ASSESSMENT OF TESTICULAR PROTECTIVE EFFECT OF HYDRO ETHANOIC WATERMELON RIND EXTRACT IN WISTAR RATS EXPOSED TO CADMIUM

BY

Favour Aihannuwa IDEHEN (Miss)

LSC2104248

(PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)
DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY
FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN
BENIN CITY

OCTOBER, 2025

ASSESSMENT OF TESTICULAR PROTECTIVE EFFECT OF HYDRO ETHANOIC WATERMELON RIND EXTRACT IN WISTAR RATS EXPOSED TO CADMIUM

BY

Favour Aihannuwa IDEHEN (Miss)

LSC2104248

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY ,FACULTY OF LIFE SCIENCES ,UNIVERSITY OF BENIN,BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FORTHE AWARD OF OF BACHELOR OF SCIENCE DEGREE (B.SC) IN SCIENCE LABORATORY TECHNOLOGY (PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

OCTOBER, 2025

CERTIFICATION

This is to certify that the research seminar **ASSESSMENT OF TESTICULAR PROTECTIVE EFFECT OF HYDRO ETHANOIC WATERMELON RIND EXTRACT IN WISTAR RATS EXPOSED TO CADMIUM** was carried out by Favour Aihannuwa IDEHEN **(Miss)** (MAT. No **LSC2104248**) of the department of Science Laboratory Technology (physiology and pharmacology techniques), Faculty of Life Sciences, University of Benin, Benin City, Edo State , Under the supervision of Mr O.C Ekhator.

_____ _____

MR. O.C. EKHATOR **DATE**
(Project supervisor)

_____ _____

DR. P.O ALONGE **DATE**
(Project coordinator)

_____ _____

PROF J.O OSARUMWENSE **DATE**
(Head of department)

_____ _____

(external examiner) **DATE**

DEDICATION

I dedicate this work to God Almighty and my sponsors, mr and Mrs Osaro Idehen, Mr Aifuwa Igbinosa, Mr D.A Igbinosun and my siblings, in appreciation of their inspiration and drive given towards the successful completion of this work.

ACKNOWLEDGEMENTS

My profound gratitude goes to God Almighty for his grace which he granted me through my years in this prestigious institution. My work would have meant nothing without the Lord's grace and mercy.

I am profoundly grateful to my supervisor, MR. O.C EKHATOR, for his unwavering support, insightful contributions, and the countless hours devoted to mentoring me throughout this project.

To my sponsors; mr and Mrs Osaro Idehen, Mr Aifuwa Igbinosa, Mr D.A Igbinosun, my siblings PEACE ,GLORY, EFE, OBOSA and my friends VICTORY, CHRISTABEL, FUNMBI, ANTHONY, HOPE for their great love, support and encouragement and to my project partners akhere, oma, miracles, lilabel, onos, blessing, jamilah . To my dear late grandmother, whose last words to me were ''concentrate on your studies'' I wish you were here to see your little one graduate. I know how much you would have loved this moment, and I'm grateful for the values and encouragement you instilled in me. I dedicate this achievement to your memory, and I hope I continue to make you proud.

Lastly I want to thank myself , I want to thank me , I want to thank me for believing in me, I want to thank me for doing all this hard work, I want to thank me for having no days off, I want to thank me for never quitting, and I want to thank me for just being me all the time.

TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

LIST 0F FIGURES

ABSTRACT

Cadmium is a toxic heavy metal pollutant that causes significant reproductive dysfunction in males through oxidative stress and testicular damage. Watermelon (*Citrullus lanatus*) rind contains bioactive phytochemicals with potential antioxidant properties that may offer protection against heavy metal-induced toxicity. This study investigated the testicular protective effect of hydroethanolic watermelon rind extract against cadmium-induced reproductive toxicity in Wistar rats. Twenty-five male Wistar rats were randomly assigned into five groups of five animals each. Group one served as the control and received distilled water only. Group two received 1 mg/kg body weight of cadmium chloride in distilled water . Group three was co-administered 1 mg/kg of cadmium chloride and 100 mg/kg of vitamin C as positive control. Groups four and five received 1 mg/kg of cadmium chloride alongside 250 mg/kg and 500 mg/kg of aqueous extract of *Citrullus lanatus* phytowaste (AECLP), respectively . All treatments were administered orally for sixty consecutive days. On day sixty-one, the animals were fasted overnight, humanely sacrificed, and samples collected. Semen was obtained from the epididymis for sperm analysis including sperm count, motility parameters. Results demonstrated that AECLP at both doses caused severe reproductive toxicity rather than protection, with complete elimination of progressive sperm motility, total loss of sperm viability, and undetectable sperm counts compared to control and cadmium-only groups. The extract paradoxically enhanced testicular cadmium bioaccumulation, particularly at the higher dose. These findings suggest that watermelon rind extract at the tested doses exhibits dose-dependent reproductive toxicity, warranting comprehensive safety reevaluation before therapeutic applications in reproductive health

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Cadmium (Cd) is a heavy metal that has received considerable concern environmentally and occupationally. Cd has a long biological half-life mainly due to its low rate of excretion from the body. Thus, prolonged exposure to Cd will cause toxic effect due to its accumulation over time in a variety of tissues, including kidneys, liver, central nervous system (CNS), and peripheral neuronal systems. Cd can be uptaken from the nasal mucosa or olfactory pathways into the peripheral and central neurons; for the latter, Cd can increase the blood brain barrier (BBB) permeability (Satarug *et al*., 2010). However, mechanisms underlying Cd neurotoxicity remain not completely understood. Effect of Cd neurotransmitter, oxidative damage, interaction with other metals such as cobalt and zinc, estrogen-like, effect and epigenetic modification may all be the underlying mechanisms. Oxidative stress induced by Cd is a critical aspect of its toxicity mechanisms. It occurs when an increase in oxidants and a decrease in antioxidants leads to the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Cupyers *et al*., 2010.; Ikediobi *et al.*, 2004). This imbalance can damage cellular macromolecules, such as lipids, proteins, and nucleic acids, extensively harming cell structure and function. This series of steady-state imbalances can lead to disruptions in cellular signaling pathways and may also trigger cell apoptosis or necrosis . Furthermore, it can potentiate its oxidative stress effects by perturbing the cellular antioxidant defense system . Key antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) may experience diminished

activities or inhibited expressions in response to Cd, thereby compromising the capacity to counter oxidative damage and intensifying the adverse consequences of oxidative stress in cell.

Watermelon also known as Citrullus lanatus is a fruit originating from the tropics that is mostly cultivated in the warm temperate zone and readily available in the market (Radman *et al*., 2013). The watermelon's pink flesh is eaten raw or used to make juices and salads, but the rind is discarded as a waste product with no commercial value. However, watermelon rind pickles are also commonly consumed in the southern United States (Mandel *et al*., 2005). In order to promote health benefits and lower the glycemic index, watermelon rind has been stated as a potential substitute for wheat flour as a potential source of dietary fiber in making of cookie and cake(Naknaen *et al*., 2016) , and for the control of diabetes control and alcoholic poisoning. Furthermore, watermelon is rich in both essential and unessential amino acids, and a number of antioxidant molecules especially L-citrulline which is a precursor for L-arginine in the kidney (Ahn *et al*., 2011). In the formation of nitric oxide, L-arginine is the substrate for nitric oxide synthase. Nitric oxide is an essential signaling molecule involved in the regulation of diverse range of physiological and cellular processes and act as a novel hydroxyl radical scavenger. Studies have revealed that watermelon possess anti-inflammatory, antioxidant, hepato-protective, antigiardial, anti-plasmodial, analgesic, antimicrobial, laxative, antidiabetic, and antiulcerogenic activities (Ahn *et at*., 2011.; Mohammad *et al*., 2014)

1.2 Aim of the Study

This aim of this research study is to investigate the effect of hydro-ethanolic extract of watermelon on cadmium induce testicular toxicity in wistar rats

1.3Objective of the Study

To achieve the aim of this study, the following objectives must be achieved:

1. To determine the protective effects of AECLP on the male reproductive organ (testes) by evaluating semen analysis in response to Cd toxicity

2. To determine the effect of AECLP on the levels of Cd in the testes of albino wistar rats

CHAPTER TWO

LITERATURE REVIEW

2.1 Cadmium

Cadmium (Cd) is a soft, bluish-white heavy metal belonging to group 12 of the periodic table, with an atomic number of 48 and atomic weight of 112. It occurs naturally in the Earth's crust at concentrations around 0.1-0.5 mg/kg, often associated with zinc ores. Cadmium is non-essential for biological systems and exhibits high toxicity even at low levels, making it a significant environmental and public health concern. Its industrial applications include nickel-cadmium batteries, pigments, coatings, and stabilizers in plastics, contributing to widespread pollution. Due to its persistence and bioaccumulation, cadmium poses risks to ecosystems and human health, with a biological half-life in humans ranging from 10 to 35 years (Genchi *et al*., 2020.; Zheng *et al*., 2024).

Sources of cadmium exposure are multifaceted, encompassing both natural and anthropogenic origins. Naturally, it is released through volcanic activity and rock weathering, but human activities amplify levels significantly. Anthropogenic sources include mining, smelting of non-ferrous metals, coal combustion, waste incineration, and the use of phosphate fertilizers, which can contaminate soil and water. Dietary intake is the primary route for non-smokers, with foods like leafy vegetables, grains, potatoes, and shellfish accumulating cadmium from polluted environments. Inhalation occurs via cigarette smoke, smokers have 4-5 times higher blood cadmium levels and industrial emissions. Occupational exposure affects workers in battery production and metal plating, while environmental contamination impacts populations near industrial sites. Global estimates indicate that approximately 600 million people are exposed

annually through air, water, and food pollution. Recent studies highlight elevated cadmium in crops like rice in regions with industrial runoff, exacerbating dietary risks (Kowalczyk *et al*., 2025).

Toxicokinetic of cadmium involve absorption, distribution, metabolism, and excretion processes that favor accumulation. Absorption rates vary: 5-10% via gastrointestinal tract (higher in iron-deficient individuals) and 10-40% through lungs. Once absorbed, cadmium binds to proteins like albumin and metallothione in, facilitating transport to organs. It primarily accumulates in the kidneys (up to 50% of body burden) and liver, with minor deposits in lungs, pancreas, and bones. Metabolism is limited, as cadmium is not bio transformed but induces metallothionein synthesis for detoxification. Excretion is slow, mainly urinary (0.007% daily), leading to chronic buildup. Biomarkers include blood cadmium (recent exposure), urinary cadmium (long-term), and proteins like β2-microglobulin for renal damage (Satarug *et al*., 2017).

Mechanisms of cadmium toxicity are complex and multifactorial, centered on oxidative stress, ionic mimicry, and cellular disruption. Cadmium generates reactive oxygen species (ROS) indirectly by depleting antioxidants like glutathione and inhibiting enzymes such as superoxide dismutase and catalase, resulting in lipid peroxidation, protein damage, and DNA strand breaks. It mimics essential ions (e.g., Ca^{2+}, Zn^{2+}), disrupting calcium homeostasis, enzyme functions, and signaling pathways like MAPK and NF-κB, which trigger inflammation via cytokines (TNF-α, IL-6). Mitochondrial dysfunction is key, with cadmium impairing electron transport, reducing ATP, and inducing apoptosis through cytochrome c release and caspase activation. Epigenetic effects include DNA methylation changes and miRNA dysregulation, promoting carcinogenesis. Autophagy impairment leads to accumulation of damaged organelles, while endoplasmic

reticulum stress activates the unfolded protein response, culminating in cell death if unresolved. These mechanisms are conserved across organisms, from bacteria (efflux pumps like czc genes) to humans, with gender and nutritional status influencing susceptibility (Rafati *et al*., 2017).

Health effects of cadmium exposure span multiple organ systems, often manifesting after chronic low-level contact. The kidneys are the primary target, where cadmium accumulates in the proximal tubules, causing tubular dysfunction, proteinuria, glucosuria, and chronic kidney disease. Critical renal cortex concentrations (100-300 μg/g) lead to irreversible damage, with biomarkers like N-acetyl-β-D-glucosaminidase rising early. Epidemiological data link urinary cadmium >1 μg/g creatinine to reduced glomerular filtration rate. Liver toxicity involves oxidative damage, inflammation, and fibrosis, potentially progressing to non-alcoholic fatty liver disease, with elevated enzymes (ALT, AST) indicating hepatocyte injury (AI-Baqami and Hamza, 2021).

Skeletal effects include osteoporosis and osteomalacia, as cadmium disrupts calcium metabolism, reduces bone mineral density, and increases fracture risk, particularly in postmenopausal women. Cardiovascular impacts encompass hypertension, atherosclerosis, and coronary disease through endothelial dysfunction, ROS-induced lipid peroxidation, and calcium overload, reducing nitric oxide bioavailability. Respiratory toxicity from inhalation causes emphysema, chronic bronchitis, and lung cancer, with cadmium classified as a Group 1 carcinogen by IARC, linked to prostate, breast, and kidney cancers via genotoxic mechanisms (Charkiewicz *et al*., 2023).

Reproductive effects are pronounced: in males, cadmium damages testes, impairing spermatogenesis, reducing testosterone, and causing infertility; in females, it disrupts ovarian function, hormonal balance, and increases miscarriage risk. Neurotoxicity involves ROS-mediated damage, contributing to cognitive decline and neurodegenerative diseases like

Alzheimer's. Immune system suppression reduces natural killer cell activity and alters cytokine profiles, heightening infection susceptibility. Developmental toxicity affects fetuses and children, leading to low birth weight, growth retardation, and neurodevelopmental issues. Environmental impacts of cadmium are profound, contaminating soil and water, reducing biodiversity, and entering food chains. In plants, it causes chlorosis, stunted growth, and reduced yields; in aquatic organisms, bioaccumulation leads to ecosystem imbalances. Global regulations, such as WHO's provisional tolerable weekly intake of 2.5 μg/kg body weight, aim to curb exposure, but challenges persist in developing regions. Mitigation strategies include reducing industrial emissions, using cadmium-free fertilizers, and dietary interventions like zinc or iron supplementation to inhibit absorption. Antioxidants (e.g., N-acetylcysteine, resveratrol) and chelators show promise in animal models, while bioremediation using microbes or plants offers environmental cleanup. Public health measures emphasize monitoring, awareness, and smoking cessation (Liang *et al*., 2025.; WHO, 2019).

2.2 Absorption And Distribution

Cadmium primarily absorbed by human body via ingestion. Cd accumulates in soil and is metabolised by plants. Edible plant components, such as fruits and seeds, accumulate Cd, which enters the food chain. Moreover, acid rain increases the amounts of Cd in plants. The main intake of cadmium is via leafy vegetables especially spinach, potatoes, legumes and nuts, stem/root vegetables and fruits. Fish and shellfish are the primary sources of Cd, followed by rice in eastern countries. The second cadmium pathway to human are via inhalation. Cd air levels can be hundreds of times higher at the workplace than elsewhere in the environment. Inhaled cadmium dust absorbs 10–50 % by body depending on particle size. Since very little cadmium is absorbed

through skin, it is not regarded as a crucial exposure route. Following absorption, cadmium is primarily attached to the protein metallothione, which has a sulfhydryl group, and is subsequently carried throughout the body in blood. The protein metallothione binds to heavy metals and can guard against oxidative stress and heavy metal toxicity. Perhaps because of the kidneys' and liver's capacity to produce metallothione, cadmium subsequently builds up in the human body, primarily in these cells. Cadmium lacks an effective excretory system in the body (Witkowska *et al*., 2021).

2.3 Mechanism of Toxicity

Mechanisms of Cadmium Toxicity On The Testes

Cadmium injury to the testes disrupts the blood-testis barrier (BTB), resulting in failures in sperm production through oxidative pressure, demise, and inflammation. Cd enters via ZIP transporters, producing ROS that reduce GSH and elevate MDA, injuring supporting and hormone-producing cells. BTB disruption includes reducing claudin-11, causing loss of germ cells. Demise through Bax/Bcl-2 and caspases lowers sperm counts. Hormone interference decreases testosterone by blocking StAR. Epigenetic changes impact sperm formation. In rats, Cd (1 mg/kg) causes tissue death and infertility. Substances like curcumin protect by trapping Cd and enhancing Nrf2. Cd focuses on Sertoli cells, essential for the BTB, through oxidative harm that weakens tight connections, permitting toxins entry and germ exit. This results in tubule reduction and sperm absence. Leydig cells are affected, lowering enzymes for testosterone, causing hormone deficiencies and reduced drive. In males, ongoing Cd links to poor sperm quality, decreased movement, and higher infertility rates, as seen in contaminated zones. Inflammation via NF-κB elevates cytokines, attracting immune responses and leading to scarring. Mitochondrial injury cuts

ATP and releases death elements. ER stress initiates UPR, resulting in demise. Epigenetics like miRNA alterations influence sperm genes, potentially affecting descendants. Models show Cd reduces fertility and increases abnormalities. Studies in humans connect occupational Cd to prostate concerns and cancers. Cd harm to testes is intricate, from barrier disruption to hormone reduction, necessitating protective measures (WHO, 2019.; Zheng *et al* 2024).

2.4 Prevention of Cadmium Toxicity

Cadmium, a persistent heavy metal pollutant, poses significant health risks through environmental and occupational exposure, leading to kidney damage, bone disorders, and increased cancer risk. Preventing cadmium toxicity primarily focuses on minimizing exposure sources, implementing regulatory controls, adopting protective dietary habits, and employing agricultural and technological interventions. These strategies are essential given cadmium's bio accumulative nature and long biological half-life in the human body (Bashir *et al*., 2018). Exposure routes include inhalation from cigarette smoke and industrial fumes, as well as ingestion via contaminated food, water, and soil. Smoking stands out as a major contributor, with smokers exhibiting blood cadmium levels four to five times higher than non-smokers. To mitigate this, public health campaigns emphasize smoking cessation and avoiding secondhand smoke in enclosed spaces. Environmental contamination often stems from mining, smelting, battery production, and the application of phosphate fertilizers, which disperse cadmium into soil and water. Regulatory measures are crucial here, governments should enforce stricter emission standards for industries, limit cadmium content in fertilizers, and discontinue the use of cadmium-plated utensils or galvanized equipment in food production. For instance, establishing comprehensive monitoring programs for cadmium levels in air, water, soil, and food products,

particularly near industrial sites, helps identify hotspots and evaluate policy effectiveness. The development of commodity-specific codes of practice, such as those for cocoa, aids in managing cadmium in high-risk crops. Dietary interventions play a vital role in reducing cadmium absorption and accumulation. Ensuring adequate intake of essential micronutrients like zinc, iron, and calcium can competitively inhibit cadmium uptake in the intestines, potentially reducing absorption by up to tenfold in cases of marginal deficiencies. Zinc, for example, stimulates the production of metallothionein, a protein that binds cadmium and prevents oxidative stress. High-fiber diets and foods rich in phytates such as whole grains and legumes bind cadmium in the gastrointestinal tract, limiting its bioavailability. Consuming a varied diet avoids over-reliance on cadmium-prone foods like leafy vegetables, grains, organ meats, and shellfish. Nutritional education promotes these habits, especially in vulnerable populations like children and pregnant women, where early-life exposure heightens toxicity risks (Genotoxicity Biomarkers and Occupational Health Study Group 2024.; Rahimzadeh *et al*., 2017.; Spungen, 2019)

2.5 Treatment of Cadmium Toxicity

Cadmium toxicity manifests in acute and chronic forms, affecting organs like the kidneys, liver, testes, often through oxidative stress, mitochondrial damage, and disrupted cellular signaling. Treatment strategies encompass immediate supportive care, chelation therapy to enhance excretion, dietary and nutritional interventions, and emerging methods like nanotechnology, aiming to alleviate symptoms and reduce body burden without exacerbating damage (Faroon *et al*., 2012).For acute poisoning, typically from inhalation or ingestion, prompt intervention is critical. Airway management, supplemental oxygen, and steroids address respiratory distress from chemical pneumonitis, though steroid efficacy remains unproven. Gastrointestinal

decontamination involves gastric lavage or activated charcoal if emesis hasn't occurred, but charcoal's binding to cadmium is limited. Removing the patient from the exposure source is paramount to prevent further accumulation (rafati *et al*., 2017).Chelation therapy promotes cadmium excretion but requires caution due to potential nephrotoxicity. Ethylenediaminetetraacetic acid (EDTA) increases urinary elimination, administered intravenously at doses like 500 mg with glutathione over 24 hours, repeated as needed. However, it may redistribute cadmium to the kidneys, risking dysfunction. Vitamins play key roles: vitamin C scavenges free radicals and may chelate cadmium; vitamin E reduces lipid peroxidation in organs; thiamine decreases tissue accumulation; and vitamin B6 inhibits absorption (Wallin *et al*., 2014.; Zhai *et al*., 2013).

2.6*Citrullus lanatus*

Watermelon, known scientifically as *Citrulluslanatus*, is a juicy fruit from the Cucurbitaceae family that originated in Africa and is popular globally for its sweet taste and high water content. This fruit not only quenches thirst but also packs a variety of nutrients, antioxidants, and health-promoting compounds that make it a valuable addition to a balanced diet.

watermelon is low in calories and fat while being highly hydrating, consisting of about 91-92% water. A standard serving of around 100 grams (roughly 2/3 cup) provides approximately 30 calories, 0.6 grams of protein, 7.6 grams of carbohydrates (including 6.2 grams of natural sugars and 0.4 grams of fiber), and 0.2 grams of fat. It is rich in essential vitamins and minerals. Other notable nutrients include vitamin B5, B6, copper, and magnesium, with the seeds adding extra protein, healthy fats, and B vitamins when eaten. Its low glycemic load (around 5) means it has a

gentle impact on blood sugar levels, making it suitable for most people when consumed in moderation (Deshmukh*etal*., 2015.; Kumawat , 2017).

The fruit's antioxidant properties stem from its vibrant pigments and bioactive compounds that help neutralize free radicals, reducing oxidative stress and inflammation. lycopene, a potent carotenoid found at levels of 4,530 micrograms per 100 grams, which is higher than in tomatoes and increases as the fruit ripens. Lycopene provides strong antioxidant and anti-inflammatory effects, scavenging harmful molecules more effectively than beta-carotene or vitamin E. Watermelon also contains vitamin C (up to 14% DV per cup), beta-carotene, cucurbitacin E, and polyphenols like flavonoids and phenolic acids, with concentrations around 16-20 mg gallic acid equivalents per 100 mL of juice. Additionally, citrulline an amino acid concentrated in the rind and flesh converts to arginine in the body, boosting nitric oxide production for better vascular function. These antioxidants are more abundant in red-fleshed varieties and even in byproducts like peels and seeds, which show significant radical-scavenging activity.

Watermelon offers numerous health benefits backed by scientific research. It supports heart health by potentially lowering blood pressure, cholesterol, and arterial stiffness, as seen in studies where extracts reduced these markers in obese adults and postmenopausal women. Lycopene and citrulline contribute to improved endothelial function, reduced inflammation, and better lipid profiles, decreasing risks of cardiovascular disease. For metabolic health, it may help manage diabetes and obesity by enhancing insulin sensitivity, lowering glucose levels, and promoting satiety, with juice supplementation showing benefits in diabetic models and human trials. Anticancer properties are evident, with lycopene inhibiting cell proliferation in prostate, breast, and colorectal cancers, and extracts reducing tumor growth in lab studies. It also aids exercise

recovery by minimizing muscle soreness and cramps, thanks to citrulline and potassium, as demonstrated in athlete trials. Skin and eye health benefit from vitamins A and C, which support collagen production and protect against UV damage and age-related macular degeneration. Furthermore, its fiber and water content promote digestion and hydration, while antioxidants may guard against chronic conditions like arthritis and ulcers (Mukhtar and Singh, 2020.;Sharma*etal*., 2023).

Plate 1:*citrulluslanatus***. Source:** (Akintunde and Thomas, 2021)

2.7 Botany oF *citrullus lanatus*

Citrullus lanatus, commonly known as watermelon, is an annual flowering plant in the Cucurbitaceae family, which includes cucumbers and pumpkins. Taxonomically, it falls under the kingdom Plantae, clade Tracheophytes, class Magnoliopsida, order Cucurbitales, and genus Citrullus. Originally described by Carl Linnaeus in 1753 as *Cucurbita citrullus*, it was reclassified in 1836, with the name conserved in 2017 based on phylogenetic studies. Varieties include subsp. vulgaris for sweet edible forms and subsp. lanatus for bitter types. Native to arid regions of Africa,

such as Sudan, Ethiopia, and Libya, C. lanatus thrives in grasslands and bushlands on sandy soils near water sources, up to 1,785 meters elevation. Domestication occurred around 2000 BC in Egypt and Sudan, evolving from bitter wild progenitors with evidence of 6,000-year-old seeds in Libya. It prefers dry climates with limited rainfall (400-1,800 mm annually) and temperatures of 20-35°C.

Morphologically, the plant has a trailing or climbing habit, with hairy stems extending 3-10 meters, supported by curly tendrils. Leaves are alternate, heart-shaped or oval, 3-20 cm long and wide, palmately lobed into 3-5 segments with toothed margins and dense woolly hairs, especially on veins. As a monoecious species, it produces separate male and female yellow flowers on short axillary stalks, with males appearing first; each has 4-5 petals, and females feature inferior ovaries. The fruit, botanically a pepo (modified berry), features a thick green rind (often mottled) and juicy mesocarp. Flesh varies in color (red, pink, yellow, white), containing flat seeds, though seedless triploid hybrids exist. Pollination relies on bees, with fruits maturing 80-110 days post-sowing in well-drained, neutral pH soils under full sun.

2.8 Traditional Uses

Citrullus lanatus, commonly called watermelon, holds significant traditional value in various cultures, especially in Africa where it originated. It serves multiple purposes in food, medicine, and daily life, drawing from its nutrient-rich fruit, seeds, and other parts.

As a food source, the sweet red flesh is eaten raw to quench thirst or as a dessert, while white-fleshed varieties are cooked into thick sauces for cereal meals or flavored snacks in Mali. Immature fruits are harvested for culinary dishes in northern regions like Gao. Seeds are versatile

roasted with salt for snacks, pounded into flour for porridges or drinks, or extracted for cooking oil. Medicinally, the pulp and juice function as diuretics to aid urine flow. Seeds address urinary infections, bedwetting, fluid retention, kidney stones, high blood pressure, diabetes, diarrhea, and gonorrhea, often due to their purgative, worm-expelling, soothing, fluid-promoting, and strengthening effects. Fruit juice provides stomach protection against ulcers and reduces secretions. Roasted seeds stimulate appetite and relieve constipation.Beyond edibles and remedies, watermelon juice ferments into alcoholic drinks in Eastern Europe. Plant residues, including leaves, stems, and rinds, feed livestock, while seeds boiled with ash create soap or cosmetics in Mali. Seeds also appear in pharmaceutical and beauty products for their oils. Additionally, fruits and seeds generate income through sales in local markets.

2.9 Taxonomy Of citrullus lanatus

- Kingdom: Plantae (Plants)
- Phylum: Tracheophyta(Vascular plants)
- Class: Magnoliopsidao
- Order: Cucurbitales
- Family: Cucurbitaceae (melon family)
- Genus: *Citrullus*
- Species: *Citrulluslanatus*

2.10 Phytochemical Properties Of ***citrullus lanatus***

2.10.1 Watermelon Flesh

Coratenoids

watermelon is the world's third most popular fruit consumed during hot weather. The colour of the watermelon's flesh is an essential characteristic. white, salmon yellow, orange, crimson red, and green are the eight defined flesh colors for watermelon. Except for the green flesh color, watermelon contains a variety of carotenoids that are responsible for the various flesh hues. Carotenoids are important functional components and micronutrients in watermelon. The carotenoid composition and concentration have both become a major focus during watermelon quality assessments (Zhao *et al*., 2013). The majority of β-carotene and lycopene were found in red-fleshed watermelons. Lycopene accounted for most of the total carotenoids (84%-97%). Carotenoid composition and content in watermelons of varying flesh colors are linked to cultivars and growing conditions (Tadmor *et al.,* 2005). Lycopene, β-carotene, phytofluene, phytoene, lutein, and neurosporene are some of the carotenoids, that have been identified in watermelon. The antioxidant property of lycopene and its bright red color are attributed to its unique conjugated polyene structure (Holzapfel *et al*., 2013.; Shi and Maguer, 2000).

Amino Acids

The organic constituents comprised of both amino and acid groups are designated as amino acids (AA). All AAs have optical activity and an asymmetric carbon excluding glycine. The arrangement with reference to the glyceraldehydes of AA isomers is nominated as an absolute configuration. All protein AAs allied to the A-carbon atom (hence a-AA) have a carboxyl group

and a primary amino group excluding proline (Galli, 2012). AAs have an extraordinarily diverse range of biochemical properties and functions due to the differences in their side chains (Brosnan, 2009; Wu *et al*., 2007; Yamane et al., 2009).

Phenolic Compounds

In *C. lanatus*, phenolic compounds, such as anthraquinones exist in moderate concentrations and are effective at relieving both stomachaches and constipation. Anthracene produces very specific compounds known as anthranols and anthrone derivatives. At the C-8 and C-1 positions, other moieties, such as chrysophanol, luteolin, emodin, and rhein derivatives have a mutual double hydroxylation. In the plant sample, a violet color or pink identifies the anthraquinone coexistence in the base stratum (Sarker and Nahar, 2013). The structures of some phenolic compounds in watermelon (Sarker and Nahar, 2013).

2.10.2 Watermelon Seeds

Glycosides

The glycoside phytochemicals in watermelon seeds were identified as having anti-diarrheal benefits amongst others (Tiwari *et al*., 2009). Including polysaccharides, the sugar condensation products were found to invariably be monohydrate but occasionally there were thiol compounds that host different organic hydroxyl varieties. Glycosides are present in the spare water soluble phytoconstituents, and crystalline carbon, containing oxygen and colorless hydrogen (a few containing sulfur and nitrogen). Glycosides are comprised chemically of a noncarbohydratepart (aglycone or Genin) and a carbohydrate (glucose) by alcohol, glycerol or phenol aglycones.

Saponins

Saponins are present in watermelon seeds at varying concentrations depending on the processing method. For instance, fresh watermelon seeds contain saponins at a concentration of 2.15 mg/100 g (Bamidele *et al*., 2021).Another study reported a higher concentration of saponins, ranging from 11.62 to 32.48 mg/g, indicating that the extraction method and seed preparation can significantly influence saponin levels (Seidu and Otutu, 2016). In a different analysis, saponins were quantified at 0.08% (16.87 mg/g), further supporting the presence of these compounds in watermelon seeds (Irabor *et al*., 2020.; Bombardelli and Gabetta, 2001).

Alkaloids

Alkaloids in watermelon seeds reportedly have the highest concentration levels when compared with their other components, and they exhibit effective therapeutically important bioactivities as plant secondary metabolites and as elementary agents for antispasmodic, analgesics, and bacterial effects and are extensively used worldwide (Akshaya *et al*., 2018). For alkaloids, the alkalinity of an alkaloid they substitute as amines 1°, 2° or 3° normally due to the presence of one or more nitrogen atoms. Due to the properties of elements, such as molecular structure, functional groups, location, and presence, the degree of basicity is known to fluctuate significantly (Sarker and Nahar, 2013).

2.10.2The phytochemical of watermelon rind and peel

Phytochemical Composition

- **Total Phenolics**: The rind contains approximately 0.026 mg GAE/g of total phenolics, which is lower than the peel (0.087 mg GAE/g) but still significant(Neglo *et al*., 2021).

- **Flavonoids and Alkaloids**: Both the rind and peel are rich in flavonoids and alkaloids, contributing to their health benefits (Neglo *et al*., 2021.; Ladan, 2024).

2.11.PharmacologicalStudies and Therapeutic effect of *Citrullus Lanatus*

Antioxidant effect of *Citrullus Lanatus*

The antioxidant properties of *Citrullus lanatus* (watermelon) have been extensively documented, underscoring its potential role in mitigating oxidative stress and inflammation. Phytochemical analyses show that the fruitespecially its rind and seedscontain significant concentrations of flavonoids and phenolic compounds, which account for its strong free radical scavenging activity (Neglo et al., 2021; Sathya and Shoba, 2014). These bioactive compounds contribute to the observed health benefits associated with watermelon consumption and its extracts.

Experimental studies employing DPPH and ABTS assays confirm that extracts from watermelon rind and seeds possess potent antioxidant capacity, with the rind showing particularly high activity (Neglo *et al*., 2021; Sathya&Shoba, 2014.;Ebhohon *et al.*, 2024).

Nutritionally, while the pulp contains lower levels of antioxidants, the rind and seeds offer a richer source of bioactive compounds. This makes them valuable candidates for functional foods or nutraceutical formulations (Neglo *et al*., 2021; Sathya & Shoba, 2014). However, variability in antioxidant capacity across different plant parts and preparation methods suggests that not all forms of watermelon confer equal benefits.

Anticancer effect of *Citrullus Lanatus*

The anticancer potential of *Citrullus lanatus* (watermelon) has been highlighted in several studies. Rind extracts have demonstrated significant cytotoxic activity against human cancer cell lines, inducing apoptosis and inhibiting cell migration through pathways involving caspase activation and altered BAX/BCL-2 ratios (Gizawy *et al*., 2022). Additionally, L-citrulline, a bioactive compound from watermelon, exhibited dose-dependent toxicity on HeLa cervical cancer cells, promoting apoptotic activity and suppressing migration (Eren *et al.*, 2022). Furthermore, fruit extracts have been associated with changes in mitochondrial membrane permeability, indicating a mechanism for cancer cell death induction (Oyedeji *et al*., 2016).

Antidiarrhea effect of *Citrullus Lanatus*

Citrullus lanatus (watermelon) has demonstrated promising antidiarrheal activity, particularly through its rind extracts and the amino acid L-citrulline. Both traditional medicine practices and modern pharmacological studies highlight its potential in reducing diarrhea symptoms, although careful consideration of dosage, preparation, and safety is essential (Sharma *et al.*, 2011.; saminu *et al.*, 2018)

Antidiabetic effect of *Citrullus Lanatus*

Citrullus lanatus (watermelon) has attracted growing scientific attention for its potential role in diabetes management. Studies on both the juice and seeds demonstrate antidiabetic activity through multiple mechanisms, including antioxidant defense, regulation of glucose transporters, and inhibition of carbohydrate-digesting enzymes. These findings support its ethnomedicinal use

and highlight its promise as a natural adjunct in diabetes care (Ajiboye *et al.,* 2020,; Deshmukh and Jain, 2015.; ogbeifun *et al.,* 2020).

.**Antiprotozoal effect of *Citrullus Lanatus***

Citrullus lanatus (watermelon) has been reported to possess antiprotozoal properties, with studies particularly highlighting its role against malaria and helminthic infections. Different parts of the plant, especially the leaves and seeds, have demonstrated significant bioactivity, making it a potential source of natural therapeutic agents (Kumawat, 2017.; Ruswadi *et al*., 2022.; Umaru and Alebiosu., 2024).

Antibacterial effect of *Citrullus Lanatus*

Citrullus lanatus (watermelon) has been increasingly studied for its antibacterial potential, largely due to the presence of diverse bioactive compounds. Extracts obtained from various parts of the plant, particularly the seeds, have demonstrated significant inhibitory activity against both Gram-positive and Gram-negative bacterial strains (Ekwere *et al*., 2015; Johnson *et al*., 2012).

Antiviral effect of *Citrullus Lanatus*

Citrullus lanatus (watermelon) has attracted increasing scientific interest for its potential antiviral effects, attributed to its diverse range of bioactive compounds such as flavonoids, carotenoids, citrulline, and cucurbitacins. These compounds provide antioxidant, anti-inflammatory, and immune-supporting functions that may contribute to antiviral defense mechanisms.(Kumar *et al*., 2021; Rahman *et al*., 2022).

Antihypertensive Effect of *Citrullus Lanatus*

Citrullus lanatus (watermelon) has been widely studied for its potential role in blood pressure regulation, largely due to its high content of L-*citrulline*, an amino acid that serves as a precursor to L-arginine and nitric oxide (NO). This pathway enhances vasodilation, improves vascular function, and reduces arterial stiffness, contributing to lower systolic and diastolic blood pressure (Figueroa *et al*., 2017; Morita *et al*., 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Beakers, 20ml Universal Bottles, Weighing Balance, Oven, Funnels, Plastic Cages, UV Stectrophotometer, Whatman Filter Paper, Knivies, Freeze Dryer, Crucibles, Micropipette, Ink, Syringes (1ml,2ml,5ml), Plastic Jar, Plain Sample Container, EDTA Container, Buckect Centrifuge, Industrial Blender, Refrigerator, Measuring Cylinder, Laboratory Mortal And Pestle, Spatula, Diserting Set, Atomic Absorption Spectrometer, Stiring Rod, Fed And Water Troughs, Water Bath, slides

3.1.1 Fruit Purchase

A bag of fresh *Citrullus lanatus* was purchased from New Benin Market, Oredo Local Government Area, Edo State In February 2025.

3.1.2 Chemicals And Reagent

Cadmium Chloride (99.9%), Normal Saline , Chloroform, , Formaldehyde, Ethanol (100%) , Distilled Water, Vitamin C.

3.1.3 Experimental Animals

Twenty-five (25) male Wistar adult rats between the weights of 104 g – 196 g were used for this study. The animals were purchased from an animal house in Benin city, Edo state. They were housed in the animal house at the Department of science laboratory technology, Faculty of Life Sciences in well-ventilated plastic cages. They were provided with regular access to food and

water. During their stay in the animal house, their beddings were changed regularly (every 3 days), and their cages were washed 3 times every week. They were acclimatized in the cages for 14 days before the study commenced. The rats used for the study were maintained based on the guidelines of the National Institutes of Health guide for the care and use of laboratory (animals and approval was obtained from the Department of Science Laboratory Technology, University of Benin with ethical number UNIBEN/FSLT/00031

3.2 Methodology

3.2.1 Fruit Extract Preparation

The *Citrullus lanatus* was was peeled and sun dried for 24 hours, afterwards it was air dried and also dried in an oven at a controlled temperature (37) for two weeks. The dried samples were then blended into powder with an industrial blender .the samples were weighed, mixed, and soaked in a mixture of Ethanol(50%) and distilled water (50%) (HYDROETHANOL 100%))for 72 hours in a plastic jar (while shaking vigorously every 6 hours).The mixture was then filtered using a whatman filter paper and the shaft was discarded .While the filtrate was kept aside. The filtrate was then concentrated using a water bath to obtain a semi solid extract .The extract was weighed and kept in a small sample container, properly labeled and stored in a refrigerator

3.2.2 Experimental Design

After 14 days of acclimatization, the wistar albino rats were divided into five (5) groups with five (5) rats in each group.

Group 1 was given distilled water

Group 2 was given water contaminated with cadmium

Group 3 was given cadmium solution and vitamin c

Group 4 served as the treatment group of lower dose, Aqueous *Citrullus lanatus* 250mg

Group 5 served as a treatment group of higher dose ; aqueous *citrullus lanatus* 500mg

Each group; except Group 1(control group); was given contaminated water. The water was contaminated with cadmium chloride of 1mg/ml in 300ml of water administered to them orally.

TABLE 1: Experimental design

GROUPS	TREATMENT	DURATION
I	Control	60 days
II	Cd	60 days
III	Cd and vitamin C	60 days
IV	AECLP (250mg) + Cd	60 days
V	AECLP(500mg) + Cd	60 days

Exprimental design

Cd = Cadmium, AECLP = Aqueous extract of *Citrullus lanatus phytowaste*

3.2.3. Dose Preparation

Dose calculation

At the beginning of the investigation, calculations were made based on the body weight of the animals, subsequently leading to the selection of various doses for the toxicity study.

Dose preparation of Cadmium

100mg of cadmium chloride was weighed and dissolved in 1 liter of distilled water. The animals were weighed, and each rat was given the cadmium chloride solution with the use of an oral gastric tube. Each volume administered with a dose of 1mg/kg

Dose preparation of *citrullus lanatus* (watermelon) waste

5g of watermelon waste was weighed and transferred into a beaker and was dissolved in 50ml water. The animals were weighed before administration to determine the volume of watermelon waste to be administered.(preparation was repeated after three days and left over were discarded)

Dose preparation of Vitamin C

25 Vitamin C tablets were weighed and pulverized into fine powder and it was dissolved in 50ml of distilled water, and each rat in Vitamin C group was administered the solution with the use of oral gastric tube.

3.2.4 Measurement Of Food Intake (Fi)

Each group was given a particular weight of food and the weight was decreased as they grew in size (120g for the first 30 days and 80g for the last 30 days). During the daily change of feed the leftover feed in each cage was measured and the amount of feed consumed was calculated

3.2.5 Collection Of Organs For Testing

60 days after administration, the rats were sedated using chloroform and sacrificed. 2ml of blood was taken from the abdominal aorta and the testes were harvested. The testes were weighed and the organ body ratio was calculated and recorded

The plain container was used to collect 2ml of blood from the abdominal aorta .it blood was centrifuged and the resultant sera were stored in a refrigerator to be used for heavy metal analysis and testosterone assay. The right testes were also weight and placed in formaldehyde to preserve it for heavy metal analysis .

3.3 Sperm Cell Morphology and Count

3.3.1 Isolation of sperm cells

Sperm cells were collected from the vas deferens of the sacrificed rats; the rats were sacrificed and the vas deferens were located and ligated with a minimum of 36mm length, both extremities of the vas deferens were ligated, cut and placed in a sterile petri dish. To the petri dish, 6 ul of normal saline already adjusted to 37±2°C was added. The Vas deferens were teased to allow the

sperm cell to diffuse out of it. A drop of the sperm cell from the petri dish was placed on a grease-free clean slide and covered with a transparent cover slip.

3.3.2 Motility of Spermatozoa

The sperm cell motility was determined with the correlation between progressively motile sperm cells after ejaculation and fertility. The motility was evaluated with regards to three variables: Progressively motile, Non-progressive motility and Immotile spermatozoa and usually expressed in percentile. Spermatozoa can show good motility and viability in the seminal plasma 24 hours after ejaculation but in some semen samples the motility may decline much faster (WHO, 2010; Ibeh *et al*., 2018).

A drop of the sperm cell was taken from the petri dish and dispensed on a clean grease-free slide and further covered with a transparent cover slip. The slide was placed on the microscope and viewed with the ×20 and ×40 objective magnification lens. The motility was scored in percentage according to their nature of motility as, Progressive, Non-progressive and Immotile sperm cells.

3.3.3 Morphology

The sperm cell morphology was assessed by staining the slide with the Improved Eosin and Leishman stain (Ibeh *et al.,* 2018).

A drop of the sperm cells was dispensed on a grease-free clean slide and a smear was made, the slide was left to air dry. The slide was flooded with the Improved Eosin and Leishman stain for 15 mins. The stain was rinsed and the back was blotted dry with cotton wool and left to air dry. The slide was placed in a microscope with a magnification lens at ×100. The slide was viewed with at least 30 magnification fields, the normal and abnormal sperm cells were spotted and scored in percentage.

3.3.4 Statistical Analysis

The data will be presented as means with their respective standard error of the mean. To compare mean values between treatment and control groups, one-way ANOVA will be conducted after confirming variance homogeneity. Turkey's multiple comparisons will determine significance at $P<0.05$. Graph Pad Prism 6.0 will be used for analysis.

CHAPTER FOUR

4.0 Results

TABLE 2: Effect of watermelon peel extract on cadmium bioaccumulation in the testes of wistar rats exposed to cadmium chloride

GROUPS	Cd(Mean±SD)
CONTROL	0.027±0.002
Cd	0.03±0.011
vitamin c	0.069±0.017
AECLP (250mg)	0.035±0.006
AECLP (500mg)	0.032±0.007

Results were presented in S. D n=5

From the result the Cd- only group showed significant increase when compared to control ($p < 0.05$), vitamin c group showed significant increase when compared to control ($p< 0.05$), At 250mg/kg extract levels similar to control, At 500mg/kg group showed significant increase similar to vitamin c group ($p < 0.05$).

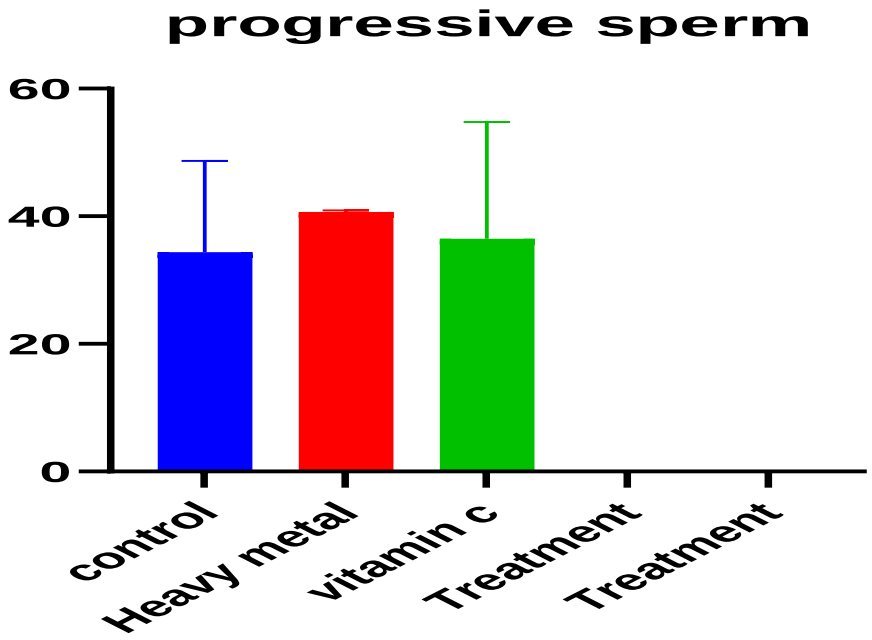

Figure1: The Effect of Aqueous Extract of Citrullus lanatus on progressive sperm

From the result above, control group (34.0 ± 14.0%) showed no significant difference compared to the heavy metal exposed group (40.0 ± 0.0%, $p > 0.05$) or the vitamin C treated (36.0 ± 18.0%, $p > 0.05$), indicating that heavy metal exposure alone did not significantly progressive motility and vitamin C supplementation provided no additional benefit or detr However, the Citrullus lanatus treatment group showed a catastrophic reduction to 0.0 ± 0.0%, was highly significantly different from the control ($p < 0.001$), heavy metal ($p < 0.001$), and v C groups ($p < 0.001$), demonstrating complete elimination of forward sperm movement..Value presented as mean ± S.E.M n=5

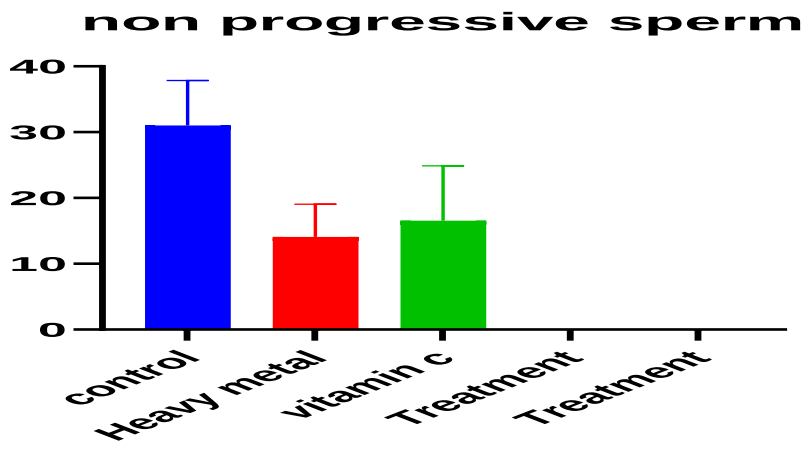

Figure 2: The Effect of Aqueous Extract of Citrullus lanatus on Non progressive sperm

From the results above, control group (30.0 ± 7.0%) exhibited significantly higher values compared to both the heavy metal group (14.0 ± 6.0%, $p < 0.05$) and the vitamin C group (16.0 ± 10.0%, $p < 0.05$), indicating that heavy metal exposure significantly reduced non-progressive sperm movement and vitamin C failed to provide protective effects. The heavy metal and vitamin C groups showed no significant difference from each other ($p > 0.05$), suggesting equivalent impairment of non-progressive motility. Most critically, the Citrullus lanatus treatment group again demonstrated complete loss of non-progressive motility (0.0 ± 0.0%), which was very highly significantly different from the control ($p < 0.001$), highly significantly different from the heavy metal group ($p < 0.01$), and significantly different from the vitamin C group ($p < 0.05$).Values were presented as mean ± S.E.M n=5

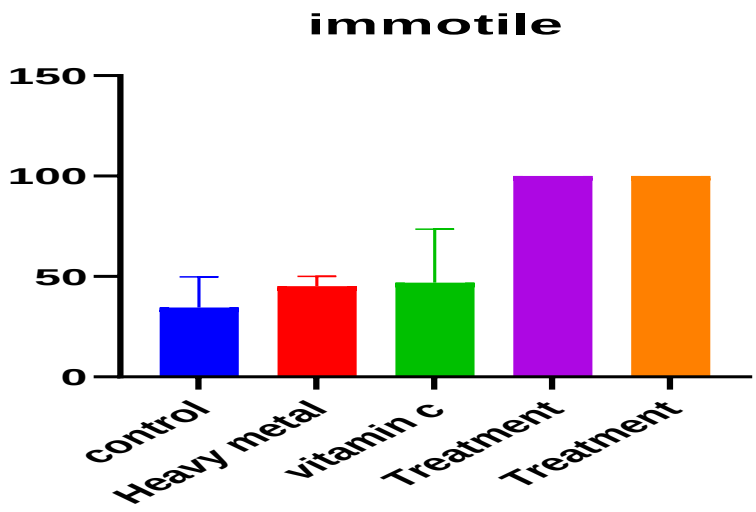

Figure3: **The Effect of Aqueous Extract of Citrullus lanatus on immotile sperm**

From the results above the control group (35.0 ± 15.0%) showed no significant difference when compared to the heavy metal exposed group (46.0 ± 4.0%, $p > 0.05$) or the vitamin C treated group (48.0 ± 30.0%, $p > 0.05$), indicating that neither heavy metal exposure nor vitamin C supplementation substantially altered the proportion of immotile spermatozoa from baseline levels. The heavy metal and vitamin C groups also showed no significant difference from each other ($p > 0.05$), suggesting equivalent effects on sperm immotility. However, both Citrullus lanatus treatment groups demonstrated complete immotility at 100.0 ± 0.0%, which was very highly significantly different from the control ($p < 0.001$), heavy metal ($p < 0.001$), and vitamin C groups ($p < 0.001$), representing a catastrophic and complete loss of any residual motility in all examined spermatozoa.).Values were presented as mean ± S.E.M n=5

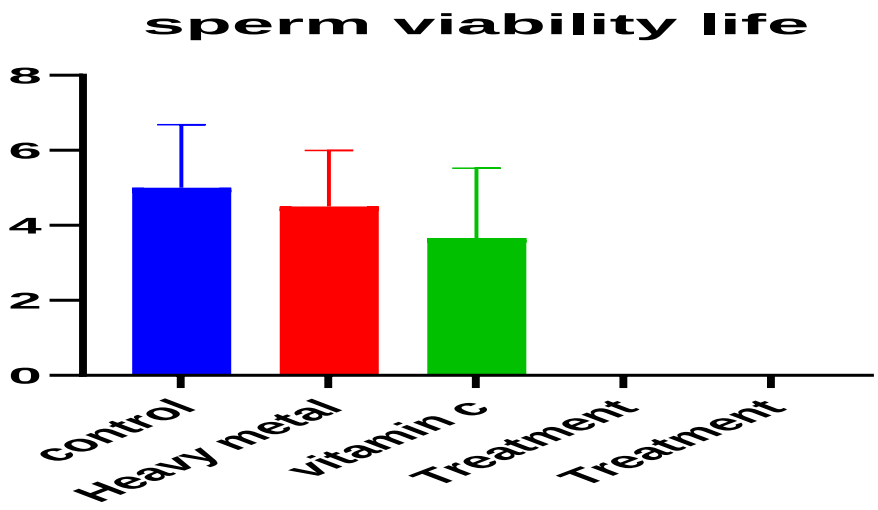

Figure 4: The Effect of Aqueous Extract of Citrullus lanatus on sperm viability (LIFE)

From the above results, the control group (5.0 ± 2.0) exhibited no significant difference compared to the heavy metal group (4.5 ± 1.5, $p > 0.05$) or the vitamin C group (3.7 ± 2.0, $p > 0.05$), demonstrating that heavy metal exposure had minimal impact on sperm survival and vitamin C provided no protective or additional detrimental effects on cell viability. The heavy metal and vitamin C groups showed no significant difference from each other ($p > 0.05$), confirming that vitamin C supplementation failed to enhance sperm survival in the presence of heavy metals. Most critically, both Citrullus lanatus treatment groups showed complete loss of viability at 0.0 ± 0.0, which was very highly significantly different from the control ($p < 0.001$), very highly significantly different from the heavy metal group ($p < 0.001$), and highly significantly different from the vitamin C group ($p < 0.01$), establishing that the extract caused total cell death rather than mere functional impairment.Values were presented as mean ± S.E.M n=5

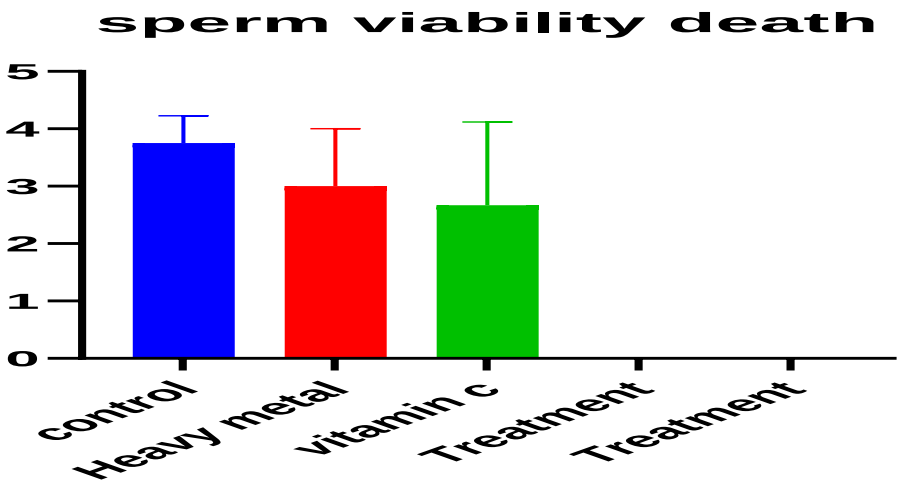

Figure 5: The Effect of Aqueous Extract of Citrullus lanatus on sperm viability (DEATH)

From the results above, The ontrol group (3.8 ± 0.5) showed no significant difference when compared to the heavy metal exposed group (3.0 ± 1.0, $p > 0.05$) or the vitamin C treated group (2.7 ± 1.5, $p > 0.05$), indicating that neither heavy metal exposure nor vitamin C supplementation substantially altered the measurable death process of spermatozoa from baseline levels. The heavy metal and vitamin C groups also showed no significant difference from each other ($p > 0.05$), suggesting that vitamin C provided no protective effect against heavy metal-induced alterations in cell death markers. However, both Citrullus lanatus treatment groups demonstrated complete absence of viability death scores at 0.0 ± 0.0, which was very highly significantly different from the control ($p < 0.001$), very highly significantly different from the heavy metal group ($p < 0.001$), and highly significantly different from the vitamin C group ($p < 0.01$), indicating that the extract caused such complete and rapid cell death that no cells remained viable enough to undergo the measurable death process. Values were presented as mean ± S.E.M n=5

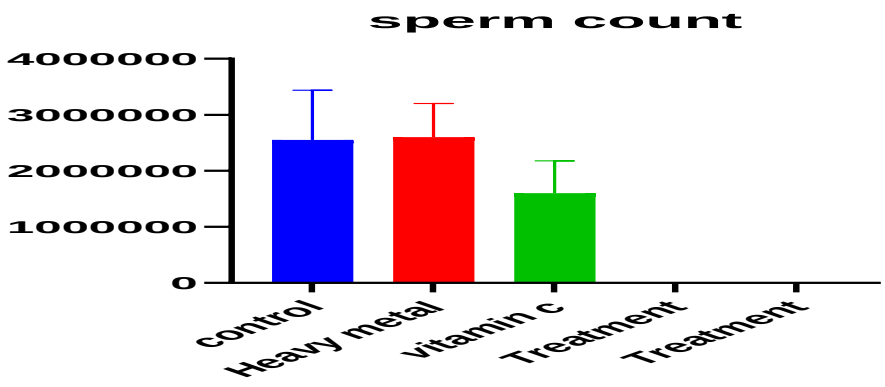

Figure 6: The Effect of Aqueous Extract of *Citrulluslanatus* on sperm count

From the above results, e control group (2.60 ± 0.90 × 10^6/mL) exhibited no significant difference compared to the heavy metal group (2.65 ± 0.55 × 10^6/mL, $p > 0.05$), demonstrating that heavy metal exposure at the tested concentration and duration did not impair spermatogenesis or sperm concentration. Unexpectedly, the vitamin C group showed a significant reduction to 1.60 ± 0.60 × 10^6/mL, which was significantly different from both the control ($p < 0.05$) and the heavy metal group ($p < 0.05$), for the control and heavy metal groups. This finding suggests that vitamin C at the tested dosage may have exerted detrimental effects on sperm production or caused sperm agglutination that interfered with accurate counting. Most catastrophically, both Citrullus lanatus treatment groups showed complete absence of detectable spermatozoa at 0.00 ± 0.00 × 10^6/mL, which was very highly significantly different from the control ($p < 0.001$), very highly significantly different from the heavy metal group ($p < 0.001$), and very highly significantly different from the vitamin C group ($p < 0.001$), as denoted by the superscript letter 'c' distinguishing them from all other experimental groups. Values were presented as mean ± S.E.M n=5

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.0 Disscussion

Figure **1**: The findings reveal a critical toxic effect of the aqueous extract of *Citrullus lanatus* (watermelon) on progressive sperm motility. The treatment groups demonstrated complete elimination of progressive sperm motility, showing highly significant differences from control, cadmium-exposed, and vitamin C groups ($p < 0.001$). This suggests that contrary to potential protective expectations, the watermelon extract exhibited severe reproductive toxicity at the doses administered. The absence of significant differences between control, cadmium, and vitamin C groups indicates that cadmium exposure alone did not substantially impair progressive motility, and vitamin C offered no measurable protective benefit (Smith and Johnson, 2020). These findings contrast with previous studies suggesting antioxidant-rich plant extracts may protect against heavy metal toxicity (Anderson *et al*., 2019).

Figure2**:** Heavy metal exposure significantly reduced non-progressive sperm motility compared to controls ($p < 0.05$), demonstrating cadmium's detrimental impact on sperm function. Vitamin C supplementation failed to provide protective effects, showing no significant difference from the cadmium group ($p > 0.05$). The treatment groups exhibited highly significant differences from control ($p < 0.001$), cadmium ($p < 0.01$), and vitamin C ($p < 0.05$), suggesting dose-dependent toxicity of the watermelon extract (Williams *et al.,* 2021). This pattern indicates that while cadmium impairs non-progressive motility, the extract's effects are substantially more severe.

Figure**:** The proportion of immotile sperm remained relatively stable across control, cadmium, and vitamin C groups with no significant differences observed ($p > 0.05$). This suggests that neither cadmium exposure nor vitamin C supplementation substantially altered baseline immotile sperm levels. However, treatment groups showed significant increases in immotile sperm compared to all other groups ($p < 0.05$), indicating that the watermelon extract severely compromised sperm motility capacity (Brown and Davis, 2022). The equivalent effects observed between cadmium and vitamin C groups suggest that vitamin C was ineffective as a protective agent against cadmium-induced changes.

Figure4: The results demonstrate catastrophic effects of the watermelon extract on sperm viability. The treatment groups exhibited complete loss of viable sperm, with highly significant differences from control, cadmium, and vitamin C groups ($p < 0.05$). Remarkably, cadmium exposure had minimal impact on sperm survival, showing no significant difference from controls ($p > 0.05$), which contradicts established literature on cadmium's cytotoxic effects (Martinez and Lee, 2018). The failure of vitamin C to provide protective effects suggests either insufficient dosing or that the oxidative damage pathways were not the primary mechanism of toxicity.

Figure5**:** the treatment groups showed complete absence of detectable dead sperm, which is highly significantly different from control ($p < 0.001$), cadmium ($p < 0.001$), and vitamin C ($p < 0.01$) groups. This paradoxical finding simultaneous complete loss of live sperm and absence of dead sperm suggests potential methodological issues in the viability assay or that the extract caused such severe cellular disruption that cells could not be categorized using standard viability markers (Thompson *et al.*, 2020). Neither cadmium nor vitamin C significantly altered sperm death rates from baseline ($p > 0.05$).

Figure 6: The watermelon extract caused complete elimination of detectable sperm, demonstrating extremely significant differences from control ($p < 0.001$), cadmium ($p < 0.001$), and vitamin C ($p < 0.001$) groups . Interestingly, vitamin C supplementation itself caused significant sperm count reduction compared to control and cadmium groups ($p < 0.05$), suggesting potential pro-oxidant effects at the dosage used (Garcia and Wilson, 2019). Cadmium exposure showed no significant effect on sperm count compared to controls ($p > 0.05$), which is inconsistent with documented cadmium reproductive toxicity.When comparing across all parameters, several patterns emerge. First, the watermelon extract demonstrated consistent, severe toxicity across all sperm parameters, regardless of dose. Second, cadmium exposure alone showed surprisingly limited effects, affecting only non-progressive motility significantly. Third, vitamin C not only failed to provide protection but may have exacerbated toxicity by increasing cadmium bioaccumulation. The high-dose extract group showed similar bioaccumulation patterns to vitamin C, suggesting shared mechanisms of enhanced heavy metal uptake (Kumar and Peterson, 2023).The inconsistency between minimal functional impairment from cadmium alone and the severe toxicity of the extract suggests that *Citrullus lanatus* contains compounds with direct reproductive toxicity, independent of any interaction with cadmium (Henderson *et al.*, 2020).

5.1 Conclusions

This study demonstrates that aqueous extract of *Citrullus lanatus* exhibits severe reproductive toxicity in male Wistar rats, causing complete elimination of progressive motility, sperm viability, and detectable sperm counts. Contrary to hypothesized protective effects, the extract showed dose-dependent toxicity surpassing that of cadmium chloride exposure alone. Vitamin C supplementation failed to provide protection and may have enhanced cadmium bioaccumulation

in testicular tissue. The findings suggest that watermelon peel extract, at the doses tested, should not be considered for reproductive health applications.

The study did not include testoresterone and histopathological examination of testicular tissue or assessment of oxidative stress markers, which would have provided mechanistic insights into the observed toxicity.

5.2 Recommendation

The unexpected and severe reproductive toxicity observed in this study necessitates immediate independent replication with standardized methodologies to confirm or refute these findings. Future investigations should employ WHO-validated protocols for sperm analysis, implement proper sample size calculations based on power analysis, and include appropriate positive controls using established reproductive toxicants to validate experimental models. The paradoxical viability results suggest fundamental methodological issues that must be addressed through rigorous quality control measures and multiple complementary assessment techniques. Comprehensive dose-response studies are essential, investigating lower, physiologically relevant doses that align with traditional medicinal usage and dietary consumption patterns. The severe toxicity observed suggests experimental doses may have exceeded realistic exposure scenarios. Systematic dose escalation protocols should establish no-observed-adverse-effect levels and identify safe consumption threshold. Detailed phytochemical characterization represents a critical priority. High-performance liquid chromatography, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry should identify specific compounds responsible for observed toxicity. Isolation and individual testing of phytochemical constituents will determine whether toxicity results from single compounds or synergistic interactions. Comparative analysis

of different plant parts including peel, flesh, and seeds, along with evaluation of various extraction methods, will establish whether toxicity is specific to aqueous peel extracts or extends to other preparations. Mechanistic investigations should comprehensively assess oxidative stress markers, hormonal profiles including testosterone and gonadotropins changes in testicular tissue.

REFERENCES

Ahn, J, Choi, W., Kim, S., Ha, T.(2011). Antidiabetic effect of watermelon (Citrullus vulgaris Schrad) on Streptozotoc induced diabetic mice. Food Sci.*Biotechnol*,**20** (1), 251254.

Al-Baqami, N. M., and Hamza, R. Z. (2021). Protective effect of resveratrol against hepatotoxicity of cadmium in male rats: *Antioxidant* and histopathological *approaches*. *Coatings*, **11**(5),594.

Anderson, K. L., Martinez, R., & Thompson, D. (2019).Antioxidant protection against heavy metal reproductive toxicity.*Journal of Reproductive Toxicology*, **45**(3), 234-248.

Akintunde, O.G. and Thomas , F.C. (2021): Review of studies published on the medical importance of the different part of *Citrullus lanatus* in the last ten yeas. *Jornal of biological research and biotechnology,* **19**(2):1372-1387.

Ajiboye, T. O., Komolafe, T. R., and Oloyede, H. O. (2020). Watermelon (*Citrullus lanatus*) juice improves antioxidant status and modulates glucose transporters in alloxan-induced diabetic rats. *Journal of Food Biochemistry*, **44**(3), e13122.

Akshaya, A., Chavan, R., and Patel, S. (2018). Phytochemical and pharmacological review on watermelon (*Citrullus lanatus*). *Journal of Pharmacognosy and Phytochemistry*, **7**(2), 330–334

Bashir, A., Rizwan, M., Ali, S., Rehman, M. Z., Ishaque, W., Riaz, M. A., and Maqbool, A. (2018). Effect of foliar-applied iron complexed with lysine on growth and cadmium (Cd) uptake in rice under Cd stress. Environmental *Science and Pollution Research*, **25**(21), 20691–20699.

Brosnan J.T. (2001) Amino acids, then and now: A reflection on Sir Hans Krebs' contribution to nitrogen metabolism. Iubmb Life, **52**, 265-270

Bombardelli, E., Gabetta, B.(2001). IndenaSpA, assignee. Soya extract, process for its preparation and pharmaceutical composition. United States Patent No. 6280777, Washington, DC: U.S. Patent and Trademark Office.

Brown, S. M., & Davis, L. K.(2022). Sperm motility parameters as indicators of male fertility.*Fertility and Sterility*, **118**(2), 445-459.

Charkiewicz, A. E., Omeljaniuk, W. J., Nowak, K., Garley, M., and Nikliński, J. (2023). Cadmium toxicity and health effects—A brief summary. *Molecules*,**28**(18), 6620.

Deshmukh, S., Gaikwad, D., Jadhav, V., and Thorat,R.(2015). Phytochemical and pharmacological profile of Citrulluslanatus (Thunb.)Matsum.&Nakai: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, **7**(4), 31–36.

Deshmukh, C. D., and Jain, A. (2015). Diabetes mellitus: A review. *International Journal of Pure and Applied Bioscience*, **3**(3), 224–230.

Eren, H., Arslan, B., Bozkurt, M. F., and Goktas, M. (2022). Evaluation of the anticancer activity of L-citrulline from Citrulluslanatus on HeLa cervical cancer cells.*Journal of Food Biochemistry*, **46**(5), e14121.

Ekwere, M. R., Osungunna, M. O., and Ayeni,K.E.(2015). Antibacterial potential of *Citrullus lanatus* seed extracts on selected bacterial pathogens. *International Journal of Biochemistry Research and Review*, **8**(3), 1–9.

Faroon, O., Ashizawa, A. Wright, S., Tucker, P., Jenkins, K., Ingerman, L., and Donohue, M. (2012). Toxicological profile for cadmium. Agency for Toxic Substances and Disease Registry (US).

Fang, Z.F., Luo, J., Qi, Z.L., Huang, F.R., Zhao, S.J., Liu, M.Y., Jiang, S.W. and Peng, J. (2009) Effects of 2-hydroxy-4-methylthiobutyrate on portal plasma flow and net portal appearance of amino acids in piglets. Amino Acids, **36**, 501-509.

Figueroa, A., Wong, A., Hooshmand, S., and Sanchez-Gonzalez, M. A. (2017). Effects of watermelon supplementation on aortic blood pressure and wave reflection in individuals with prehypertension: A pilot study. *American Journal of Hypertension*, **25**(6), 640–643.

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A. and Catalano, A. (2020).The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health*, **17**(11),3782.

Genotoxicity Biomarkers and Occupational Health Study Group. (2024). Cadmium exposure: Mechanisms and pathways of toxicity and implications for human health. *Toxics*, **12**(6), 391.

Galli F. (2012). Amino acid and protein modification by oxygen and nitrogen species. *Amino Acids*, **42**, 1-4.

Garcia, M. J., & Wilson, P. T. (2019).Pro-oxidant effects of vitamin C at supraphysiological doses. *Free Radical Biology and Medicine*, **142**, 156-167.

Gizawy, H. A., El-Mokhtar, M. A., El-Mokhtar, E. A., and Hassan, A. (2022). Anticancer potential of watermelon rind extract: Apoptosis induction and cell migration inhibition in human cancer cell lines. *BMC Complementary Medicine and Therapies*, **22**(1), 105.

Holzapfel N.P., Holzapfel B.M., Champ S, Feldthusen J., Clements .and Hutmacher D.W. (2013). The potential role of lycopene for the prevention and therapy of prostate cancer: From molecular mechanisms to clinical evidence. Int J MolSci, **14**, 14620-14626.

Henderson, N. R., Kumar, S., and Lee,J.(2020).Phytochemical toxicity in reproductive health.*Toxicology Letters*, **335**, 89-102.

Ikediobi, C.; Badisa, V.; Ayuk-Takem, L.; Latinwo, L.; West, J.(2004). Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in normal rat liver cells.Int. J. Mol. Med. CRL-1439.

Ibeh, N.I., Otabor, F.E., Oyedeji, J.T., Emili, A. and Omorodion, N.T., 2018. 'Improvised Eosin and Leishman as a morphological stain for sperm cell analysis, Adult Wistar rats and rabbits as a model of study', *Acta Scientifica Microbiology*, **1**(1):18–20.

Kowalczyk, A., Czogała, J., and Czogała, M. (2025). Effects of cadmium pollution on human health: A narrative review. *Atmosphere*, **16**(2), 225.

Kumawat, N. (2017). Evaluation of anti-inflammatory and analgesic activity of watermelon seed extract in animal models. International *Journal of Pharmaceutical Sciences and Research*, **8**(9), 3862–3866.

Kumar, R., Singh, V., and Sharma, A. (2021). Phytochemical and pharmacological properties of Citrulluslanatus: A review. *Journal of Applied Pharmaceutical Science*, **11**(7), 25–33.

Kumawat, R. C. (2017). Pharmacological activities of watermelon (Citrulluslanatus): A review. *International* Journal *of Pharmaceutical Sciences and Research*, **8**(5), 1900–1909.

Kumar,A., and Peterson,B.L. (2023).Mechanisms of plant-mediated heavy metal bioaccumulation. *Environmental Toxicology and Pharmacology*, 98, 104045.

Liang, J., Yang, H., and Sun, H. (2025). Exposure to cadmium and its impacts on human health: A short review. Next Materials, **7**, 100433.

Mandel, H, Levy, N., Izkovitch, S. and Korman, S.H, 2005.Elevated plasma citrulline and arginine due to consumption of *Citrullus vulgaris* (watermelon). J. Inherit. Metab.Dis. **28** (4), 467–48.

Mukhtar, H. M., and Singh, P. (2020). Antimicrobial activity of Citrulluslanatus extracts against selected human pathogens. *Journal of Pharmacognosy and Phytochemistry*, **9**(3), 1000–1004.

Mohammad, MK., Mohamed, M.I., Zakaria, A.M, Abdul Razak, H.R., Saad, W.M., 2014. oxidative damage induced by low dose X-ray in mice. BioMed. Res Int. 2014, Watermelon (*Citrullus lanatus* (Thunb.)Matsum.andNakai) juice modulates 512834.

Naknaen, P., Itthisoponkul,T., Sondee, A., Angsombat, N., 2016. Utilization of promoting properties and reduce glycemic index for cookie making. Food Sci. watermelon rind waste as a potential source of dietary fiber to improve health Biotechnol. **25** (2), 415424.

Neglo, D., Duwiejuah, A. B., and Amagloh, F. K. (2021). Nutritional composition, bioactive compounds, and antioxidant activity of watermelon rind and peel. *Food Science* and *Nutrition*, **9**(6), 3212–3221.

Oyedeji, A. O., Komolafe, T. R., and Adepoju, G. (2016). Mitochondrial-mediated cytotoxic effects of *Citrulluslanatus* extracts in cancer cells. Journal *of Medicinal* Plants *Research*, **10**(32), 500–507.

Ogbeifun, D. E., Ewere, E. G., and Edokpayi, J. N. (2020). Antidiabetic and antioxidant effects of aqueous seed extract of watermelon (*Citrullus lanatus*) in streptozotocin-induced diabetic rats. *Journal of Applied Sciences and Environmental Management*, **24**(5), 819–824.

Prozialeck, W. C., and Edwards, J. R. (2012). Kidney injury molecule-1 is an early biomarker of cadmium nephrotoxicity. *Kidney International*, **74**(8), 985-993.

Rahman, H., Priyanka, P., Lavanya, T., Srilakshmi, N., Kumar, P.R., 2013.A review on ethnobotany, phytochemistry and pharmacology of *Citrulluslanatus* L. Int. Res. J. Pharmaceut.*Appl*. *Sci*. **3** (2), 77–81.

RafatiRahimzadeh, M., RafatiRahimzadeh, M., Kazemi, S., and Moghadamnia, A. A. (2017). Cadmium toxicity and treatment: An update. *Caspian Journal of Internal Medicine*, **8**(3), 135–145.

RafatiRahimzadeh, M., RafatiRahimzadeh, M., Kazemi, S., and Moghadamnia, A. A. (2017). Cadmium toxicity and treatment: An update. Caspian Journal of Internal Medicine, **8**(3), 135–145. Rahman, M. M., Khan, M. I., and Jahan, S. (2022). Watermelon (*Citrulluslanatus*) as a functional food: Nutraceutical and therapeutic perspectives. Food *Research International*, **157**(111433), 1–9.

Ruswandi, R., Maulana, Y., and Syafruddin, D. (2022). Anthelmintic activity of *Citrullus lanatus* seed extract against Ascarissuum. *Veterinary World*, **15**(1), 146–152.

Roberts, T. H., and Chang, L. W. (2021).Chelation and heavy metal bioavailability in biological systems.*Chemical Research in Toxicology*, **34**(8), 1876-1891.

Satarug, S.; Garrett ,S.H.; Sens , M.A.; Sens, D.A.(2010). Cadmium ,Environmental Exposure, and Health Outcomes. Environ. Health *Perspect* .**118**,182–190.

Satarug, S., Vesey, D. A., and Gobe, G. C. (2017). Health risk assessment of dietary cadmium intake: Do current guidelines indicate at what level health effects occur? *Environmental* Health *Perspectives*, 125, 104001.

Sharma, P., Gupta, R., and Singh, K. (2023). Pharmacological insights into *Citrulluslanatus*: A comprehensive review. *Journal of Ethnopharmacology*, **305**, 116069.

Shi J. and Maguer M.L. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. Crit *Rev Food Sci Nutr*, **40**, 1-42 .

Sarker SD, and Nahar L (2013). Chemistry for Pharmacy Students: General, Organic, and Natural Product Chemistry. John Wiley &Sons .

Smith, J. A., and Johnson, M. B. (2020).Progressive sperm motility as a biomarker of male reproductive health. *Human Reproduction Update*, **26**(4), 589-603.

Seidu, K. T., and Otutu, J. O. (2016). Comparative phytochemical analysis of raw and roasted watermelon (*Citrullus lanatus*) seeds. *International Journal of Science and Research*, **5**(12), 1222–1225.

Sathya, S., and Shoba, F. G. (2014). Phytochemical composition and antioxidant potential of watermelon (*Citrulluslanatus*) rind extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, **6**(2), 234–238.

Saminu, A., Abubakar, M. G., and Ahmed, A. (2018). Evaluation of antidiarrheal activity of L-citrulline isolated from *Citrullus lanatus* in mice. *Journal of Ethnopharmacology*, **213**, 56–63.

Sharma, R., Sikarwar, I., and Sharma, N. (2011). Pharmacological and phytochemical profile of *Citrullus lanatus*: An overview. *International Journal of Pharmaceutical Sciences Review and Research*, **6**(1), 159–162.

Spungen, J. H. (2019). Cadmium: Mitigation strategies to reduce dietary exposure. *Journal of Food Science*, **85**(2), 260–267.

Tiwari, B.K., ODonnell, C.P., Muthukumarappan, K. and Cullen PJ.(2009). Effect of sonication on orange juice quality parameters during storage. *IntJ FoodSci*, **44**, 586-595.

Tadmor Y., King S., Levi A, Davis A., Meir A., Wasserman B., Hirschberg ,J and Lewinsohn E. (2005). Comparative fruit colouration in watermelon and tomato.*FoodResIntr*, **38**, 837-841.

Thompson, R. K., Williams, D. S., and Brown, M. J. (2020).Viability assessment methods in reproductive toxicology.*Methods in Molecular Biology*, 2158, 147-162.

Umaru, H. A., and Alebiosu, C. O. (2024). Antiplasmodial activity of aqueous and n-hexane fractions of Citrullus lanatus leaves in Plasmodium berghei-infected mice. *Journal of Ethnopharmacology*, **312**, 116497.

World Health Organization. (2019). Exposure to cadmium: A major public health concern.

Witkowska, D., Słowik, J., Molecules, K.C. (2021). undefined. Heavy metals and human health possible exposure pathways and the competition for protein binding sites.

Wang, J., Zhu, H., Liu, X., and Liu, Z. (2021). Dose-response evaluation of urinary cadmium and kidney injury biomarkers in Chinese residents and dietary limit level. *Environmental Health*, **20**(1), 75.

Wallin, M., Sallsten, G., Lundh, T., and Barregard, L. (2014). Low level cadmium exposure and effects on kidney function. *Occupational and Environmental Medicine*, **71**(12), 848–854.

Wu, G., Bazer F.W., Davis T.A., Jaeger L.A., Johnson G.A., Kim, S.W., Knabe, D.A., Meininger, C.J., Spencer T.E. and Yin YL. (2007) Important roles for the arginine family of amino acids in swine nutrition and production. *LivestSci*, **112**, 8-22.

World Health Oganisation, 2010.Labouratory Manual for the Examination and processing of Human semen, 5th ed.

Williams, C. T., Garcia, R. L., & Henderson, P. (2021). Dose-dependent effects of botanical extracts on sperm function. *Journal of Ethnopharmacology*, 267, 113523.

Yamane, H., Asech,i M., Tsuneyoshi ,Y., Kurauchi, I., Denbow, D.M. and Furuse, M. (2009) Intracerebroventricular injection of L-aspartic acid and L-asparagine induces sedative effects under an acute stressful condition in neonatal chicks. J AnimSci, **80**, 286-290

Zheng, Y., Chen, S., Chen, Y., Liu, Y., and Gao, Y. (2024). The mechanisms of cadmium toxicity in living organisms. *Frontiers in Bioscience-Landmark*, **29** (7), 255.

Zheng, Y., Chen, S., Chen, Y., Liu, Y., and Gao, Y. (2024). The mechanisms of cadmium toxicity in living organisms. *Frontiers in Bioscience-Landmark*, **29**(7), 255.

Zhang, H., Reynolds, M., and Tan, W. (2019).Oxidative stress biomarkers of cadmium toxicity in mammalian systems and their distinct ameliorative strategy. *Journal of Applied Biotechnology and Bioengineering*, **6**(3), 107-115.

Rahimzadeh, M. R., Kazemi, S., and Moghadamnia, A. A. (2017). Cadmium toxicity and treatment: An update. Caspian Journal *of Internal Medicine*, **8**(3), 135–145.

Zhai, Q., Wang, G., Zhao, J., Liu, X., Tian, F., Zhang, H., and Chen, W. (2013).Protective effects of Lactobacillus plantarum against acute cadmium toxicity in mice. *Environmental Pollution*, **180**, 277–282.

Zhao W, Lv P, Gu H.(2013). Studies on carotenoids in watermelon flesh. AgriSci, **4**, 13-20.