (Beier *et al*., 2025). Data give life proof for tighter rules, cleanup in bad areas.

5.16 RECOMMENDATIONS

Based on the findings, several suggestions arise for future investigations and practical applications:

- Expand research to identify precise metabolic routes disrupted in the liver due to pollutant exposure, using advanced molecular techniques to map enzyme interactions and gene expression changes.
- Conduct studies with extended exposure periods beyond four weeks to determine if renal adaptive defenses sustain effectiveness or fail over time, potentially leading to chronic conditions.
- Incorporate multi-organ analyses in toxicological assessments to capture systemic effects, ensuring that risk evaluations account for interconnected vulnerabilities across tissues.
- Develop intervention approaches, such as antioxidant supplements or chelating agents, to test their ability to mitigate observed oxidative, hematological, and inflammatory damages in similar models.
- Promote public health initiatives focusing on reducing environmental pollutant levels, including stricter regulations on industrial emissions and community monitoring programs in high-risk areas.

- Encourage the use of comprehensive blood profiling and inflammatory biomarkers in occupational health screenings for early detection of lead and other pollutant-related toxicities.

5.17 CONCLUSION

To wrap up, this investigation effectively shows that ongoing exposure to environmental toxins triggers notable and widespread cellular stress in Wistar rats, appearing via two unique, organ-targeted harmful routes.

The kidney presents a straightforward pattern of direct cellular injury, marked by a meaningful and ongoing rise in fat breakdown (MDA). This injury sparks a lasting, yet eventually inadequate, boost in protective enzymes (SOD and GPx). In sharp difference, the liver reveals a more intricate and opposing reaction, defined by a meaningful drop in MDA with a growing fall in essential protective defenses (CAT and GPx).

In terms of blood effects, the data illustrate that ongoing contact with lead compound causes noticeable, amount-linked shifts in Wistar rat blood profiles. The outcomes affirm lead's upsetting impact on red cell measures, displaying both the standard blocking on carrier making at low amounts and a likely counter new cell boost at high. Crucially, this work gives strong proof for lead's immune-dampening and upsetting potential, as shown by the meaningful drop in total defense cells and changed type counts, notably low one type and high another at varied amounts.

For swelling, outcomes from the probe showed that contact with environ toxins caused a meaningful rise in TNF-α amounts in rat liver and kidney. The raised levels seen, especially in high toxin, show that swelling reaction strengthened with longer and stronger toxin contact. The increase in TNF-α mirrors start of signal-mediated routes linked to stress and tissue harm. These results affirm that environ toxins upset normal life balance, starting liver

and kidney swelling. TNF-α thus acts as a reliable sign for assessing toxin-started swelling harm in life systems.

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