POTENTIAL OF *Cocos nucifera* L. WATER IN ALLEVIATING CADMIUM INDUCED ANTIFERTILITY IN MALE WISTAR RATS

BY

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DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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AN UNDERGRADUATE PROJECT 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.

SEPTEMBER, 2023.

CERTIFICATION

This is to certify that this research titled "**Potential of *Cocos nucifera* L. water in alleviating cadmium induced antifertility in male wistar rats**" was carried out by **"Excellence Naomi Ohihon-Joseph"** 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 Bachelor of Science degree in Environmental Management and Toxicology.

___________________________ ___________________________

DR. B.J. ENAGBONMA **Date**

Project Supervisor

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Prof. A.A. ENUNEKU **Date**

Head of Department

DECLARATION

I "**EXCELLENCE NAOMI OHIHON-JOSEPH**" declare that "**Potential of *Cocos nucifera* L. water in alleviating cadmium induced antifertility in male wistar rats**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

EXCELLENCE NAOMI OHIHON-JOSEPH (Miss)

…………………………

Date

DEDICATION

This project is dedicated to God almighty for His Grace, Strength, Mercies, Wisdom, and Knowledge during this period of studying this course and being able to complete my studies and also carrying out this research.

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ABSTRACT

Cadmium (Cd) a toxic non-essential transition metal that poses a health risk for both humans and animals. With many reviews recommending the use of plant extracts in abating heavy metal toxicity due to its rich medicinal properties. This study aimed to investigate the protective effects of *Cocos nucifera L.* water in abating cadmium-induced toxicity in male wistar rats. Twenty (20) sexually matured male wistar rats were randomly distributed into four groups (n=5). Group A received with 2ml of cadmium chloride, Group B received 2ml of cadmium chloride and 4ml of *Cocos nucifera* L. water, Group C received 2ml of cadmium chloride and 6ml of *Cocos nucifera* L. water and Group D received *Cocos nucifera L.* water for 7 days. Thereafter, rats were sacrificed to obtain the blood and the testis were used for testosterone and histopathological analysis. Result showed that the cadmium chloride significantly decreases ($p<0.05$) body weight and testosterone level in group A however, the coadministration of *Cocos nucifera L.* water with cadmium chloride significantly increases ($p>0.05$) testosterone level both in Group B and C. Histopathological analysis showed that cadmium chloride caused mild intestinal edema in both in Group A when compared with Group D but no significant changes occurred when compared with cotreated groups (Group B and C). From this investigation, *Cocos nucifera* water showed abating potential in cadmium toxicity due to its polyphenol content and antioxidant properties, however, more studies in recommended.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Reproduction is a crucial biological property and a vital part of all living things. It is crucial for species survival and the continuation of their evolutionary history (Inhorn, 2015). As a result, differentiation of gametes and their generation is a crucial component of successful reproduction (Kumar, 2019). Heavy metal pollutants like cadmium, which cause hormonal changes, spermatogenic modification, and endocrine disruptor chemicals that ultimately cause infertility, have a significant negative impact on reproductive success, particularly in industrialized nations (Bhardwaj, 2021).

A poisonous and cancer-causing contaminant that is widely present in the environment is cadmium (Cd). Through smoking, drinking water that has previously been tainted with cadmium, etc., cadmium enters the human body. The kidney has been identified as the most sensitive target of chronic cadmium exposure due to the link between cadmium buildup in the kidney and cadmium excretion via urine. Cadmium also has an impact on the brain, neurological system, and respiratory system. As a biomarker of exposure to cadmium (Cd), urinary cadmium (UCd) has been employed.

The International Agency for Research on Cancer (IARC) has categorized cadmium (Cd) as a Group I human carcinogen (IARC, 2018). Cadmium (Cd), a heavy metal example, has recently been substantial research due to its toxicological effects on the human reproductive system (Luo, 2020). The hypothalamus-pituitary-testis (HPT) axis, the seminiferous tubules, the blood-testis barrier, and the testicular endothelium are all affected by cadmium, which reduces the quantity and quality of spermatozoa

(SPZ), which prevents humans and animals from reproducing (Zhu, 2020). All of these take place at the cellular and molecular level, increasing oxidative stress, enhancing tissue necrosis and apoptosis, and switching between steroidogenesis and spermatogenesis (Wright, 2020). The reduction or low generation of normal SPZ morphology and motility was the result of all of these factors working together, which ultimately caused a reproductive issue (Cho, 2019).

Recent research has urged the use of dietary supplements to shield cells and tissues from Cd toxicity (Zhai et al. 2019). This call has increased interest in medicinal plants' therapeutic potential for preventing tissue damage brought on by free radicals (Roy et al. 2019). Recent studies have highlighted several types of medicinal plants, including *Monodora myristica*, *Allium sativum*, and *Anthocleista djalonensis*, for their ability to detoxify or neutralize toxins and shield cells and tissues from the damaging effects of cadmium. But because of this, scientific research has supported the use of Cocos nucifera water for medical purposes (Ogunrinola *et al.* 2019). In light of this, the water from *Cocos nucifera L.* water has been chosen as a potential ameliorating agent in the current study on cadmium toxicity utilizing a rat model.

The green coconut (*C. nucifera L.* water) includes more bioactive enzymes like tannins in its water than other varieties of coconut. Green *Cocos nucifera* L. water is frequently thought to be able to break down and remove toxins from the body because tannins are anti-toxic compounds (Candra, 2016). According to local knowledge, green *Cocos nucifera* L. water is one of the traditional treatments that can remove toxins from the body.

1.2 RATIONALE OF THE STUDY

Due to human activities including mining, industrial operations, and agricultural practices, cadmium (Cd), a highly hazardous metal, is present in large quantities in the environment. Due to the serious health risks associated with cadmium pollution, several researchers have called for the use of medicinal herbs to reduce cadmium toxicity in cells and tissues. It has been noted that *Cocos nucifera L*. water is a strong source of antioxidants, but there is no information on their capacity to reduce cadmium toxicity. For this reason, it was chosen as the protocol in this study to determine its capability to reduce cadmium toxicity in wistar albino rats.

1.3 AIM AND OBJECTIVE OF THE STUDY

The aim of this study is to investigate the potential of *Cocos nucifera L* water in abating cadmium-induced toxicity in male wistar rats. The objectives of the study were to:

1. To access the impact of cadmium exposure on the testis
2. To examine the histopathological changes due to cadmium toxicity in albino rats
3. To investigate the potential effects of *Cocos nucifera L.* water against cadmium-induced testicular toxicity

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION TO HEAVY METAL POLLUTION AND ITS EFFECT BOTH IN THE ENVIRONMENT AND MAN

The environment is the setting in which people, animals, plants, and microorganisms reside or operate. It is made up of the Earth's surface, its atmosphere, and its ocean. The four spheres—the biosphere (living things), the atmosphere (air), the lithosphere (land), and the hydrosphere (water)—that make up the Earth's system all coexist harmoniously with one another. Chemicals that are more prevalent in one area of the environment than another are known as environmental contaminants. (Masindi *et al.,* 2019). During the last hundred years, industrialization has grown at a fast rate. It has thus increased the demand for exploitation of the Earth's natural resources at a careless rate, which has exacerbated the world's problem of environmental pollution (Gautam, 2019). The environment has been seriously polluted by several pollutants such as inorganic ions, organic pollutants, organometallic compounds, radioactive isotopes, gaseous pollutants and nanoparticles (Walker, 2020). The roles and chemical characteristics of the group of elements known as heavy metals are diverse. In the Periodic Table, heavy metals primarily fall under the transition element category. According to Leonard (2019), heavy metals are substances that have a particular mass of over 5 g cm^3. According to ASTDR's 2019 definition of heavy metals, these metals must be at least five times as dense as water. Mo, Mn, Cu, Ni, Fe, and Zn are essential heavy metals, whereas Cd, Ni, As, Hg, and Pb are non-essential heavy metals. Essential metals support human metabolism, such as copper (Cu), which is required for the production of hemoglobin and the digestion of carbohydrates but damages cells when present in excess (Bharathi, 2019). Similar to humans, plants require a variety of heavy metals because they operate as cofactors, catalyze enzyme reactions, exhibit ductility and conductivity, and provide cation stability (Stohs, 2021). These metals exhibit toxicity when present in greater amounts than necessary. Essential heavy metal deficiency has an impact on both agricultural productivity and human health. Even in small amounts, non-essential metals are hazardous. They did not degrade in the environment or undergo metabolization into other intermediate molecules. Heavy metals are released into the ecosystem as a result of human activity such as industrial, residential, agricultural, medical, technical, or natural occurrences such volcanic eruptions and rock weathering. Hazardous, non-biodegradable, and persistent in the environment are heavy metals. They have a detrimental influence on people, plants, and animals, which causes them to become contaminated and turn into the biggest problem. Industry, atmosphere, soil, water, food, and human beings all contribute to the cyclical chain of heavy metal contamination in the ecosystem (Holmes, 2021). Heavy metals can enter the body of a human in a number of ways, including through contaminated food, water, skin, or breathing. These metals are usually water soluble and are absorbed through the colon before being circulated to other organs. However, at low concentrations, heavy metals affect the respiratory tract and many cells like endothelial, epithelial, etc (EPA, 2020). Heavy metals pollutants also restrict the growth of plants. Metals present in higher concentration in the soil, affecting the plant system's seed germination, plant growth, production, physiological, biochemical, and genetic elements (Guidotti, 2019). Toxicity in plants varies with the concentration of soil composition, pH and with plant specifics.

2.1.1 Sources of Heavy Metals

These heavy metals are found naturally on the Earth's crust since the Earth's formation. Due to the astounding increase of the use of heavy metals, it has resulted in an imminent surge of metallic substances in both the terrestrial environment and the aquatic environment (Gautam, 2019). Heavy metal pollution has emerged due to anthropogenic activities which is the prime cause of pollution, primarily due to mining the metal, smelting, foundries, and other industries that are metal-based, leaching of metals from different sources such as landfills, waste dumps, excretion, livestock and chicken manure, runoffs, automobiles and roadworks. Heavy metal use in the agricultural field has been the secondary source of heavy metal pollution, such as the use of pesticides, insecticides, fertilizers, and more. Natural causes can also increase heavy metal pollution such as volcanic activity, metal corrosion, metal evaporation from soil and water and sediment re-suspension, soil erosion, geological weathering (Shallari and Herawati, 2021).

2.1.2 Properties of Heavy Metals

Given that they frequently form covalent bonds, metalloids have toxicological characteristics. They are able to bond covalently with organic groups, which has two significant effects. As a result, they can produce harmful effects when they attach to nonmetallic components of cellular macromolecules and form lipophilic ions and compounds. The distribution of metalloids in the biosphere and their hazardous response differs from those of the same element's simple ionic forms as a result of being lipophilic. Tributyltin oxide and hazardous methylated versions of arsenic are examples of lyophilic chemicals. The sulphydryl groups of the protein can bind lead and mercury as examples of nonmetallic elements. Ingestion of tainted food, airborne inhalation, drinking tainted water, and skin contact from industrial, residential, pharmaceutical and agricultural regions are the four main ways that heavy metals can enter a human (Masindi, 2019; Walker, 2020).

Metals are not biodegradable and cannot be broken down. By enclosing the active component in a protein or storing them in intracellular granules in an insoluble form to be expelled in the organism's feces or for long-term storage, organisms can detoxify metal ions. The heavy metals bioaccumulate in our systems after being ingested or absorbed into them. As a result, they are considered dangerous. Biological and physiological issues are brought on by this bioaccumulation. Some heavy metals are referred to as essential elements because they are needed for a number of biochemical and physiological processes. However, when present in excessive quantities, they can be harmful (Duffus, 2019). They have been extensively employed in agriculture, industry, medicine, and other industries, with the result that they have gotten into our soils, rivers, and atmosphere (Wang, 2020).

The three categories of essential elements are the principal elements required by the body, macro-minerals, and trace elements. The fundamental components of the majority of biological matter consist of four crucial main elements. In order of atomic number, these are hydrogen, carbon, nitrogen, and oxygen. The ionic balance of structural components, amino acids, and nucleic acids is maintained by the macro-minerals, a group of seven additional important elements. According to their atomic number, these are sodium, magnesium, phosphorus, sulfur, chlorine, potassium, and calcium. According to their atomic number, the thirteen elements that make up the last group, known as trace elements, are silicon, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, and iodine. The maintenance of skeletal structure creation, acid-base equilibrium regulation, and colloidal system maintenance all depend on the necessary elements. As components of critical enzymes, structural proteins, and hormones, they are also crucial. For example, selenium is necessary for the glutathione peroxidase enzyme, iron is required for hemoglobin, and zinc is a component of several enzymes (Villanueva, 2019). Although non-essential metals play no significant roles in the body, they can nonetheless be harmful because they can change the body's levels of essential elements (Walker, 2020).

2.1.3 Effect of Cadmium on the Environment and Man

The earth's crust contains naturally occurring heavy metals that are neither destroyed nor degraded. Life requires both organic and inorganic components, hence it cannot exist without metal ions. Numerous metals, including Fe, Co, Cu, Se, and Zn, are necessary metals. They support plant development and metabolism and are found at low amounts. However, hazardous consequences result when metals are present in amounts that are higher than what plants need. The soils are contaminated with heavy metals from both natural and man-made sources. Heavy metals are absorbed by plant roots from the soil and then transferred to other plant sections, where they have a variety of negative effects on the plants.

A non-essential element called cadmium has an impact on how plants grow and develop. Since cadmium is readily soluble in water and causes harm even at low concentrations, it is regarded as a highly significant contaminant. According to Wagner's calculations, the Cd concentration in unpolluted soil ranges from 0/04 to 0/32 mM, while the Cd concentration in polluted soil ranges from 0/32 to around 1 mM (Garbisu, 2019). The three primary signs of Cd toxicity are stunting, chlorosis, and leaf rolling. Cd increases the generation of oxygen free radicals and decreases enzymatic and non-enzymatic antioxidants. Because heavy metals are more concentrated in plant roots, phytotoxic symptoms such leaf redness and brownish discolouration, browning of the roots, and leaf chlorosis are more frequently seen there. Cd has an impact on plant growth by raising the dry to fresh mass ratio (DM/FM) across the board. High Cd accumulations in plants cause a variety of problems that hinder their growth and development, including stomatal closure, reduced nutrient intake, decreased enzyme activity, and problems with photosynthesis and respiration. Reactive oxygen species (ROS) are produced in plant cells as a normal byproduct of respiration and photosynthesis, but at greater quantities, ROS is exceedingly damaging. High concentrations of it oxidize a variety of proteins, including lipids and ascorbate peroxidase and catalase, which results in mutagenesis and changes to cell structure. However, unlike other heavy metals, cadmium does not appear to have a direct impact on ROS formation (Ishibashi, 2019). The protein phosphoenol pyruvate carboxylase is crucial for the plant's C4 and CAM cycles, however Cd poisoning prevents the protein's synthesis from taking place (James, 2019). Leaf senescence, a drop in carotenoid content, and occasionally even mortality were caused by cadmium. Heavy metals impair photosynthetic enzyme functions and obstruct the photosynthetic electron transport chain, which results in a decrease in the amount of chlorophyll in plants.

2.1.4 Effect of Cadmium on Man

The ecosystem naturally contains heavy metals. When present in small amounts, metals are beneficial to humans. As a result, they are referred to as vital metals. For example, in humans, Fe aids in the development of hemoglobin, Cu aids in oxygen and electron transport, Co aids in cell metabolism, Mn governs enzyme regulation, Se aids in the manufacture of hormones and antioxidants, and Ni aids in cell growth. However, when heavy metal concentrations are higher, they have hazardous effects on people. However, humans are more at risk when heavy metal concentrations are higher. Living close to a site where these metals are improperly disposed of, drinking water, and eating foods contaminated by heavy metals are just a few of the ways that heavy metals enter the body of a person and have negative effects on them. Heavy metals are also absorbed through breathing.

An extremely dangerous heavy metal is cadmium. The US National Toxicology Program and the International Agency for Research on Cancer have classified cd as a human carcinogen (Congeevaram, 2019). Cd prevents the creation of proteins, RNA, and DNA, and it changes healthy epithelial cells into cancerous ones, making it a carcinogen. Long-term exposure to Cd in people damages the kidneys and causes lung cancer. In addition to causing functional and morphological alterations in numerous organs, interactions between Cd and critical elements including An, Fe, Ca, Mg, and Se also disrupt secondary metabolism. In severe situations, Cd can result in death since it causes pneumonia, overall weakness, fever, and chest pain. Women suffer more severely than males by the deposition of high concentrations of Cd in urine, blood, and kidney cortex because dietary Cd absorption in women's intestines is higher (Sandana, 2020). According to Cervantes (2019), Cd causes the Itai-Itai illness, which primarily affects women, impairs tubular and glomerular processes, and results in repeated fractures of the bones owing to osteoporosis and osteomalacia (where calcium is released from the bones). When Cd combines with hydrochloric acid in the stomach, it produces chlorides that cause immediate intestinal inflammation (Kao, 2020). Because Cd primarily targets T cells (cytotoxic K (killer) cells), NK (natural killer cells), macrophages, and B cells, it results in immune system dysfunction (Mishra, 2019). According to Rodriguez (2019), when Cd attaches to Metallothionein (a protein that controls zinc homeostasis and free radical scavenger activity), hypertension and an increase in the production of reactive oxygen species (ROS) result, which is inappropriate for the cardiovascular system. Respiratory Distress Syndrome results from cadmium inhalation. By reducing the kidney's capacity to reabsorb vitamins, minerals, and nutrients, Cd leads to renal irregularity, which also causes diabetes and hypertension. High levels of Cd in the air can lead to anosmia, respiratory stress, and persistent rhinitis. By interfering with testicular and prostate function, changing hormonal activity, and increasing male fertility, cd has a negative impact on reproductive function (Choo, 2019).

2.2 CADMIUM TOXICIY AND CHEMICAL METHODS IN REMEDIATING ITS EFFECT

According to (Shanmugaraj *et al*., 2019), cadmium is a contaminant that has been released into the environment as a result of the modern economy's rapid industrialization. It is greatly absorbed by contaminated water, food, and air. Crustaceans, bivalve mollusks, oysters, cephalopods, and crabs all have high levels of Cd, as do oil seeds, cocoa beans, some wild mushrooms, and offal products like liver and kidney (Sandana, 2020). Depending on the degree of soil contamination, foods made from plants typically have higher quantities of Cd than meat, eggs, milk, and dairy products. In comparison to other plant-based foods, rice and wheat, green leafy vegetables, potatoes, carrots, and celery can all have higher metal content beyond permitted limits, i.e., 3–30 ppm for plants and 8 ppm for agricultural soils (Ismael *et al.,* 2019).

2.2.1 Cadmium's Effects on Mitochondria and Cd^{2+} Induced Apoptosis

Through oxidative phosphorylation (OXPHOS), which produces the energy required for life—adenosine triphosphate (ATP)—the mitochondrial respiratory chain plays a crucial role in preserving energy balance. Furthermore, amino acid, lipid, and phospholipid production, ion balance, motility, and apoptosis (programmed death) are all dependent on mitochondria. Animal tissues and cells have different numbers and types of mitochondria depending on their energy requirements as well as in reaction to modifications in their physiological or environmental conditions. Ageing and a variety of illnesses, including cancer, are linked to dysfunctional mitochondria (Modica-Napolitano, 2021). The outer and inner membranes, the intermembrane gap, and the matrix make up the four sub-compartments that make up a mitochondria. Additionally, post-translational changes including phosphorylation, ubiquitination, and sumoylation affect the mitochondrial morphological machinery (Soubannier, 2020). The genome of mitochondria is a tiny double-stranded circular DNA molecule (mtDNA), which is found within each cell.

Thirty-seven genes (16,569 base pairs) are found on the human mitochondrial chromosome, including 13 that code for respiratory chain proteins. The remaining 24 genes encode for rRNA and tRNA, which are necessary for the production of proteins by DNA-binding proteins that are distinct from nuclear histones (Bogenhagen, 2020). Mammalian mtDNA is more susceptible to oxidative damage and is exposed to a greater mutation rate than nuclear DNA because DNA repair activities are limited, and ROS generation is significant (Venkatesh, 2019).

With inhibiting respiratory chain enzymes (Wang, 2022) and causing mitochondria to swell and collapse, cadmium may change the activity of many mitochondrial proteins (enzymes and transporter systems across the outer and inner membranes). Cadmium may directly raise permeability and lower mitochondrial membrane potential, which results in the release of cytochrome C when the caspase pathway is activated (Figure 1). Cadmium also increases the levels of ROS and lipid peroxidation by inhibiting the activities of ATPase, lactate dehydrogenase (LDH), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Cannino, 2019). Fenton reactions seem to involve cadmium. Although it doesn't serve as a catalyst for the Fenton reaction, it can still generate ROS by indirectly removing an endogenous Fenton metal (like Fe2+) from proteins and therefore raising the concentration of free redox-active metals. By interacting with exogenous and endogenous antioxidants like glutathione GSH, cadmium may change the cellular redox status (Cuypres, 2019). Cadmium may harm mitochondria and interfere with Ca2+ signaling, as well as increasing the production of ROS within the mitochondria (Biagioli and Belyaeva, 2021). In contrast to their effects on complex I (NADH dehydrogenase or NADH: ubiquinone oxidoreductase) and complex IV (cytochrome oxidase), cadmium ions operate as xenobiotics by suppressing the activity of complex II (succinate dehydrogenase) and complex III (cytochrome bc1 complex) of the electron transfer chain. The complex III appears to be the primary location of ROS production, and an accumulation of ROS alters the potential of the mitochondrial membrane and triggers a series of events, including apoptosis (a genetically encoded and programmed form of cell death) (Chatterjee, 2020).

There are two distinct apoptotic processes: the intrinsic or mitochondrial-mediated system and the extrinsic or death receptor-mediated pathway. The molecules in one of the two pathways can affect those in the other, and the two pathways are related to one another. The intrinsic pathway causes apoptosis in reaction to internal stimuli, such as DNA damage, whereas the extrinsic pathway causes apoptosis in response to external stimuli. Through increases in Ca^{2+} and ROS, the mitochondrial pathway is connected to harmful stimuli such ROS, UV radiation, ionizing radiation, Ca^{2+}, and Cd^{2+}. These stress-related stimuli cause a discharge of cytochrome C from the intermembrane space of the mitochondria into the cytosol, which in turn activates caspase-8 and results in apoptosis. A cellular stress signal, which is produced when DNA damage activates caspase-9, initiates the intrinsic route. Apoptosis can also be brought on by Ca^{2+}-calpain coupled processes or caspase independent events in addition to Cd poisoning. A significant stage in the intrinsic process of apoptosis is caused by excessive ROS generation, which causes a free radical attack on the phospholipids in the mitochondrial membrane and mitochondrial membrane depolarization (Ott and Gobe, 2021). The initiators of each route, such as the intrinsic pathway's caspase-9 and the extrinsic pathway's caspase-8, then activate the executioner's caspase-3 and caspase-7. With cysteine in the active site, caspases (cysteine-aspartic proteases) exhibit proteolytic activity and can cleave proteins after an aspartate residue.

Characteristic cytomorphological characteristics of the execution pathway include cell shrinkage, chromatin condensation, the development of cytoplasmic blebs, and the creation of apoptotic bodies. Protein breakage, protein cross-linking DNA breakdown, and phagocytic identification are further features of apoptotic cells. In addition to apoptosis, high levels of ROS can cause phospholipid free radical damage, macromolecule oxidation, mitochondrial membrane disruption, mitochondrial membrane depolarization, and mtDNA mutation (Plate 1) (Ott, 2021). The production of ROS, modification of the mitochondrial membrane potential, and activation of caspase-9 are the three main components of mitochondrial apoptosis. In vivo, cadmium causes apoptosis in a number of organs, including the kidneys and liver. It causes the mitochondrial apoptosis pathway to be activated in cultured cells, which is the result of a cellular stress response (Thévenod, 2019). It takes at least 15 to 24 hours after Cd^{2+} exposure for intact cells to experience Cd^{2+}-induced mitochondrial damage and apoptosis (Lee, 2020).

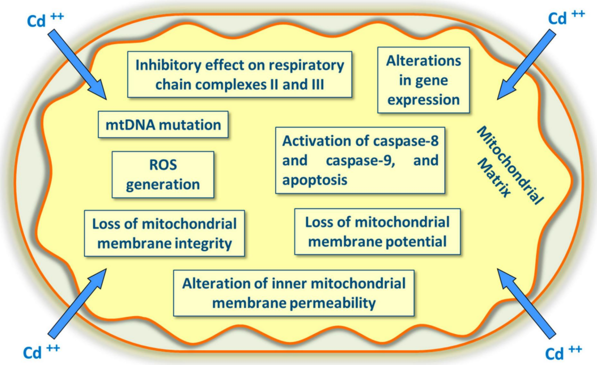

Plate 2.1: The main target in cadmium intoxication. Cadmium acts on mitochondria by inducing oxidative stress and generating reactive oxygen species (ROS), activating apoptosis, mutating mtDNA, altering gene expression, inhibiting respiratory chain complexes, reducing ATP synthesis, and altering the inner mitochondrial permeability.

2.2.2 The Epigenetic Effects of Cadmium Exposure

The term "epigenetics" describes heritable gene expression changes without a corresponding alteration in the DNA's nucleotide sequence. DNA methylation, histone post-translational modifications, small non-coding RNA molecules (microRNA, miRNA), which can obstruct gene transcription and/or translation, and DNA packaging around nucleosomes are the main mechanisms governing epigenetic regulation of gene expression. DNA methyltransferase, histone methyltransferase, histone acetyltransferase, and histone deacetylase are some of the enzymes involved in epigenetic processes. Numerous environmental factors may have an impact on these systems, and their dysregulation is linked to a number of disorders (Baccarelli and Koturbash, 2019). Growing data suggests that heavy metal toxicity may be mediated by miRNA. A variety of pathophysiological situations and signaling pathways have been directly linked to aberrant changes in the endogenous miRNA, which ultimately result in numerous cancers and diseases (Wallace, 2020).

When DNA methyltransferase and S-adenosyl methionine (SAM), which acts as a methyl group donor, are present, a methyl group is covalently joined to the cytosine to create 5-methylcytosine. DNA methylation controls biological functions such as gene transcription, chromosomal integrity, and genomic imprinting. The chromatin structure and gene expression are affected by post-translational covalent processes that happen to the N- and C-terminal tails of H3 and H4 histones. These reactions include methylation, acetylation, phosphorylation, ADP-ribosylation, ubiquitination, and sumoylation.

MiRNAs are small non-coding molecules of 20–25 nucleotides, which are involved in the post-transcriptional regulation of protein expression by degrading their target mRNA and/or inhibiting their translation, which is based on the degree of complementary base pairing. MiRNAs are transcribed from DNA but not translated in proteins. MiRNA's main function is to down-regulate gene expression by interfering with messenger RNA (mRNA) functions (Pillai and Huang, 2019).

Epigenetic changes can be triggered by environmental factors, such as the type of nutrition and exposure to xenobiotics, due to the dynamic state of the epigenome (Szyf, 2020). Cadmium exposure may alter gene expression profiles and change epigenetic components in three features: DNA methylation, histone post-translational modifications, and miRNAs. DNA methylation levels seem to be associated with the time of exposure to cadmium. In fact, Cd exposure for a short time (24 h–1 week) induces hypomethylation, while longer times (8–10 weeks) induce hypermethylation (Takiguchi, 2021). In vitro exposure to cadmium of TRL1215 rat liver cells for only one-week inhibited DNA methyltransferase activity (up to 40%), ending in DNA hypomethylation, while longer exposures to Cd (10 weeks) of the same cells resulted in DNA hypermethylation because of an increase in the activity of DNA methyltransferase. (Benbrahim-Tallaa *et al.,* 2019) indicated that Cd-induced DNA hypermethylation occurred in association with the malignant transformation of human prostate epithelial cells.

MiRNAs are initially produced as 70-nucleotide precursors, which are then broken down into miRNA (20–25 nucleotide products) by the enzyme Dicer endoribonuclease. Lin-4 was the first miRNA to be found in the nematode Caenorhabditis elegans (Lee, 2019). Several miRNAs have so far been identified in all types of species as epigenetic regulators, including vertebrates, plants, flies, worms, and viruses (Cullen, 2019). It is not unexpected that miRNAs control a wide range of biological functions, including metabolism, cell division, proliferation, differentiation, and death. Additionally, miRNA expression has been linked to the development of cancer, with certain miRNA acting as tumor suppressors and others as oncogenes.

Given that cadmium telluride (CdTe) quantum dots have the capacity to alter gene expression after the initial signal has been blocked, the epigenetic elements of their toxicity are receiving increased attention. Furthermore, the fact that epigenetic pathways play a role in miRNA production raises the possibility that miRNAs contribute to the cytotoxicity of CdTe quantum dots. According to SOLiD (sequencing by oligonucleotide ligation and detection) sequence data, CdTe quantum dot exposure significantly alters the expression patterns of miRNAs, leading to cell death that resembles apoptosis. Additionally, after subjecting NIH/3T3 cells to exposure to CdTe quantum dots, Fe2O3 nanoparticles, and multi-walled carbon nanoparticles (MW-CNTs), the scientists acquired the miRNA expression profiling using the SOLiD sequence approach (Li, 2021).

2.2.3 Chemical Method in Remediating Cadmium Toxicity

Reagents and chemicals are used in this remediation method for heavy metals. It mostly uses the following techniques:

- **Soil washing:** In this method, chemicals and reagents are used to leach the heavy metals out of the soil. To immobilize hazardous components in a less accessible form, chelators like EDTA are used. This method involves excavating the contaminated area of the soil and treating it with the proper extractants (Peng, 2021). The soil and the heavy metal to be extracted from determine the best extractant to use. The extractant and soil are then thoroughly combined. Additionally, the soil is moved from the solid phase to the liquid phase by processes like precipitation, absorption, or chelation, among others (Zhu, 2021). Due to its ability to remove the poisons quickly and fully, soil washing is a commonly utilized heavy metal cleanup technique. There are a ton of chemicals and reagents utilized, including cyclodextrins, organic acids, EDTA, and surfactants. For the extraction of heavy metals, EDTA and coal ash have grown to be common materials. For paddy fields with cadmium contamination, the soil wash technique was created (Wang, 2020).
- **Immobilization techniques:** By utilizing immobilizing agents, heavy metals are contained in soil. For the immobilization process, a variety of techniques have been used, including adsorption, precipitation, and complexation. Heavy metals in contaminated soil can be immobilized using both organic and inorganic substances.

Clay, zeolites, minerals, cement, etc. are the primary components. To lessen the availability of heavy metals in soil, they might be immobilized on solid particles. Biosolids and animal manures are the two most commonly employed organic agents and additives. The application of biosolids to the soil was reported to have negative consequences (Xia, 2019). It is the best adsorbent for stabilizing heavy metals in soil, hence there are also benefits (Zhu, 2021).

2.3 *Cocos nucifera L.* Water

The plant known as the coconut (*Cocos nucifera L.* water) is a common sight in tropical and subtropical areas. Coconut is a monocotyledon plant and a member of the Arecaceae (Palmae) family. Malaysia, the Philippines, and Indonesia are the top three producers (Halim *et al.*, 2019). Coconuts can reach a height of 30 m (98 ft) and have pinnate leaves that are 4-6 m (13–20 ft) long (Aniekpeno *et al*., 2019). In order to use coconut in food processing, it is often processed into coconut milk or coconut oil. Almost every component of a coconut, notably the fruit, can be used in daily life. Ripening of coconuts typically takes 11 to 12 months. Based on the chemical makeup of the water, the fruit during this time can be classified as immature or young (6–8 months), mature (9–11 months), and overripe or mature (12 months or more). Each fruit's *Cocos nucifera* L. water volume and composition were determined to be at their peak between 6 and 9 months of age (Burns *et al*., 2020).

The majority of *Cocos nucifera* L. water's nutritional contents include the following:

- **Vitamins:** Young *Cocos nucifera* L. water includes trace levels of the B vitamins thiamine (B1) and pyridoxine (B6) as well as the B vitamins nicotinic acid (B3) (0.64 g/mL), pantothenic acid (B5) (0.52 g/mL), biotin (0.02 g/mL), riboflavin (B2) (0.01 g/mL), and folic acid (0.003 g/mL). Additionally, young *Cocos nucifera* L. water has vitamin C (15 mg/100 mL), which can stop the production of lipid peroxidase and halt the oxidation of blood cells. Additionally, vitamin C boosts the body's ability to absorb iron and serves as a cell protector against free radical damage (Zulaikhah *et al.,* 2019).

- **Amino Acid:** According to (Rukmini *et al.*, 2017), young *Cocos nucifera* L. water contains full of nutrients, including vital amino acids like lysine, leucine, cysteine, phenylalanine, tyrosine, histidine, and tryptophan. Amino acids, according to (Joseph *et al.*, 2019), not only aid in the synthesis of energy but also aid in the production of lymphocytes, or white blood cells, which enhance immunological function. Nutritional digestion and absorption can be enhanced by lymphocytes. L-arginine is a particular kind of amino acid that can be utilized to lessen the symptoms of heavy metal poisoning and is crucial to the body's antioxidant system, according to (Zulaikhah, 2019).
- **Minerals:** Young *Cocos nucifera* L. water contains both organic and inorganic ions, which are important to the body's antioxidant system because they normalize cellular activity, boost antioxidant activity, increase bone formation, increase hemoglobin, regulate gene expression, and metabolize amino acids, fats, and carbohydrates (Zulaikhah, 2019). Young *Cocos nucifera* L. water is a natural hydrating beverage that can be used in place of manufactured isotonic drinks due to its high potassium content (Halim *et al*., 2018). Young *Cocos nucifera* L. water's electrolyte concentration causes an osmotic pressure comparable to that in blood but has no impact on plasma coagulation. Young *Cocos nucifera* L. water's high potassium content has been shown to lower blood pressure (DebMandal and Mandal, 2019). Young *Cocos nucifera* L. water is a vital source of calcium and can balance calcium levels in the body, preventing nutrient rickets, according to (Joseph *et al*., 2019). Young *Cocos nucifera* L. water can be administered intravenously in an emergency

because it includes electrolytes that keep the body's osmotic pressure stable (Zulaikhah, 2019). Young *Cocos nucifera* L. water plays a significant part in the treatment of diarrhea as a result of its special composition, which hydrates users and guards the digestive system against various illnesses (Prado *et al.*, 2020).

- **Sugar:** In emergency surgical procedures, blood plasma can be substituted with young *Cocos nucifera* L. water as a supply of sugar (Aniekpeno *et al*., 2019). Young *Cocos nucifera* L. water is beneficial for diabetics since it has a slightly lower glucose content than fasting blood sugar levels (blood sugar levels measured after going without food for eight hours Joseph *et al.*, (2019). Sugar, which is the major source of energy for humans, is crucial as an ergogenic aid in addition to adding to the sweetness of young *Cocos nucifera* L. water. When people engage in an intense exercise, they require sugar for rapid glycogen recovery and energy replenishment (Halim *et al.,* 2018). Mannitol or one of the sugar alcohols found in young *Cocos nucifera* L. water can stop oral bacterial metabolism.

2.4 WISTAR ALBINO RATS

An outbred albino rat is called a Wistar rat. At a time when laboratories mainly used the house mouse (Mus musculus), this breed was created at the Wistar Institute in 1906 for use in biological and medical research. It is noteworthy that this breed was the first rat created to serve as a model organism. The initial colony started by physiologist Henry Herbert Donaldson, scientific manager Milton J. Greenman, and geneticist/embryologist Helen Dean King is the source of more than half of all laboratory rat strains (Clause, 2019).

Currently, one of the most often utilized rats in lab research is the Wistar rat. It is distinguished by its broad head, lengthy ears, and a tail that is always shorter than its body. The WISTAR rat represents an outbred strain that is employed in all biological and medicinal research disciplines. It is the perfect candidate for ageing studies because to its lifespan and high rate of spontaneous tumors. Albino and hooded rats have been primarily utilized in research labs since the middle of the 19th century. Particularly, albino rats have been utilized in laboratories on a larger scale. As a result, "laboratory rat" became associated with the albino rat. Although the term "albino rat" is frequently used nowadays, the rat has also been known by the names white rat, Daikoku rat, and ratte. The Wistar rat is a versatile model that can be utilized in oncology, aging, toxicology, and safety and efficacy research.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental animals

Twenty (20) male wistar albino rats (*Rattus norvegicus*), with mean body weight of 135g were purchased from the breeding facility of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Benin City. They were housed in a plastic cage with a wire mesh cover and fed regularly with rat pellets obtained from the University of Benin Teaching Hospital (UBTH) Shopping Complex, Edo State, and water for a period of 7 days (acclimatization).

3.2 Procurement of Cadmium Chloride

The source of the cadmium chloride ($CdCl_2$) was Pyrex Chemicals in Benin City, Edo State. Cadmium chloride was chosen because it readily dissolves in water and when in water it is clear and colourless. This will make the rats easily take it as water.

3.3 Procurement and extraction of *Cocus nucifera L.* water

Cocos nucifera L. water was purchased from the New Benin Market, Benin City. *Cocos nucifera L.* water was then carefully extracted at the point of administration through the eyes of the *Cocos nucifera L.* water.

3.4 Experimental Design

Twenty (20) healthy wistar male rats (*Rattus norvegicus*), with mean body weight of 135g, were obtained and weighed using an Ohaus Scout weighing balance (model Sp-602). The rats were then divided into 4 groups, each having 5 rats, and given the labels Group A (cadmium alone), Group B (2ml of cadmium + 4ml of *Cocos nucifera*

L. water), Group C (2ml of cadmium + 6ml of *Cocos nucifera L.* water), and Group D (received only *Cocos nucifera L.* water). A 2ml syringe and a gavage needle were used to administer the toxicant and protocol each day for a period of 7 days. At the end of the 7 days, three rats (which serve as triplicates) were sacrificed in order to get the necessary blood sample and testis for further analysis at Quality Immunodiagnostic Laboratory and Histopathology Laboratory of University of Benin Teaching Hospital.

3.5 Collection of body weight

To monitor changes in the rats' body weight prior to administration, the weight of each rat was recorded using an Ohaus Scout weighing balance (model Sp-602) each day at a specific time.

3.6 Collection of samples for testosterone and histopathology analysis

The testicles taken from the dissected rats was placed inside a universal bottle and 10% formalin was added and delivered to the Department of Histopathology Laboratory at the University of Benin Teaching Hospital for analysis.

3.7 Procedure for analyzing the testosterone

The blood of the dissected rats was collected with a syringe and kept inside a plain bottle. The blood sample was sent to the laboratory for analysis. Then the sample was centrifugated which separate the liquid portion of the blood into (serum or plasma) from the cellular component.

3.8 Histopathology procedure

The organ (testis and penis) obtained from the dissected specimens were fixed in 10% formalin to arrest metabolic activity in the tissues, to avoid autolysis and protein precipitation thus preventing enzymatic digestion of dead tissues. The fixed tissues were passed through several changes of alcohol, 70% alcohol for 24 hours and 90% alcohol for 12 hours and through absolute alcohol to remove water from the fixed tissues and allowed complete infiltration of tissues by paraffin. The tissues were then passed through xylene for 3 hours to prevent shrinkage and tissue brittleness in paraffin. After tissue processing, tissues were embedded or blocked out using the leukhand embedded mould. The L-pieces are arranged on an aluminum base to form a rectangle. The molten paraffin was then poured into the moulds and the selected surfaces of the tissues embedded with the aid of a pair of blunt end forceps and allowed to set. The embedded tissues were separated into different blocks and then attached to wooden blocked with the aid of an electric spatula. The blocks were then trimmed using a rotary microtome and knife. At the end of each trimming, the blocks were arranged on ice trays in order to cut thin sections using the rotary microtone at a thickness of 3 micron. Section are then collected with help of a camel hair brush and then placed on the slide. Flood picked section with 20% alcohol in other to spread out fold on the sections and then floated out on a water bath with a temperature 5-10 degree centigrade below the melting point of the wax used. The sections were picked and floated on a water bath and then picked with a pre-labeled slide. The slides were dried on a hot plate at a temperature of a 5-10 degree centigrade above the melting point of wax used. These were left on the hot plate for 15 minutes. The staining methods employed in staining the sections were haematoxylin and eosin method to demonstrate general tissue structure and Masson's trichrome method for the demonstration of connective tissue fibre. Dewax in two bathes of xylene for 2 minutes each and hydrate in descending grades of alcohol, absolute: 90%, 70% for 2 minutes each and then wash in water. Stain with enrich haematoxylin for 15 minutes. Wash excess stains with tap water and differentiate in 1% acid alcohol briefly. Wash in running tap water and blue for 10 minutes then counter stain in 1% aqueous eosin for 3 minutes then section washed off in water. Dehydrate through 70% alcohol, 90% alcohol and absolute for 2 minutes each. Clear in 2 baths of xylene for 2 minutes and mount with Canada balsam devoid of air bubbles.

CHAPTER FOUR

4.0 RESULTS

4.1 Body Weight of Rats

The lowest mean body weight value of 156.71 ± 6.63 g was measured in the category of rats administered with cadmium chloride while the highest mean value of 169.30 ± 5.20 g was recorded in the group of rats administered *Cocos nucifera L.* water only. The mean body weight values for the group treated with 2ml Cd + 4ml *Cocos nucifera L* water and 2ml Cd + 6ml *Cocos nucifera L.* water were 165.43 ± 5.46 g and 159.43 ± 4.42 respectively (Figure 1).

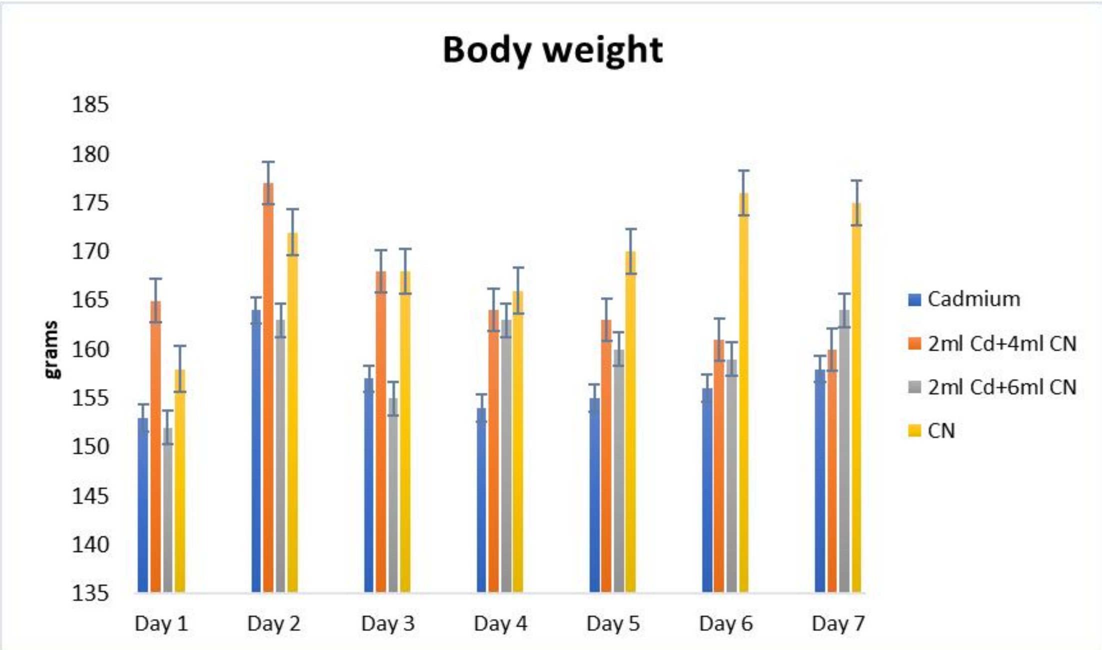

Figure 4.1: Mean ± SE body weight (g) of Wistar albino rats exposed to cadmium metal and the dietary protocol.

4.2 Testosterone

The mean testosterone value of 0.45 ± 0.05 g/l was measured in the category of rats administered with cadmium chloride only while the highest mean value of 2 ± 0.08 g/l was recorded in the group of rats administered with 2ml Cd + 6ml *Cocos nucifera* L. water. The mean testosterone value for the group treated with both 2ml Cd + 4ml *Cocos nucifera* L. water are 0.93 ± 0.61 g/l and 1.93 ± 0.45g/l respectively (Figure 2).

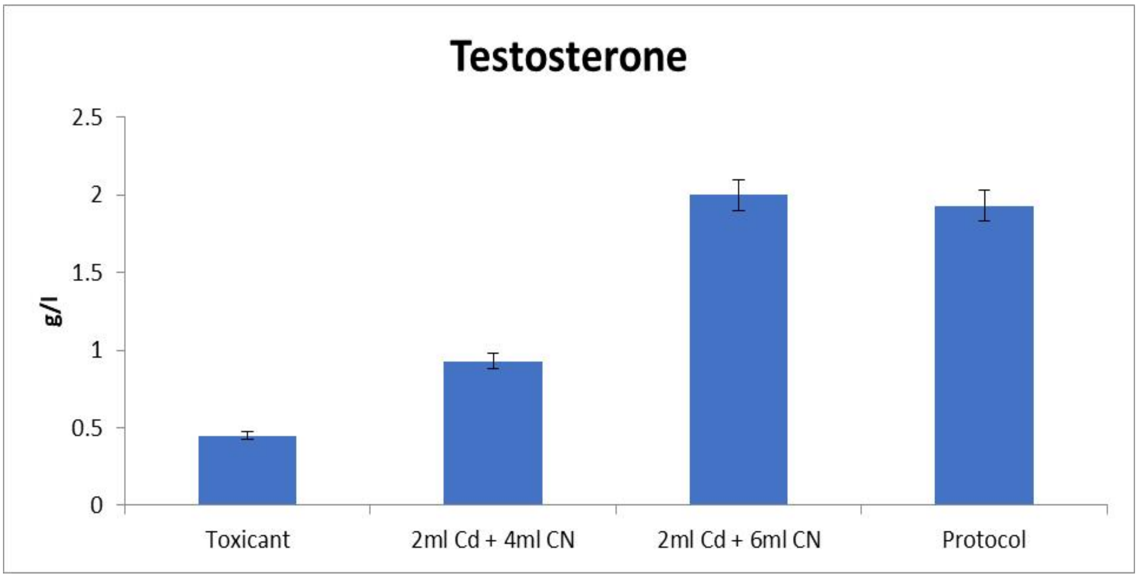

Figure 4.2: Mean ± SE values of chemical analysis in the testis and penis of albino rats exposed to lead metal and the dietary protocols.

4.3 Result of histopathology in testis of albino rats

4.3.1 Testis of albino rats in the group administered with 2ml of cadmium only

Section of the testis of albino rats exposed to cadmium chloride only showed normal seminiferous tubules (blue arrow) containing spermatids (green arrow) and mild intestial edema (black arrow) as represented in Plate 2.

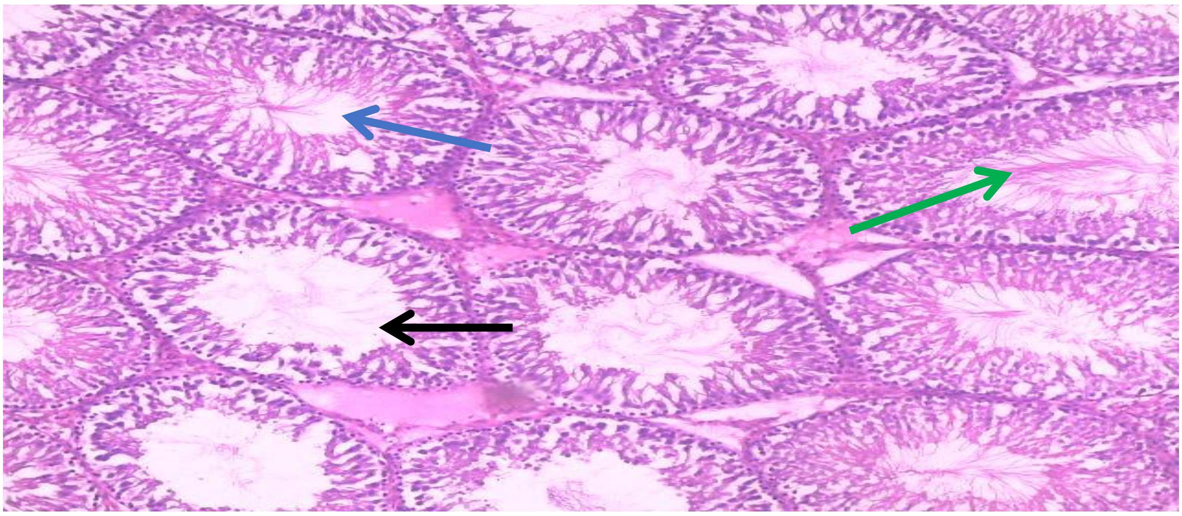

Plate 4.2: Section of the testis showing normal seminiferous tubules (blue arrow) containing spermatids (green arrow) and mild intestinal edema (black arrow). X 400magn

4.3.2 Testis of albino rats in the group administered with 2ml Cd + 4ml *Cocos nucifera L.* water

Section of the testis of albino rats exposed to 2ml Cd + 4ml *Cocos nucifera L.* water only showed mild intestinal edema. Plate 4

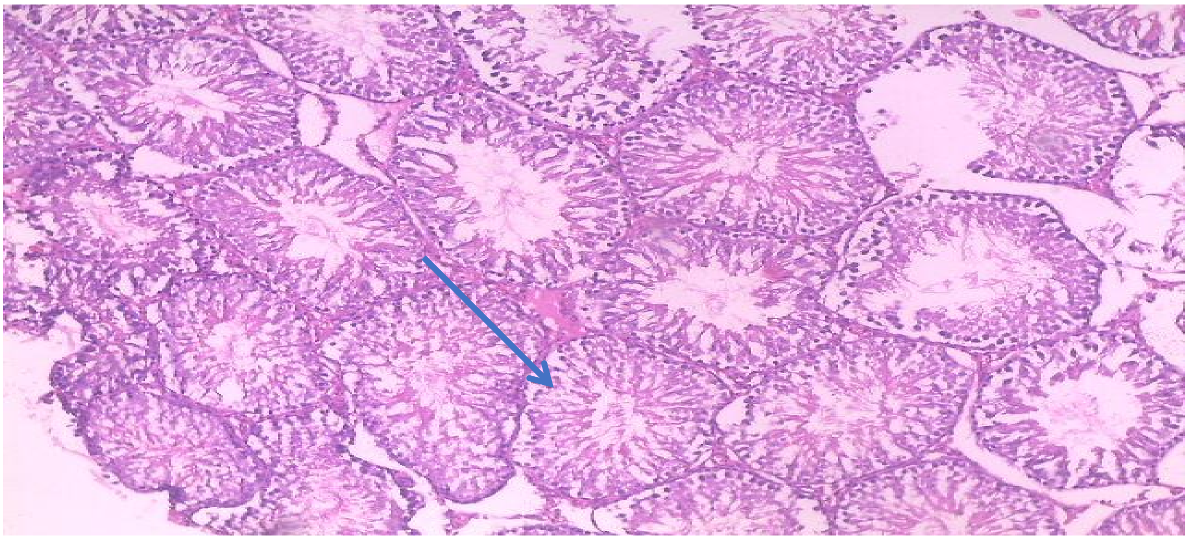

Plate 4.3: Shows mild intestinal edema. X 400magn

4.3.3 Testis of albino rats in the group administered with 2ml Cd + 6ml *Cocos nucifera L.* water

Section of the testis of albino rats exposed to 2ml Cd + 6ml *Cocos nucifera L.* water only showed mild intestinal edema.

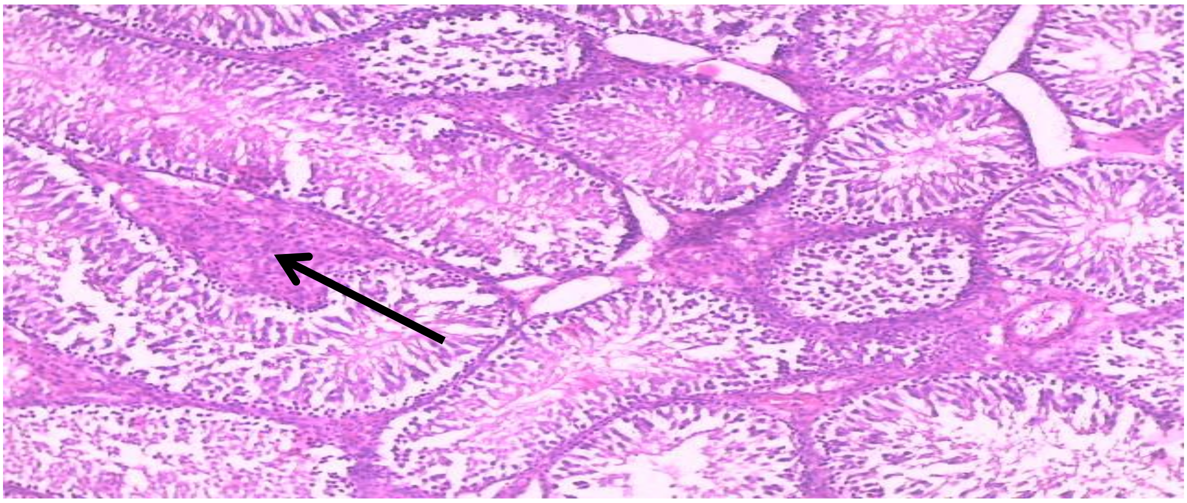

Plate 4.4: Section of the testis showing mild intestinal edema. X 400magn

4.3.4 Testis of albino rats in the group administered with *Cocos nucifera L.* water

Section of the testis of albino rats exposed to *Cocos nucifera* L. water only showed normal seminiferous tubues lined by thr cells of the spermatogenic series.

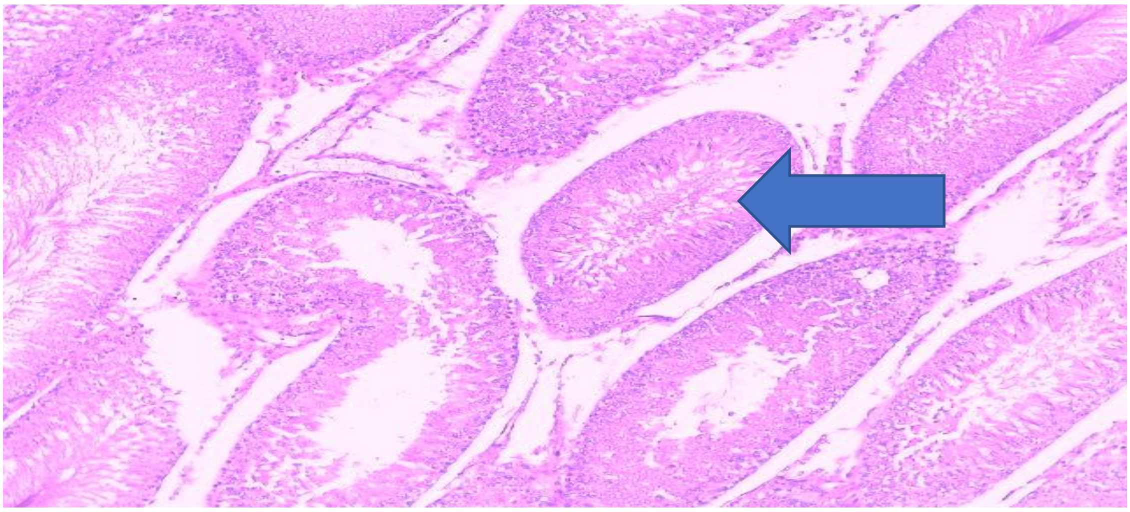

Plate 4.5: showing different sizes of normal seminiferous tubules lined by cells of the spermatogenic series. X 400magn

CHAPTER FIVE

5.0 DISUCSSION

5.1 DISCUSSION

According to (Wilhelm, *et al.,* 2019), the testis is the primary organ for male sexual development and fertility. It generates sperm for reproduction and hormones to support male-specific features. Seminiferous tubules, which act as physical barriers and nutrient sources for sperm survival and maturation, are the main structural elements of the testes. Sertoli cells, which resemble epithelial cells, and peritubular myoid (PM) cells, which resemble myofibroblasts, form seminiferous tubules.

According to the findings, as compared to the weight of rats in the other groups, which ranged from 165.43 ± 5.46 g to 169.30 ± 5.20 g, the mean body weight of the testis in the rats in Group A were (156.71 ± 6.63 g) substantially ($p<0.05$) (Figure 1). In the group (Group A) that received only cadmium chloride, the rats' weights fluctuated throughout the days. According to the study done by (Mamoun, 2020), the variation in body weight may be explained by the individual differences in the rats' reactions to cadmium chloride. Some rats might be more sensitive to cadmium than others, or they may absorb it differently. Their reaction to coconut extract may also differ. Different weight outcomes for the rats may be the result of this natural variability.

In this investigation, administration of cadmium chloride led to a decrease in the mean value of testosterone (0.45 ± 0.05 g/l) of the rat model when compared with the mean value of the category of rats administered with only *Cocos nucifera L*. water only as seen in (Figure 2). Adult rats given a high dose of cadmium (0.45 mg/kg, sc) had lower blood testosterone levels, according to (Waseem, 2022). Additionally, in vitro studies show that cadmium suppresses the production of testosterone and the DNA integrity of Leydig cells. Comparing the group of rats given only cadmium chloride (Group A) to the group given *Cocos nucifera* L. water along with cadmium chloride, there was a significant increase ($p > 0.05$) in the mean value of the testosterone from (0.45 ± 0.05 g/l to 0.93 ± 0.61 g/l. This is because of the vitamin C and minerals (Zulaikhah *et al*., 2019).

According to (Creasy & Lanning, 2019), histopathology is thought to be the most accurate parameter for identifying toxic effects on male fertility. The testis from Group A (where only cadmium chloride was administered), Group B (where only 2ml of cadmium chloride and 4ml *Cocos nucifera L.* water was administered), Group C (where only 2ml of cadmium chloride and 6ml *Cocos nucifera L.* water was administered) and Group D (where only *Cocos nucifera L.* water was used) showed up in the histopathological analysis. The group administered with cadmium chloride and coadministration of *Cocos nucifera L.* water showed mild intestinal edema. Leydig cells (LCs) secrete androgen and peptide hormones like insulin-like 3 (INSL3) in the interstitial compartment of the mammalian testis to control the development of the male reproductive tract, the descent of the testis, and the spermatogenesis (Rebourcet *et al*., 2019).

Both in humans and in animals, cadmium (Cd) builds up in the male reproductive organs (Benoff, 2019). The testis is more susceptible to Cd than other significant organs, according to numerous research (Foote, 2019). Inhibiting oxidative stress (Turner, 2019) causes an increase in germ cell apoptosis and/or distorting the blood-testis barrier causes germ cell loss, testicular edema, and hemorrhage (Cheng, 2019), which are all symptoms of Cd-induced testicular toxicity (Porto, 2019). Rats in the

Cocos nucifera L. water group only displayed typical seminiferous tubules surrounded by spermatogenic series cells. The results of some researchers, (Ogedengbe, 2016) who reported that the adjuvant treatment of *Cocos nucifera L.* water had a protective effect on the seminiferous epithelium. The histological study showed that the group of rats administered with *Cocos nucifera L.* water had enhanced spermatogenesis. This finding is consistent with the findings of (Fischer *et al*., 2019), who also noted that Wistar rats treated with *Cocos nucifera L.* water did not exhibit any histological abnormalities as they transitioned from primordial cells to spermatids and then to spermatozoa. According to (Brucefife, 2019), coconut oil has an antioxidant impact. Antioxidants strengthen gonadal function by stabilizing testicular membranes and presumably reducing aberrant sperm and lipid peroxidation (Emanuele, 2019).

5.2 CONCLUSION AND RECOMMENDATION

Although coconut water is often touted for its possible health benefits, there is little scientific data to back up its ability to reduce cadmium toxicity in particular. Therefore, this study took a brave step in looking into the potential of *Cocos nucifera L.* water in reducing cadmium-induced antifertility in albino rats. The results of this investigation showed that the water from *Cocos nucifera L*. water could potentially reduce albino rats' infertility caused by cadmium chloride. This was demonstrated by the fact that rats cotreated with cadmium chloride and *Cocos nucifera L.* water had improved testosterone levels and body weight compared to rats simply given cadmium chloride, however there was no appreciable change in the testis' histology. Overall, I advise conducting more research to confirm these facts.

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