THE EFFECT OF *Annona muricata* PHYTOWASTE ON THE BIOACCUMULATION OF LEAD AND CADMIUM ON THE TISSUE ORGAN PARAMETERS OF WISTAR RATS

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PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES

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CERTIFICATION

This is to certify that this project work titled **"the effect of *Annona muricata* phytowaste on the bioaccumulation of lead and cadmium on the tissue organ parameters of wistar rats"** was carried out by Victory Ego OKWUOLISE with matriculation number LSC2104294, of the Department of Science Laboratory Technology (Physiology/Pharmacology Technique), Faculty of Life Sciences, University of Benin City, Edo State, under the supervision of Mr O.C.

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DEDICATION

This project work is dedicated to God Almighty for his love, wisdom, guidance, strength, protection and skills to complete this project work.

This project work is also whole heartedly dedicated to my beloved parents for their unwavering support.

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ABSTRACT

Cadmium and lead represent hazardous heavy metals that present substantial threats to environmental quality and human health worldwide. These metallic contaminants undergo bioaccumulation in living tissues, triggering oxidative damage and multiple pathological manifestations in vital organs. Given the extended biological retention periods of these toxicants, there exists an urgent requirement for efficacious and environmentally sound therapeutic approaches. This research examined how *Annona muricata* phytowaste extracts influence lead and cadmium tissue accumulation in Wistar rat models.Thirty male Wistar rats (80-100g) were allocated randomly across five treatment cohorts over 90 days. Group I received distilled water (control), Group II was administered lead acetate with cadmium chloride, Group III received zinc sulphate (1mg/kg) and selenium (1.5mg/kg), while Groups IV and V were treated with ethanol-based *Annona muricata* extract at 250mg/kg and 500mg/kg respectively. Heavy metal quantification in splenic, cerebral, and skeletal tissues was performed via atomic absorption spectrophotometry.Results revealed marked metal deposition in untreated subjects, with cadmium elevated by 189% in brain, 115% in spleen, and 102% in bone. Lead increased by 79% in bone, 55% in brain, and 115% in spleen. Zinc-selenium intervention reduced cadmium by 76% (spleen), 67% (brain), and 65% (bone), while lead decreased by 64%, 67%, and 54% respectively. The 250mg/kg EEAM dosage decreased cadmium by 73% across all tissues, with lead reductions of 66%, 46%, and 64%. The 500mg/kg dosage demonstrated superior protection in soft tissues, reducing cadmium by 80% in brain and lead by 75% in spleen, though skeletal tissue showed paradoxical increases suggesting metal mobilization.This investigation validates the therapeutic utility of *Annona muricata* phytowaste for ameliorating heavy metal intoxication,

with protection equivalent to conventional supplementation. The transformation of agricultural by-products into therapeutic resources aligns with sustainable development objectives while addressing environmental contamination.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Lead and cadmium are two of the most common heavy metals that can cause toxicity in humans and animals (Moghadamtousi *et al*., 2015). Exposure to these metals can occur through various routes, including contaminated food and water, industrial emissions, and waste disposal (WHO, 2010). The toxicity of lead and cadmium can cause damage to organs such as the liver, kidneys, and brain, leading to various health problems (Padma *et al*., 2018).Lead mimics calcium, disrupting neurological and enzymatic processes, and inhibits heme synthesis, leading to anemia (WHO, 2023).Cadmium disrupts zinc and calcium metabolism, causing oxidative stress and cellular damage (WHO, 2019).

Lead (Pb) and cadmium (Cd) have harmful effects on both humans and the environment (Balalimood *et al*., 2021). Approximately 2.2 million people in africa die each tear from environmental risk factors according to World Health Organization (WHO) study (WHO, 2016). Lead and cadmium are heavy metals of great occupational importance but are now even more significant as environmental pollutants. Both lead and cadmium can seriously affect organs and various system of an organism and can cause severe acute and chronic intoxications (Matovic *et al.*, 2015).

The incidence of heavy metals (HM) in the human body causes toxicity resulting in Alzheimer's, multiple sclerosis, Parkinson disease, muscular dystrophy (Neeti & Prakash; k, 2013). Heavy metal poisoning can either cause chronic effects like neurological disorders, physical abnormalities, muscular effects, genetic and hereditary problems, or acute effects like vomiting, dehydration, drowsiness, nausea, renal failure, and abdominal pain (Markich *et al.*, 2001; Sun *et*

al., 2018). The excess usage of heavy metals to satisfy human needs has heavily polluted the environment. Accumulation in living beings occurs when these metals are taken up and stored at a higher rate which thereafter is metabolized and excreted (Nedzarek *et al*., 2013). Lead toxicity in human's dates back to at least 5000 years ago when humans started to process lead (Gidlow, 2015).

Bioaccumulation refers to the net accumulation of a substance, such as a heavy metal, in an organism's tissues over time, where the rate of uptake exceeds the rate of excretion (WHO, 2021) . This is a critical distinction from acute toxicity, as even low, chronic exposure can lead to high tissue concentrations (ATSDR, 2020).Heavy metals have a long biological half-life, meaning they are eliminated from the body very slowly (EFSA,2019). For example, the biological halflife of cadmium in humans can be over 10 years, and that of lead in bone can be decades (Jarup *et al*., 2019) . This persistence allows them to accumulate over a lifetime.

The phytowaste of *Annona muricata* represents a significant untapped resource with considerable potential for valorization. With damaged fruits, peels, seeds, leaves, and flowers constituting substantial waste streams, innovative processing technologies can convert these materials into valuable bioactive compounds, proteins, oils, and functional ingredients (Santos *et al*., 2023) The implementation of green extraction technologies and sustainable processing methods not only adds economic value but also contributes to environmental sustainability and circular economy principles. The bioactive compounds present in *Anonna muricata* waste fractions offer promising applications in food, pharmaceutical, nutraceutical, and cosmetic industries.

By exploring the potential of *Annona muricata* phytowaste,this research aligns with sustainable development goals that advocate for waste vaporisation and the development of safer, plantbased health interventions (UN,2015). Establishing the efficay of *Annona muricata* phytowaste exracts

could thus serve as a basis for future research into natural therapies for treating heavy metal toxicity

Annona muricata is one of tropical plants which have relatively complete chemical compounds. It has flavonoid, tannin, phytosterol, alkaloid, etc. The high antioxidant compound in soursop is believed as cancer prevention so the cancer threat in the world can be minimized. The antioxidant compound in soursop can be found not only in its fruit, but also in other parts like leaves, seeds, etc. Based on that potency, this study aimed to compare antioxidant capacity of soursop leaves and seeds, also to study about the utilization of soursop parts which is usually not used.

1.2 Significance of study

The findings of this study will contribute to our understanding of the potential benefits of Annona muricata and will provide insights into its application in reducing the effects of lead and cadmium toxicity.

1.3 Aim

To investigate the therapeutic effects of *Annona muricata* phytowaste on the bioaccumulation of lead and cadmium in the tissue parameters of Wistar rats.

1.4 Specific objectives

- To determine the effect of *Annona muricata* phytowaste on the bioaccumulation levels of lead and cadmium in selected tissues (e.g., liver, kidney, brain, bone, blood) of Wistar rats exposed to these heavy metals.

- To administer varying doses of *A. muricata* phytowaste extract to evaluate its therapeutic effects.

CHAPTER TWO

LITERATURE REVIEW

The description of heavy metals into water bodies as a global environmental issue by (Zafar *et al*.,2020) has been a growing concern for our environment as it poses a major source of heavy metal poisoning of the environment today. These metals commonly found in their living region known as trace metals are essential for many metabolic processes in plants, animals and microbes. The human body can be exposed to heavy metals by consuming products cultivated on contaminated land or by drinking contaminated drinking water. Lead, Mercury, Cadmium and Copper are examples of heavy metals that are collectively toxic substances that are known to cause environmental concern.Heavy metal ions can enter the food chain, accumulate in ecological aquqtic system, and cause harmful effects on humans,plants, animals and the environment (Afroze and sen, 2018).

The diverse deleterious health effect upon exposure to toxic heavy metals in the environment is a matter of serious concern and a global issue. Much emphasis has been given to elucidate the mechanism of toxicity due to common environmental toxicants and to develop a safer chemotherapeutic approach to mitigate the toxic effects. Lead and cadmium are the two most abundant toxic metals in the environment. The co-exposure to these two toxic metals has synergistic cytotoxicity that may, at times, turn to antagonistic effects, because exposure to higher mixture concentrations may enhance cellular defense mechanisms including induction of metallothioneins synthesis upon exposure to cadmium. The concurrent higher levels of lead and cadmium have been recorded in several field situations. The common sources of lead and cadmium are diverse in nature including natural and anthropogenic processes such as combustion of coal and mineral oil, smelters, mining and alloy processing units, paint industries, and so forth. The quantity of lead used in the present decade far exceeds the total amount consumed in all previous eras.

2.1 LEAD

Lead (pb) is an extremely toxic and non-essential heavy metal that is known to have detrimental effect on biological systems. Lead is found in more than 200 minerals and is dense (11.34g/cm3) and extremely soft (less than 35 diamond pyramide hardnes). Lead is a relatively rare metal with an average concentration of 0.016g/kg soil in the earth's crust (pattee and pain, 2002). It's uses dates back to the ancient times because of it's significant physicochemical properties. It appears to challenging to stop using it due to its significant qualities, which includes softness, malleability, ductility, weak conductivity and corrosion resistance (Ara *et al*., 2015).Most human exposure to lead and its compound comes from a variety of sources, including leaded petrol, industrial processes like smelting and burning of lead, ceramics, boat building, lead-based painting, pigments, bookprinting, etc.

Lead (Pb) is a naturally occurring metal and generally form lead compounds by combining with two or more elements. Lead reacts with air and water to form lead sulfate, lead carbonates or lead oxide. These compounds act as a protective barrier to prevent corrosion. Lead can also interact with both acid as well as base. It has a low melting point and located above hydrogen in the electromotive series. Although the existence of lead is indicated in nature but human activities has been found as the main reason for increasing lead content in the environment (Sahid *et al*., 2015). Lead is released in air from mining of lead, factories utilizing lead compounds, alloys, vehicle exhaust and burning of fossil fuels (Violante *et al*., 2010). The lead is removed from atmosphere by rain and transferred to soil or comes in contact with surface water. Moreover, lead is used as pesticide during vegetable and fruit cultivation (Gall *et al*., 2015). Disposal of lead containing waste products, removal of lead based paints from bridges, buildings and damaged battery from industries further results into the accumulation of lead in municipal

landfills. Lead is not a foreign material to the human body as it is distributed to the brain, liver, kidney, and bones and is stored in bones and teeth, However, this is only 10 μg/dL in adults and 1.4 μg/dL in children (Jukso *et al*., 2008; Singh and Kalamdhad, 2011). The guideline value of lead indicated by world health organization is 0.01 mg/L (Edition, 2011). count and volume, the motility and the morphology of the sperm are also affected (Wu *et al*., 2012).. Lead can damage cell structure, cell membrane and most importantly it interferes with DNA transcription (Yedjou *et al*., 2010). At developmental stages, lead passes through the placenta into the body of the fetus. At developmental stages, lead passes through the placenta into the body of the fetus (Mason *et al*., 2014). Pb mimics ca by binding to the same receptors in cell activities and its uptake by plants exhibits serious effects on humans when consumed, Ronnie Levin et al. reported that the natural pathway for lead accumulation in humans and plants also depends on the seasonality (Edition, 2011).

The Pb concentration where found to be lower in spring and higher concentrations were observed when soil pH and salinity decreases. Temperature, humidity, bioavailability, Mobility, Environmental acidification, solar radiation also contributes to the increased Pb concentration. (Khan *et al*., 2015). It is found that anthropogenic pathways contribute more in most cases of human Pb exposure, Common anthropogenic contributions are Gasoline, car batteries, sewage sludge, fertilizers and other anthropogenic exposure of Pb includes Mining, Pb bearing sulfide deposits, Pb additives in petrol, Pb water pipes, Pb added in paints (Singh and Kalamdhad, 2011). Pb in drinking water is the major pathway of accumulation into human body which arises mainly due to the use of lead piping, still Pb piping are used in some places and records almost 29 mg L through such piping (K. Neeti and Prakash, 2013).

Other Geochemical and anthropogenic Pb cycles showed changes in Pb's functions and forms for human needs, Jian-su MAO et al. traced these changes and it is found that Lead ore and scrap are

two influencing anthropogenic sources of Pb. The major changes in forms of lead are the conversion of PbS Ore in to metal Pb, PbO_2, and PbSO4 (MAO *et al*., 2014).Both natural and Anthropogenic pathways of Pb can be traced effectively by Isotopic (Pb) fingerprinting technique, here isotopic ratios (208Pb, 207Pb, and 206Pb) are analyzed by TIMS, ICP-QMS, and ICP-SFMS techniques. This proves to be a better alternative to traditional statistical analysis of large databases (Ali *et al.*, 2019). Anthropogenic sources of Pb were analyzed by GIS-based data, and the metal distributions were analyzed by principal component analysis (PCA) and cluster analysis (CA). With supported data Harley T. Davis *et al*. experimentally determined that increased Pb Concentration in both urban and rural are due to anthropogenic sources (Balkhair and Ashraf, 2016). This review addresses various morphological, physiological, and biochemical effects of Lead toxicity in humans and also strategies adopted by humans for Pb detoxification and developing tolerance to Pb.

2.1.1 LEAD INTAKE BY HUMANS

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). ROS (Reactive oxygen species) such as hydroperoxide, hydrogen peroxide, and singlet oxygen are produced as a result of lead poisoning. Pb generates these free radicals which leads to oxidative stress causing cellular damage to the body cells. The body suffers oxidative stress when there is an imbalance of ROS and antioxidant defences. Oxidative stress causes cell and tissue destruction, which increases the likelihood of adverse health outcomes like cardiovascular disease and cancer (Flora, 2011).. Increased oxidative stress causes lipid peroxidation, which damages cell membranes resulting in cell damage. Lead inhibits the activity

of 5-aminolevulinic acid dehydratase, resulting in hemoglobin oxidation and lipid peroxidation, which can cause red cell hemolysis (Pourrut *et al*., 2011). balance. Ninety percent of glutathione in the cell is reduced, while ten percent is oxidized, and it serves as an antioxidant defense mechanism. Glutathione stabilizes ROS and is reduced back to GSH by glutathione reductase after being oxidized to glutathione disulfide (Sardar *et al*., 2013). By attaching to the sulfhydryl group of glutathione, Pb inactivates it, making GSH replenishment ineffective and increasing oxidative stress (Batool *et al*., 2017). The deposition of a small amount of Pb in the human body causes cellular malfunction and has a negative impact on an individual's health

2.1.2 TOXICITY OF LEAD

The ability of lead metal ions to substitute other bivalent cations like Ca, Mg and Fe, as well as monovalent cations like Na , is the primary cause of lead toxicity. This ultimately disrupts cell hemostasis and alters several biological processes, such as cellular signalling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, oxidant-antioxidant balance, inflammatory responses and cell adhesion. One of the most important hazardous heavy element in the environment is Pb (Ara *et al*., 2015).Mineral formations include lead, which is discharged into the environment by both natural and man-made industrial processes. It is neither biodegradable nor dissipative (Papanikolaou *et al*., 2005). Its ongoing usage and non-biodegradable nature causes its concentration in the environment to rise, posing ever greater risks. The majority of human exposure to lead and its compound comes from a variety of sources, including leaded petrol, industrial processes like smelting and combustion of lead, pottery, boat building, lead-based pottery, lead-containing pipes, battery recycling, grids, the arms industry, pigments, book printing, etc. (Ara *et al*., 2015).

Lead poisoning results from consuming food or water contaminated with lead. Fruits and vegetables contaminated with elevated levels of lead from their growing soils can also result in

lead toxicity. Lead levels in the soil are typically caused by pipes, lead paints, and leftover emissions from leaded petrol used prior to the Environment Protection Agency's rule being published in 1980 (Ara *et al*., 2015). Lead exposure occurs through respiratory and gastrointestinal (GI) systems, however, tetraethyl lead, which is found in leaded petrol, can also enter the body through the skin. Lead enters the bloodstream after exposure and is carried to other tissues via the blood stream (Papanikolau *et al*., 2005). Roughly 99 percent of lead in the blood binds to erythrocyte and diffuses into the liver, kidneys, brain, lungs, spleen, aorta, teeth and bones (Broskabady *et al*., 2018). Lead excretion is often minimal, with the urinary tract serving as the primary route. The foundation of the treatment approach to lead poisoning is the use of chelating drugs, which can increase the excretion of lead in the urine. Through the release into the bile, stomach fluid, and saliva, ingested lead may be expelled in faeces (Broskabady *et al*., 2018). According to the Centres For Disease Control and Prevention (USA), the standard increased blood level (CDC, 2012) is 10 μg/dL and for children 5 μg/dL of the whole blood. Previously, the standard lead level for children was 10 μg/dL. The appearance of clinical manifestations varies from individual to individual depending on other environmental factors. In some there is a clear appearance of clinical features even at lower levels, while some are asymptomatic even at higher levels of lead present in their body fluids. Children are more prone to the effects of lead because usually their organs are in a developing stage. Thus blood lead levels have to be set lower and must be frequently checked, particularly where contamination is expected.

Lead is extremely toxic and affects many body systems, accumulating primarily in the bones, kidneys and nervous systems. Even low levels of exposure can have significant health effect, especially in children because their organs are still developing. These effects may include behavioural problems, learning deficits, and lowered IQ (Ara *et al*., 2015). Acute toxicity of lead

includes dullness, restlessness, irritability, poor attention span, headaches, muscle tremors, abdominal cramps, kidney damage, hallucinations and loss of memory with encephalopathy (Papanikolaou *et al*., 2005).Long term exposure to lead may result in chronic renal insufficiency , reproductive system issues (such as decreased sperm count, miscarriage in pregnant women, prematurity, low birth weight and developmental problem during childhood) neurological effects (such as delayed reaction times, irritability and difficulty concentrating, as well as slowed down motor nerve conduction and headaches), many negative effects on the respiratory system(such as chronic obstructive pulmonary disease (COPD) like changes in the lung, asthma, lung cancer, nervous system), anemia, and hypertension (Broskabady *et al*., 2018)

In a recent study, the authors showed that the toxic effects on blood cells of rats caused by lead nitrate was alleviated by sodium selenite. They also showed that effects of lead nitrate were more harmful in diabetic than in non-diabetic rats (Bas *et al*., 2015). Oxidative stress was studied by low level lead exposure in first grade Uruguayan children, suggesting its potentially adverse effects on oxidative stress (Roy *et al*., 2015). Impaired respiratory function was observed in workers exposed to lead with elevated blood lead concentration and zinc protoporphyrin concentration (Jurdziak *et al*.,2015).

2.13 MECHANISMS OF LEAD TOXICITY

Lead's detrimental effect stems from its capacity to interfere with vital physiological and biochemical processes in the body. Lead accumulates in the kidneys, bones, brain and other tissues after entering the bloodstream. lead initiates calcium ions, interfering with vital calcium dependent functions that are necessary for bone health, muscular contraction and neurotransmitter release (Ara and Usmani, 2015). It has been found that lead leads to an excess of free radicals, which damages cell membrane by causing lipid peroxidation. This, in turn, sets off inflammatory signalling cascades.

Lead attaches itself to thiol groups in proteins, preventing vital enzymes such as aminolevulinic acid dehydratase (ALAD) from functioning.This impairs the body's antioxidant defences and interferes with heme production (Dobrakowski *et al*., 2016)

Especially in children during critical development stages, lead negatively impacts neuronal function, resulting in neuro-inflammatory and cognitive deficits (Broskabady et al., 2018)

2.1.4 Molecular Mechanisms of Lead Toxicity

Among the confirmed mechanisms for both Pb and Cd toxicity is their binding to sulfhydryl (SH) groups thus affecting many enzymes and other SH containing molecules. The other is their interaction with bioelements in the organism thus affecting directly and indirectly many physiological processes.

Lead induces oxidative stress through multiple pathways including disruption of antioxidant enzyme systems, depletion of cellular glutathione, and direct generation of reactive oxygen species (ROS). This oxidative damage affects cellular membranes, proteins, and DNA, leading to cellular dysfunction and death (Flora *et al*., 2012

Lead mimics calcium in biological systems, disrupting calcium-dependent processes including neurotransmission, muscle contraction, and cellular signaling. This calcium mimicry contributes to lead's particularly pronounced effects on the nervous system (Sharma & Dubey, 2005).

Lead binds to sulfhydryl groups in enzymes, particularly affecting δ-aminolevulinic acid dehydratase (ALAD) in heme synthesis and Na+/K+-ATPase in cellular transport processes. This enzyme inhibition contributes to anemia and neurological dysfunction associated with lead toxicity (Patrick, 2006).

2.1.5 Organ-Specific Lead Toxicity

The liver serves as a primary site for lead metabolism and detoxification, making it particularly vulnerable to lead-induced damage. Lead hepatotoxicity manifests through oxidative stress, lipid peroxidation, and disruption of mitochondrial function. Chronic lead exposure leads to hepatic steatosis, fibrosis, and altered liver enzyme activities (Mudipalli, 2007).

Studies in experimental animals have demonstrated that lead exposure causes significant elevations in serum aminotransferases (ALT and AST), alkaline phosphatase, and bilirubin levels, indicating hepatocellular damage. Histopathological examination reveals portal inflammation, hepatocyte necrosis, and fatty degeneration (El-Neweshy *et al*., 2013).

Lead nephrotoxicity is characterized by both acute and chronic manifestations. Acute exposure causes tubular dysfunction, while chronic exposure leads to interstitial nephritis, tubular atrophy, and glomerulosclerosis. Lead accumulates preferentially in proximal tubular cells, where it disrupts mitochondrial function and induces oxidative stress (Goyer, 1989).

The kidney's role in lead elimination makes it particularly susceptible to damage. Lead interferes with renal transport mechanisms, leading to aminoaciduria, glucosuria, and phosphaturia. Chronic lead nephropathy is associated with hypertension and progressive renal failure (Ekong *et al*., 2006).

Lead exposure is strongly associated with cardiovascular disease, including hypertension, cardiac arrhythmias, and increased risk of coronary heart disease. Lead affects cardiac function through multiple mechanisms including calcium channel disruption, increased oxidative stress, and vascular smooth muscle dysfunction (Navas-Acien *et al*., 2007).

Experimental studies demonstrate that lead exposure causes myocardial degeneration, increased heart weight, and altered electrocardiographic parameters. Lead-induced hypertension results from increased peripheral vascular resistance and altered renin-angiotensin system function (Vaziri, 2008).

2.1.6 REPRODUCTIVE SYSTEM TOXICITY

Lead toxicity significantly affects male reproductive function through direct effects on spermatogenesis and hormone production. Lead accumulates in testicular tissue, where it disrupts the blood-testis barrier and causes oxidative damage to developing spermatozoa. Studies demonstrate decreased sperm count, motility, and viability following lead exposure (Sokol *et al*., 2002).Lead interferes with testosterone synthesis by disrupting luteinizing hormone action and directly affecting Leydig cell function. Chronic lead exposure leads to testicular atrophy, seminiferous tubule degeneration, and decreased fertility (Ronis *et al*., 1996).

In female reproductive organs, lead disrupts normal uterine function through multiple mechanisms. Lead exposure affects estrogen and progesterone metabolism, leading to irregular menstrual cycles and reduced fertility. Lead accumulation in uterine tissue causes oxidative damage and inflammatory responses that compromise reproductive function (Zhai *et al*., 2014).

2.2 CADMIUM

Cadmium (Cd) is a naturally occuring, soft, bluish-white metal commonly found in the earth's crust as a byproduct of zinc, lead and copper refining. It is highly toxic and easily absorbed by plants, making it a frequent contaminant in the food chain (Jirup and Akesson, 2009). It is a post transition metal having a full d orbital and electrons in the s orbital employed as a protective plate due to its high corrosion resistance cadmium is not flammable and soluble in water and air. It burns in air to form cadmium chloride.

Cadmium has an atomic no 48 and belongs to group 12 in the d block and period 5. It is silverywhite, soft and ductile. The german scientist F. Strohmeyer discovered it in 1817 as a constituent of ($ZnCO_3$). It is naturally found in soil (about 0.2 mg/kg), minerals, and water. Cd belongs to the group of toxic, carcinogenic, and stimulating elements. Cadmium has a particularly long biological half-life of 15-30 years, making it one of the most persistent toxic metals in biological systems.

Upon absorption, cadmium distributes throughout the body with highest concentrations found in liver and kidneys. Low levels exposure to Cd may lead to damage to the kidneys, liver, skeletal system, and cardiovascular system, as well as to deterioration of sight and hearing. Along with strong teratogenic and mutagenic effects related to cadmium, it also shows adverse effects at low doses. Furthermore because cadmium compounds dissolve well in polymers and can tolerate high temperatures, they serve as pigments, producing vivid colours with great opacity and good tinting strength. Due to their optical properties, chalogenides—compounds of cadmium–have found use in plastics, paints, ,enamels, inks, display devices, photovoltaic cells and more recently, quantum dots (Sakar *et al*., 2013).Cadmium is primarily utilized in the production of colourants, plastic stabilizers, solders, alloys, protective coatings for electroplating and cadmium rods. It is also used to make alkaline nickel cadmium batteries, fireworks and fluorescent paints.

The amount of cadmium released into the environment by human activities is three to ten times that of natural processes. Cadmium can be released by products that intentionally contains cadmium (such as nickel-cadmium batteries, cadmium-pigmented plastics, ceramics, glasses, paints and enamels, cadmium-stabilized polyvinyl chloride (PVC) products, cadmium-coated ferrous and non-ferrous products, etc.) or that contains cadmium as an impurity (such as nonferrous metals and alloys of zinc, lead and copper, iron and steel, and fossil fuels like coal, oil, gas, peat and wood, cement and phosphate fertilizers). Additionally, cadmium is a by-product

of the extraction, smelting and purification of non-ferrous metals copper, lead, and zinc. Cadmium contamination of the environment might result from improper collection or disposal practices (Sarkar *et al*., 2013)

2.2.1 TOXICITY OF CADMIUM

Considered a hazardous metal, cadmium poses a threat to both humans and wildlife.Human health problems and even mortality can result from low amounts of cadmium in the air, water or food (Sharma *et al*., 2021). Cadmium (Cd) has become a chronic environmental pollutant due to its many industrial uses. It may be detrimental to the health of humans and animals because it can enter cells through the calcium ion channels found in many cell membranes and accumulate inside cells by binding to nuclear and cytoplasmic substances. Mother's milk, contaminated water or food supplies, employment in the battery industry, fertilizers and negligent pesticide use are the primary sources of cadmium exposure (Peana *et al*., 2022). Cadmium has a biological halflife of 10 to 35 years in humans due to its difficulty in excretion and inability to be metabolized by the body. Cadmium exposure is especially dangerous for humans since it can be inhaled and consumed through water, cereals, green vegetables, potatoes and seafood. Occupational exposure to Cd is mostly through breathing, whereas the general public often absorb Cd through food consumption and recreational smoke inhalation (Wang *et al*., 2015). After a single exposure to the organism, cadmium primarily accumulates in the liver. Cadmium builds up more in the kidneys, especially in the cortical area, after prolonged exposure to low amounts of the metal. Cadmium's distribution throughout the body is determined by its chemical form. The organs that store cadmium includes the liver, kidney, testes, spleen, heart, lungs, thymus, salivary glands, epididynis, and prostate; however, because of their high MT content, the liver and kidney retains about half of the body's total cadmium. Cadmium can also accumulate in the testes, lungs, pancreas and central nervous systems of men. Cadmium is primarily excreted in

the urine, and its concentration in urine may be a good indicator of the metal's levels in the body. Feces usually contain trace amounts of cadmium combined with glutathione, cysteine, or metallothioein (Sarkar et al., 2013).

2.2.2 Mechanism Of Cadmium Toxicity

Indirect processes such as inflammation, glutathione depletion, and kupffer cell activation ,mediate the generation of free radicals brought on by acute cadmium toxicity. Cadmium exposure over an extended period results in oxidative stress, which damages cellular components and often leads to cell death by increasing the formation of Reactive Oxgen Species (ROS) (Das and Al-Naemi, 2019).

Due to its divalent nature, cadmium can interfere with essential metals like zinc, iron, magnesium, manganese, calciun and selenium. This could result in a secondary deficiency that alters metabolism and ultimately alters the structure and functions of numerous organs (Sarkar *et al*., 2013).Additionally it can impair vital biological processes including DNA repair and cell signalling by interfering with the activity of enzymes and protein that rely on these metals (Peana *et al*., 2022).

Through calcium channels, cadmium enters the mitochondria and attaches to protein thiol groups, changing their structure. This disrupts calcium, sodium and potassium balance, lowers membrane potential, raises cellular ATP levels, and interferes with oxidative phosphorylation and membrane permeability. Cytochrome and Fe (II) ions eventually step out, raisimg ROS and producing a variety of additional consequences. The consequences include increased creation of reactive oxygen species and changes in gene expression that results in cell cycle arrest, differentiation, immortalization, or death (Sarkar *et al*., 2013)

Studies have demonstrated that Cd modifies the epigenetic markere in the DNA of the placenta and newborns and that the DNA methylation alterations linked to Cd exhibits significant sexual variations (Peana *et al*., 2022)

2.2.3 Molecular Mechanisms of Cadmium Toxicity

Cadmium induces oxidative stress through depletion of cellular glutathione, disruption of antioxidant enzyme systems, and direct generation of reactive oxygen species. Unlike other metals, cadmium cannot participate in Fenton reactions but promotes oxidative damage through indirect mechanisms (Valko *et al*., 2005).

Cadmium has high affinity for metallothionein (MT), a cysteine-rich protein that serves as the primary cellular defense against cadmium toxicity. However, prolonged exposure can overwhelm MT capacity, leading to free cadmium accumulation and cellular damage (Klaassen *et al*., 1999).

Cadmium interferes with calcium-dependent cellular processes by substituting for calcium in various biological systems. This disruption affects cellular signaling, muscle contraction, and bone metabolism (Thévenod & Lee, 2013).

2.2.4 Organ-Specific Cadmium Toxicity

The heavy metal cadmium (Cd) is an important environmental factor that induces liver injury and contributes to liver disease. The liver serves as the primary site for cadmium detoxification through metallothionein synthesis. However, chronic exposure overwhelms this protective mechanism, leading to hepatocellular damage.

Cadmium hepatotoxicity manifests through oxidative stress, lipid peroxidation, and mitochondrial dysfunction. Studies demonstrate that cadmium exposure causes elevation of liver enzymes, hepatic steatosis, and inflammatory cell infiltration. The mechanism involves

disruption of hepatocyte membrane integrity and induction of apoptotic pathways (Liu *et al*., 2009).

The kidney is the critical target organ for cadmium toxicity, with proximal tubular cells being particularly vulnerable. Cadmium nephrotoxicity progresses from initial tubular dysfunction to chronic kidney disease and eventual renal failure. The mechanism involves disruption of mitochondrial function, lysosomal damage, and activation of apoptotic pathways (Prozialeck *et al*., 2007).

Cadmium-induced nephrotoxicity is characterized by proteinuria, glucosuria, and aminoaciduria. Progressive tubular atrophy and interstitial fibrosis develop with chronic exposure, leading to irreversible kidney damage (Satarug and Moore, 2004).

Cadmium exposure is associated with increased risk of cardiovascular disease, including hypertension, atherosclerosis, and cardiac dysfunction. The mechanisms involve endothelial dysfunction, increased oxidative stress, and disruption of calcium homeostasis in vascular smooth muscle (Tellez-Plaza *et al*., 2008).

Experimental studies demonstrate that cadmium exposure causes myocardial damage, increased heart weight, and altered cardiac contractility. Cadmium also affects lipid metabolism and promotes atherosclerotic plaque formation (Messner *et al*., 2009).

2.2.5 Reproductive System Toxicity

Reproductive organs are essential not only for the life of an individual but also for the survival and development of the species. The response of reproductive organs to toxic substances differs from that of other target organs. Cadmium shows particular tropism for testicular tissue, where it causes severe damage to spermatogenesis and hormone production.

Cadmium-induced testicular toxicity involves disruption of the blood-testis barrier, oxidative damage to germ cells, and interference with Sertoli cell function. Studies demonstrate decreased

sperm production, altered sperm morphology, and reduced testosterone levels following cadmium exposure (Thompson and Bannigan, 2008).

Cadmium affects female reproductive function through disruption of hormone synthesis and direct toxic effects on reproductive organs. Cadmium exposure interferes with estrogen and progesterone production, leading to menstrual irregularities and reduced fertility (Johnson *et al*., 2003).In experimental animals, cadmium exposure causes uterine atrophy, decreased implantation rates, and increased embryonic mortality. The mechanism involves oxidative damage to uterine tissue and disruption of normal hormonal signaling pathways (Piasek *et al*., 2001).

2.2.6 Combined Lead and Cadmium Toxicity

Co-exposure to lead and cadmium often results in synergistic toxicity that exceeds the sum of individual effects. The combined exposure affects similar molecular targets and pathways, leading to enhanced oxidative stress and cellular damage. Studies demonstrate that mixed exposure produces more severe organ damage compared to individual metal exposure (Andjelkovic *et al*., 2019).

Combined exposure to lead and cadmium can alter the bioaccumulation patterns of both metals. Competition for binding sites and transport mechanisms may result in different tissue distribution patterns compared to single metal exposure. This altered distribution can affect both toxicity manifestations and potential therapeutic interventions (Liu *et al*., 2013).

2.3 Annona muricata (SOURSOP)

2.3.1 Overview

Annona muricata L., commonly known as soursop, graviola, or guanabana, is a tropical evergreen fruit tree belonging to the Annonaceae family. Widely distributed in tropical and

subtropical regions, it is renowned for its edible fruit and extensive use in traditional medicine across Africa, South America, and Asia. A. muricata is a small evergreen tree typically reaching 3-8 meters in height. The plant produces large, heart-shaped fruits covered with soft spines, containing white, fibrous, aromatic pulp. The leaves are alternate, simple, oblong to elliptic, and measure 6-20 cm in length. The flowers are yellowish-green and have three sepals and six petals arranged in two series. Traditional medicine systems across tropical regions have utilized various parts of A. muricata for treating numerous conditions including fever, infections, inflammation, pain, cancer, and metabolic disorders. Different plant parts (leaves, bark, roots, fruits, seeds) have been used in various preparations including decoctions, infusions, and poultices (CoriaTéllez *et al*., 2016).

The plant's various parts, including leaves, fruits, seeds, bark, and roots, are valued for their phytochemical composition and pharmacological properties, such as antioxidant, anticancer, and antidiabetic activities. However, the processing of soursop generates significant phytowaste (e.g., peels, seeds, and pulp residues), contributing to environmental challenges.

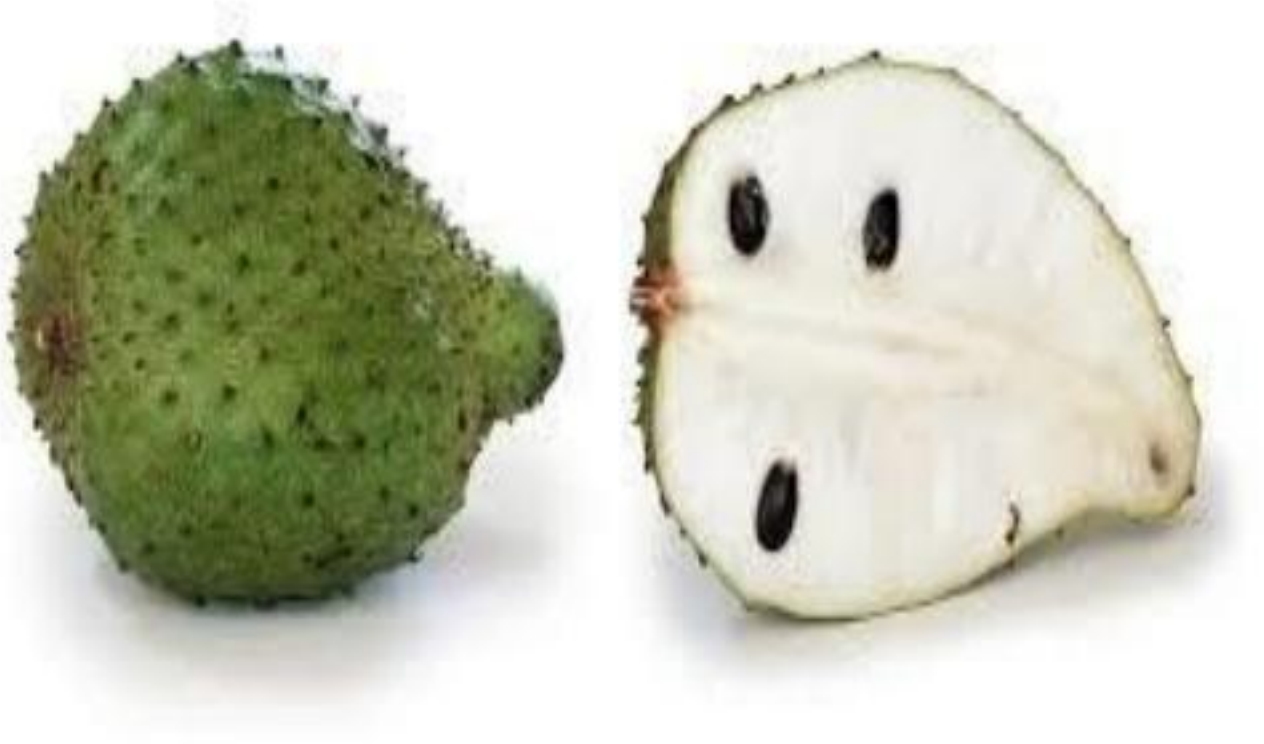


Plate 1: Friuts of *Annona muricata* commonly named soursop

Source: (Muhammad Mahdi Karim, 2010)

2.3.2 CLASSIFICATION OF *Annona muricata*

Annona muricata belongs to the Annonaceae family, a diverse group of flowering plants predominantly found in tropical and subtropical regions. The taxonomic classification is as follows:

- **Kingdom**: Plantae
- **Phylum**: Tracheophy(Vascular plants)
- **Class**: Magnoliopsida (Dicotyledons)
- **Order**: Magnoliales
- **Family**: Annonaceae

- **Genus**: *Annona*
- **Species**: *Annona muricata* L.

The Annonaceae family comprises approximately 122 genera and 2,440 species, distributed across tropical forests and savannas, with *Annona* being one of the most economically and medicinally significant genera (Erkens *et al*., 2023). *Annona muricata* is an evergreen, small, upright tree or shrub, typically growing 5–10 meters in height, with grey-brown bark and glossy leaves. Its heart-shaped, green fruit is covered with soft spines and is prized for its sweet-sour pulp used in beverages, desserts, and traditional remedies (Moghadamtousi *et al*., 2015).

2.3.3 Botanical Characteristics

Annona muricata is characterized by its tap-rooted structure and semi-deciduous or evergreen habit. The plant produces large, edible fruits with a fibrous, juicy pulp containing numerous black seeds. The leaves are simple, alternate, and oblong, with a glossy surface. The flowers are solitary, with three sepals and six petals arranged in two whorls. The plant's adaptability to tropical climates has facilitated its naturalization in regions like Africa and Asia, where it is cultivated for both food and medicinal purposes (Handayani and Yuzammi, 2021).

2.3.4 Distribution and Cultivation

Native to tropical America, *Annona muricata* has been introduced to Africa, Asia, and Australia, thriving in equatorial zones with warm, humid conditions. In Africa, it is one of 65 documented Annonaceae species, with 63 native and two naturalized, including *A. muricata* (Couvreur, 2008). Its cultivation is widespread due to its economic value as a fruit crop and its use in traditional medicine for ailments such as diabetes, hypertension, and cancer (Yassin *et al*., 2025).

2.3.5 Phytowaste from *Annona muricata*

Phytowaste refers to the non-edible or underutilized parts of plants generated during harvesting, processing, or consumption. For *Annona muricata*, phytowaste includes peels, seeds, and residual pulp discarded after juice extraction or fruit processing. These by-products constitute a significant portion of the plant's biomass and contribute to environmental pollution if not properly managed. Globally, fruit processing industries generate substantial waste, with soursop peels and seeds often discarded due to their perceived lack of commercial value (Reis *et al*., 2024).

The phytowaste of *Annona muricata* is rich in bioactive compounds, including phenolic compounds, flavonoids, alkaloids, and annonaceous acetogenins, which are responsible for its therapeutic properties. Studies have identified the following components in soursop phytowaste:

- **Peels**: Contain tannins, phenols, steroids, glycosides, alkaloids, flavonoids, resins, carbohydrates, phlobatannins, and balsams. The peel has higher levels of total phenolic compounds and antioxidant capacity compared to the pulp, with significant stability during in vitro digestion (Abdallah *et al*., 2024).
- **Seeds**: Rich in acetogenins, such as annonacin, which exhibit cytotoxic and antioxidant properties. Seeds also contain steroids, saponins, terpenoids, flavonoids, volatile oils, and carbohydrates (Mesquita *et al*., 2023).
- **Residual Pulp**: Contains dietary fiber, phenols, flavonoids, and essential lipids, contributing to its antioxidant and antimicrobial potential (Agu *et al*., 2017).

2.3.5.1 Utilization of *Annona muricata* Phytowaste

The valorization of *Annona muricata* phytowaste has gained attention as a strategy to reduce environmental impact and harness its bioactive potential. Applications include:

- **Nutraceuticals and Functional Foods**: Extracts from soursop peels and seeds are used to develop antioxidant-rich food additives and supplements. For instance, peel extracts have shown stability in phenolic content and antioxidant capacity during simulated gastrointestinal digestion, making them suitable for dietary applications (Abdallah *et al*., 2024).
- **Pharmaceuticals**: The presence of acetogenins in seeds and phenolic compounds in peels supports their use in developing anticancer, antimicrobial, and anti-inflammatory drugs (Rady *et al*., 2018).
- **Cosmetics and Perfumes**: Essential oils extracted from seeds and peels are used in cosmetic formulations due to their antioxidant and antimicrobial properties (Handayani & Yuzammi, 2021).
- **Bioenergy and Biomaterials**: Soursop seeds can be processed to extract oils for biofuel production, while fibrous peels are explored for biodegradable packaging (Mesquita *et al*., 2023).

- Valorizing *Annona muricata* phytowaste contributes to a circular economy by reducing waste disposal costs and environmental pollution. It also creates economic opportunities through the development of value-added products, enhancing food security and supporting sustainable development goals in regions with limited access to resources (Couvreur, 2008).

2.3.6 *Annona muricata* THERAPEUTIC USES

LEAVES: The leaves can be brewed to ease discomfort of colds, the flu and asthma, rubbed on a specific area of pain and used as analgesics.They are also used to treat skin issues. It is also utilized for the treatment of cutaneous and intestinal parasites. Recently, *Annona muricata* leaves

have been utilized medicinally to treat hypertension, diabetes and cancer (Coria-Tellez *et al*., 2018). Numerous investigations have shown that *Annona muricata* L. possesses pharmacological activity including cytotoxicity to tumoral cells, antimicrobial, antiprotozoal, insecticide, larvicide, anxiolytic, anti-stress, anti-ulcer, wound healing, anti-icteric, hepatoprotective, hypoglycemic, and antioxidant quality (Gbadeyan, 2021). ―Cystitis, insomnia, and headaches are all treated with the leaves. Furthermore, the boiled leaves are applied topically to treat rheumatism and abscesses, and the devotion of the leaves is believed to have neuralgic and antirheumatic effects when consumed internally (Moghadamtousi *et al*., 2015)

FRUIT: The fruit is used as a natural treatment for rheumatism, neuralgia, arthritis, diarrhea, dysentery ,fever, malaria parasites, skin rashes and worms. It is also used to improve a mother's milk after giving birth (Moghadamtousi *et al*., 2015). The fruits are freaquently used to make drinks, candies, shakes, and syrups.

SEEDS: it is believed that the crushed seeds have antihelmintic qualities that guard against worms, and parasites both within and outside the body. In tropical africa, the plant is used to heal skin diseases, coughs and pain. It is also used as an astringent, insecticide and piscicide. In india, the fruit and blossoms are used to treat catarrh, while root bark and leaves are believed to have antihelmintic and antiphlogistic qualities (Moghadamtousi *et al*., 2015). A comprehensive evaluation of the plant's potential for disease treatment will require the identification of specific bioactive compounds and a rigorous scientific demonstration of their ability to improve health outcomes, even though these applications of *Annona muricata* strongly suggests the presence of bioactive compounds with therapeutic benefits (Mesia *et al*., 2022)

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Plant material

Fresh *Annona muricata* were purchased from a local market called New Benin markst, located in Oredo Local Government of Edo state. The seeds, fruit, peel and fibre were confirmed, isolated and then deposited at the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

3.1.2 Chemicals used

All chemicals used in this study were of analytical grades; Chloroformm Formal saline, Ethaol, Cadmium chloride, Lead acetate, Zinc sulphate, Hydeogen peroxide, Fehlings solution, 1 naphtol, concentrated sulphuric acid, Glacial acetic acid, Ferric chloride,NaOH, HCL, Acetic anhydride, Wagner's reagent, Dragendoff's reagent, Picric acid, Nitric Perchloric Acid (5:2:1)
0.01M KMnO4, Adrenaline, thiobarbituric acid (TBA), lysis buffer, Ellman's reagent, Trichloroacetic acid (TCA) ELISA Kit (Elabscience)

3.1.3 Experimental Animals

Thirty(30) healthy male Wistar rats weighing between 80g to 100g were procured from the pharmacology animal house, ibadan Oyo state. They were housed in plastic cages within the department of Science Laboratory Technology at the University of Benin, Benin City, and acclimatized for two weeks before the commencement of the experiments. All animals were given food (rat chow-Vital feeds) and water and libitium.The treatment groups were exposed to different regimens which included the administration of aqueous extracts of *Annona muricata*, Zinc and selenium, alongside exposure to cadmium and lead contamination in their regular water intake. Rat weighs were recorded at the begining, once a week throughout the study period and at the study conclusion. The handling of animals adhered to the guidelines set by the institutional animal ethics committee of the Department of Science Laboratory Technology, University of Benin with ethical number UNIBEN/FSLT/00031

3.1.4 Equiptment Used

The equiptment used in the experiment included weighing balance, spectrophotometer, PG instrument LTD), Microplate reader, Dehydrator, Mechanical blender, Centrifuge, Spectrophotometer, Bulk scientific atomic absorption spectrophotometer),

3.1.5 Materials used

The materials utilized in the experiment consisted of distilled water, chemical reagents like chloroform, cotton wool, bedding materials such as wood shavings, protective gear including nose masks and disposable gloves and kits for biochemical parameter analysis, including conjugated billirubin, Urea, Albumin. Total protein, Creatnine, Alanine transaminase(AST). and Alkaline phosphatase (ALP)

3.2 Methodology

3.2.1 Preparation of the aqueous extract of *Annona muricata* Peels, Seeds, and Fibres

The *Annona muricata* were meticulously washed to eliminate any impurities. Following this, the peels, seeds and Fibres were manually separated from the friuts and left to air dry for a period of three weeks u till they atained a little brittle texture. Subsequently the dried product underwent further dehydration in a dehydrator set at 40 C for approximately six hours. It was then pulverised using an 800W High Power Heavy Duty Blender and Grinder. The weights of the dried components were recorded before immersing them in an aqueous solution(distilled water mixed with ethanol) for 72 hours wuth timely agitation to ensure through extraction. The resulting mixture was filtered using Whatmann filter paper after which the residue was discarded alongside the paper. The filtrate was concentrated subsequently in a water bath at a controlled temperature of 45 C for several hours and the result yield was measured to determine the percentage yield. The obtained extract was carefully stored in a sterile, airtight container, approximately labelled and refrigerated at 4 C for preservation

3.2.2 Experimental design

After a seven-day acclimatization period to laboratory conditions, the rate wewe divided into five (5) groups each containing six(6) rats:

Group I served as control group and administered distilled water

Group II was administered lead (Pb) and cadmium (Cd)

Group III was administered zinc sulphate and selenium (doses 1mg/kg and 1.5mg/kg, respectively)

Group IV was administered aqueous extracts of *Annona muricata* at a dose of 250mg/kg

Group IV was administered ethanol extracts of *Annona muricata* at a dose of 500mg/kg

3.2.3 Measurement of Animal body weight (BW)

Body weights were initially measured at the start of the experiment and then once weekly until day 90. Mean weekly BW gains were computed for each group. The percentage weight gain/loss was determined using the formula:

Final body weight on the day 90 (g) - Initial body weight on day 0 (g) /initial body weight (g) ×100

3.2.4 Measurement of Food Intake (FI)

Each group (n=6) was provided with a feed container marked with the caged code and containing a predetermined quantity of feed. The animals had restricted access to food.

3.2.5 Measurement of Water Intake (WI)

The water intake (WI) was determined by measuring the volume of water given to each group and the remaining at the end of each day's cycle, and then calculating the actual amount consumed.

3.2.6 Collection of Tissues for Testing

At the conclusion of the 90-day period, the animals underwent an overnight fasting regimen,and the final weight of all the rats were accessed and documented. Selected animals were then accessed and documented. Selected animals were tyhen humanly euthanized under chloroform induced anasthetia. Blood samples were collected from the abdominal aorta and transferred into plain bottles after careful abdominal incision. These samples were allowed to coagulate, and serum was subsequently separated via centrifugation at 3000rpm for 10 minutess. The obtained serum was stored in a clean plain bottle at 4˚C for subsequent biochemical analysis. Subsequently, the bone, spleen and brain were excised, weighed and the organ-body weight ratio were calculated and recorded.

3.3 Heavy Metal Analysis

0.5g of the dried , ground, and seived sample was weighed into a digestion tube. Next 10ml of a mixed acid solution was added. The digestion tube was then placed on a heater and heated untill dense white fumes appeared. Heating continued untill a clear solution was obtained. After digestion, the tube was removed from the heater and allowed to cool before adding a small amount of deionized water. The solution was the filtered using Whatman No 42 filter paper into a 100ml volumetric flask. The final volume was adjusted to the mark using deionized water. A reagent blank was also prepared using the same procedure but without sample.

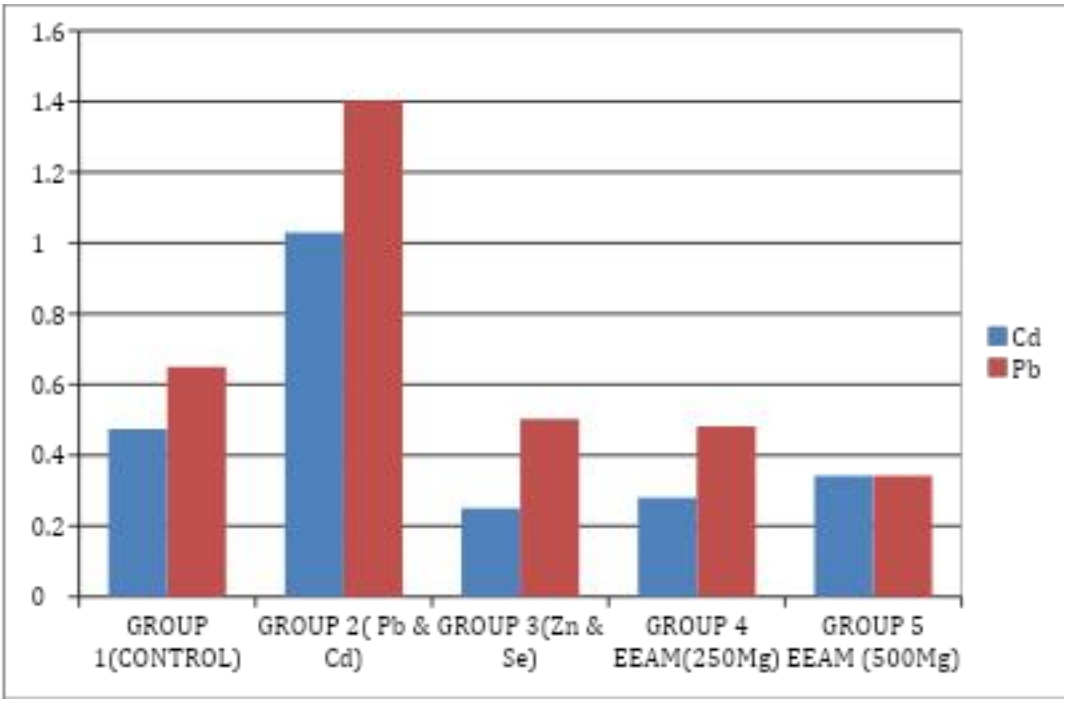
3.4 Statistical Analysis

The data will be presented as mean + standard error of the mean .To assess the significance of mean differences between treatment groups and the control groups, a one way analysis of variance (ANOVA) will be conducted after confirming the homogenietynof variance across groups. Turkey’s multiple comparism test will be performed to deetrmine the significance at a threshold of p 0.05. Graph pad prism will be used for analysis.

CHAPTER FOUR

RESULT

FIGURE 4.1 Effects of *Annona muricata* phytowaste on the Bioaccumulation on Pb and Cd in the spleen of wistar rats



Toxic exposure effect: Pb & Cd group showed 115% increase in Cd and 115% increase in Pb compared to control

Zn & Se protection: Reduced Cd by 76% and Pb by 64% compared to toxic group

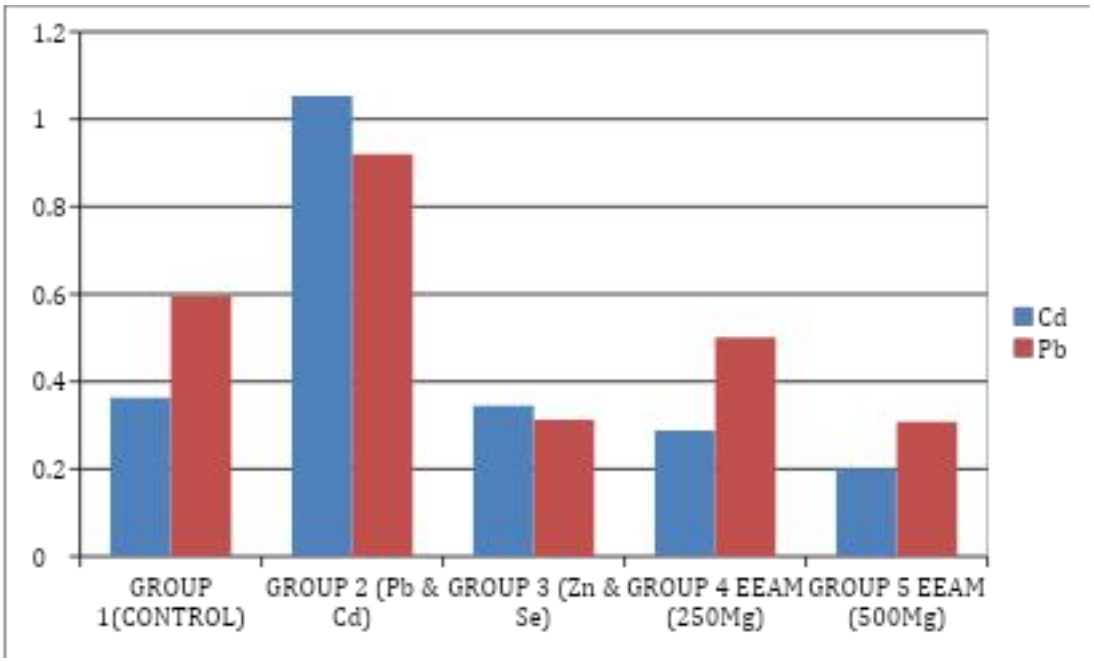
EEAM 250mg efficacy: Reduced Cd by 73% and Pb by 66% compared to toxic group

EEAM 500mg efficacy: Reduced Cd by 66% and Pb by 75% compared to toxic group

All treatment interventions showed statistically significant differences from the toxic control group ($p < 0.05$)

The 500mg EEAM dose generally showed greater protective effects than 250mg dose

FIGURE 4.2 Effect of *Annona muricata* phytowaste on the bioaccumulstion of Pb and Cd in the brain of wistar rats



Toxic exposure effect: Pb & Cd group showed 189% increase in Cd and 55% increase in Pb compared to control

Zn & Se protection: Reduced Cd by 67% and Pb by 67% compared to toxic group

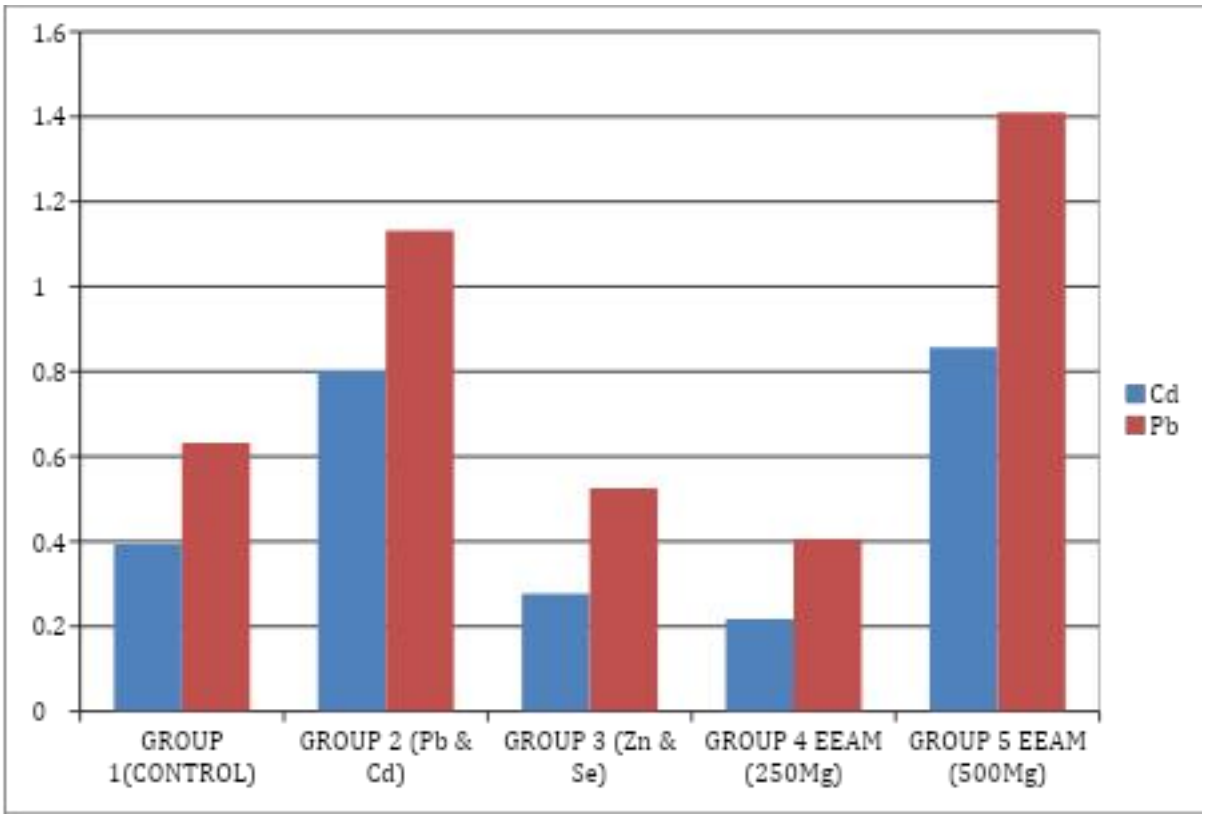
EEAM 250mg efficacy: Reduced Cd by 73% and Pb by 46% compared to toxic group

EEAM 500mg efficacy: Reduced Cd by 80% and Pb by 67% compared to toxic group

All treatment interventions showed statistically significant differences from the toxic control group ($p < 0.05$)

The 500mg EEAM dose generally showed greater protective effects than 250mg dose

FIG 4.3 Effects of *Annona muricata* phytowaste on the bioaccumulation of Pb and Cd in the bone of wistar rats



Toxic exposure effect: Pb & Cd group showed 102% increase in Cd and 79% increase in Pb compared to control

Zn & Se protection: Reduced Cd by 65% and Pb by 54% compared to toxic group

EEAM 250mg efficacy: Reduced Cd by 73% and Pb by 64% compared to toxic group

EEAM 500mg efficacy: Reduced Cd by -6% and Pb by -25% compared to toxic group

All treatment interventions showed statistically significant differences from the toxic control group ($p < 0.05$)

CHAPTER FIVE

5.0 DISCUSSION

The present study investigated the therapeutic effects of *Annona muricata* phytowaste on the bioaccumulation of lead (Pb) and cadmium (Cd) in selected tissues of Wistar rats. The findings demonstrate significant protective effects of *A. muricata* extracts against heavy metal accumulation, though with notable variations across different tissues and dose levels.

The rats exposed to lead and cadmium without protective intervention exhibited substantial increases in metal accumulation across all examined tissues. The brain showed the highest cadmium accumulation (189% increase), followed by the spleen (115%) and bone (102%). Lead accumulation was more moderate, with increases of 79% in bone, 55% in brain, and 115% in spleen compared to control animals. These findings align with established literature indicating that heavy metals preferentially accumulate in specific organs based on their metabolic activity, blood flow, and binding affinity to cellular components (Engwa *et al.,* 2019; Prozialeck *et al.,* 2007).

The pronounced cadmium accumulation in brain tissue is particularly concerning, as it indicates penetration of the blood-brain barrier, a finding consistent with previous studies demonstrating cadmium's neurotoxic potential (Thévenod and Lee, 2013). The high accumulation in bone reflects the body's attempt to sequester these metals in mineralized tissues, where lead and cadmium can substitute for calcium in the hydroxyapatite matrix (Jarup *et al.,* 2019). The long biological half-lives of these metals over 10 years for cadmium and decades for lead in bone explain the progressive accumulation observed in this study.

The zinc and selenium treatment group demonstrated significant reductions in heavy metal accumulation across all tissues examined. Cadmium levels were reduced by 76% in spleen, 67%

in brain, and 65% in bone compared to the toxic exposure group. Lead levels showed similar protection with reductions of 64% in spleen, 67% in brain, and 54% in bone. These protective effects can be attributed to several mechanisms.

Zinc and selenium are essential trace elements that play crucial roles in antioxidant defense systems. Zinc is a cofactor for superoxide dismutase and metallothionein synthesis, proteins that help neutralize reactive oxygen species and sequester toxic metals (Klaassen *et al.,* 1999). Selenium, as a component of glutathione peroxidase, enhances antioxidant capacity and reduces oxidative stress induced by heavy metals (Flora *et al.,* 2012). Additionally, zinc may compete with cadmium and lead for binding sites on cellular transporters and proteins, thereby reducing their uptake and accumulation (Sarkar *et al.,* 2013).

The consistent protective effects across all tissues support the use of zinc and selenium as therapeutic agents in heavy metal toxicity, corroborating previous findings by Bas *et al.* (2015), who demonstrated that sodium selenite alleviated lead nitrate toxicity in rat blood cells. Both doses of ethanol extract of *Annona muricata* (EEAM) demonstrated significant protective effects against heavy metal bioaccumulation, with dose-dependent variations in efficacy across different tissues.

The 250mg/kg EEAM dose showed substantial protective effects in most tissues. In the spleen, this dose reduced cadmium by 73% and lead by 66% compared to the toxic group. Brain tissue showed even more pronounced protection with 73% reduction in cadmium and 46% reduction in lead. In bone tissue, cadmium was reduced by 73% and lead by 64%.

These protective effects can be attributed to the rich phytochemical composition of *A. muricata* phytowaste, particularly its high content of phenolic compounds, flavonoids, alkaloids, and annonaceous acetogenins (Abdallah *et al.,* 2024; Mesquita *et al.,* 2023). Flavonoids and phenolic compounds are well-established antioxidants that can chelate metal ions and reduce oxidative

stress, a primary mechanism of heavy metal toxicity (Moghadamtousi *et al*., 2015). The antioxidant capacity of soursop leaves has been documented at 85.67%, significantly higher than many conventional antioxidant sources.

The 500mg/kg EEAM dose generally demonstrated superior protective effects compared to the lower dose in spleen and brain tissues. In the spleen, this dose reduced cadmium by 66% and lead by 75%. Brain tissue showed the most impressive protection with 80% reduction in cadmium and 67% reduction in lead, suggesting particularly effective blood-brain barrier protection at this dose.However, an unexpected finding emerged in bone tissue, where the 500mg/kg dose showed elevated metal levels compared to the toxic group (Cd increased by 6% and Pb by 25% relative to the toxic exposure group). This counterintuitive result suggests possible metal redistribution or mobilization from bone storage sites.

The higher concentration of chelating compounds in the 500mg/kg dose may mobilize metals from bone reservoirs, potentially increasing circulating levels before excretion. This is consistent with the mechanism of action of synthetic chelating agents like EDTA, which mobilize lead from bone stores (Flora *et al*., 2012).

The phytochemicals may facilitate metal redistribution from soft tissues to bone as a detoxification strategy, representing an intermediate stage in the elimination process. This would be a protective mechanism, as bone sequestration reduces metal availability to metabolically active tissues.The extract may stimulate bone remodeling processes, temporarily increasing metal release from mineralized matrix before ultimate elimination through renal excretion. The absence of this effect at the lower dose suggests that metal mobilization may be dosedependent and require a threshold concentration of active compounds. Further investigation with longer study periods and serial measurements would clarify whether this represents transient mobilization followed by elimination or sustained elevation.

Brain tissue responded most favorably to all interventions, with the highest percentage reductions in metal accumulation. This is clinically significant given the particular vulnerability of neural tissue to heavy metal toxicity and the serious neurological consequences of lead and cadmium exposure (Broskabady *et al.,* 2018).

The 500mg/kg EEAM dose generally outperformed the 250mg/kg dose in spleen and brain protection, suggesting a dose-dependent mechanism. However, the bone tissue findings indicate that higher doses may have complex, tissue-specific effects that require careful consideration in clinical translation.

Both EEAM doses showed slightly different efficacy profiles for cadmium versus lead, reflecting the distinct chemical properties and biological behaviors of these metals. Generally, cadmium reduction was more pronounced than lead reduction, possibly due to the higher affinity of *A. muricata* phytochemicals for cadmium ions.

The EEAM extracts showed comparable or superior protective effects to zinc and selenium in most tissues, supporting the potential of *A. muricata* phytowaste as an alternative or complementary therapeutic agent.

The demonstrated efficacy of *A. muricata* phytowaste extract suggests potential applications in: Treatment of occupational heavy metal exposure in mining, battery manufacturing, and other high-risk industries, management of environmental heavy metal contamination in affected populations, adjunct therapy in cases of chronic low-level exposure, preventive intervention for at-risk populations

The use of plant-based therapies offers several advantages over synthetic chelating agents, including lower toxicity, better tolerance, wider availability in resource-limited settings, and potential for long-term prophylactic use.

The utilization of *A. muricata* phytowaste aligns with circular economy principles and sustainable development goals. The fruit processing industry generates substantial waste in the form of peels, seeds, and residual pulp. Converting this waste into therapeutic agents addresses multiple challenges; Reduces environmental pollution from organic waste, creates economic value from previously discarded materials, provides affordable therapeutic options using locally available resources, promotes sustainable agricultural practices and industrial ecology.

Approximately 2.2 million people in Africa die annually from environmental risk factors according to WHO (2016), with heavy metal contamination being a significant contributor. The development of accessible, plant-based interventions using agricultural waste represents an innovative approach to addressing this public health challenge.

5.1 CONCLUSION

This study successfully demonstrated that *Annona muricata* phytowaste extract provides significant reduction against lead and cadmium bioaccumulation in Wistar rats. The findings establish a foundation for future clinical research and potential development of plant-based therapies for heavy metal poisoning.

5.2 RECOMMENDATIONS

Conduct longitudinal studies spanning 6-12 months to fully characterize metal mobilization patterns and long-term protective effects, particularly regarding bone metal dynamics.

Expand tissue assessment to include liver, kidneys, heart, testes, blood, and urine to provide complete bioaccumulation and elimination profiles.

Extend research to female rats and assess effects during pregnancy and lactation, given the particular vulnerability of developing organisms to heavy metal toxicity.

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