HARNESSING WATERMELON RIND EXTRACT AS A HEPATOPROTECTIVE AGENT IN CADMIUM EXPOSURE: HISTOPATHOLOGICAL PERSPECTIVE

BY

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LSC2009857

PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN,

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILMENT FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) SCIENCE LABORATORY TECHNOLOGY OF THE UNIVERSITY OF BENIN, BENIN CITY

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CERTIFICATION

This is to certify that this project work was carried out by **Blessing NDUKA** with matriculation number, **LSC2009857** of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

I dedicate this WORK to GOD ALMIGHTY, who has blessed and sustained me through this work and has been my source of inspiration and strength.

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ABSTRACT

Cadmium (Cd) is a toxic heavy metal with a long biological half-life that accumulates in vital organs, particularly the liver, causing oxidative stress and liver damage. Conventional treatments are expensive and often have side effects, creating a need for safer, affordable alternatives. This study evaluated the hepatoprotective potential of hydro-ethanolic extract of watermelon (*Citrullus lanatus*) rind against cadmium-induced liver toxicity in Wistar rats. Twenty-five Wistar rats (104-196g) were divided into five groups: negative control (distilled water), positive control (cadmium chloride), standard treatment (cadmium + vitamin C), and two treatment groups receiving cadmium plus watermelon rind extract at 250 mg/kg and 500 mg/kg respectively. After 60 days of oral administration, liver tissues were examined for histopathological changes, and body weight, hepatic cadmium levels, and relative liver weights were measured. Histological examination revealed normal liver architecture in all groups, with hepatocytes showing normal appearance without necrosis or inflammation. Extract-treated rats, especially at 500 mg/kg, maintained body weights similar to controls, suggesting protection against cadmium induced weight loss. Hepatic cadmium levels were lowest in the 500 mg/kg extract group, indicating enhanced metal elimination. Relative liver weights in extract-treated groups were similar to controls, preventing cadmium-induced liver enlargement. These findings demonstrate that watermelon rind extract provides hepatoprotection against cadmium toxicity, likely through antioxidant activity and metal chelation. The study supports watermelon rind as a cost effective, sustainable natural alternative for managing heavy metal toxicity and promotes the use of agricultural waste for therapeutic purposes.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Heavy metal pollution, particularly cadmium (Cd), poses a significant threat to public and environmental health due to its extremely long biological half-life (approximately 10-30 years in humans) and low rate of excretion from the body. Cadmium is absorbed in significant quantities from cigarette smoke, food, water, and air contamination and is known to have harmful effects in both humans and animals. Cadmium is used for anti-corrosion coating of steel sheets and other metals. They are used by the petroleum, automotive, electric, and electronic industries. It acts as an excellent protective coating in an alkaline environment and is also commonly used to produce low-melting alloys such as wood's metal used in fire protection systems (Tchounwou *et al*., 2012). Studies have demonstrated that cadmium uptake occurs mainly via inhalation through the respiratory system (approximately 13-19% absorption) and via ingestion through the digestive system (10-44% absorption) when dust particles are swallowed (Charkiewicz *et al*., 2023; Rasin *et al.*, 2025). This element accumulates most often in the lungs, liver, pancreas, kidneys, testicles, adipose tissue and skin inhibiting the activities of sulphur containing enzymes (Tchounwou *et al.,* 2012; Genchi *et al.,* 2020; Qing *et al.,* 2021). Toxic effects of cadmium include carcinogenicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive dysfunction, and oxidative stress (Rani *et al*., 2014).

Watermelon (*Citrullus lanatus) is a member of the Cucurbitaceae family, which originates from the tropics and is cultivated in warm temperature zones*. It is divided into three parts: the pulp, seed, and rind. The pulp or flesh of watermelon is commonly consumed raw or used in juices and salads, while the seeds and rind are often discarded as waste due to their bland taste and limited

awareness of their nutritional value. However, watermelon is known for its antioxidant properties, attributed mainly to its high vitamin C content. Studies have shown that vitamin C levels in the fruit range from 2.50 to 8.30 mg/100g, while the rind contains even higher concentrations, exceeding 5.35 mg/100g (Abu- Hiamed, 2017).

Citrullus lanatus (Watermelon) rind accounts for almost 40% of the total weight (Kataria and Kaur 2023). It contains a wide range of nutritional components and bioactive compounds, including flavonoids, phenolic acids, citrulline, saponins, carotenoids, and cucurbitacins, which exhibit antioxidant, anti-inflammatory, and detoxifying effects.

1.2 Statement of Problem

Cadmium exposure remains a primary environmental and health concern due to its widespread presence in industrial waste, contaminated food, and water. Its persistent accumulation in biological systems leads to oxidative stress and damages vital organs, especially the liver and kidneys, affecting overall cellular function. Although chelating agents are used to manage cadmium toxicity, they are often costly, not consistently effective, and may cause harmful side effects. This highlights the need for safer, affordable, and more accessible treatment options. While plant-based remedies are gaining attention for their antioxidant potential, the therapeutic benefits of watermelon rind, a rich source of natural phytochemicals, remain largely underexplored in combating cadmium-induced toxicity.

1.3 Significance of Study

This study investigates the therapeutic potential of hydro-ethanolic watermelon rind extract, a natural, cost-effective, and eco-friendly agricultural byproduct, in mitigating cadmium-induced hepatotoxicity in Wistar rats. Given cadmium's harmful effects and the limitations of conventional treatments, this research could provide scientific evidence for a sustainable plant-

based alternative, reducing reliance on synthetic drugs. The outcome could support the development of plant-based detox agents, encourage the sustainable use of biowaste, and offer practical solutions to reduce the health risks associated with heavy metal exposure, benefiting both medical science and ecological conservation.

1.4 Aim of Study

The study aims to evaluate the hepatoprotective potential of the hydroethanolic extract of watermelon rind against cadmium-induced liver toxicity in Wistar rats, using histopathological assessments .

1.5. Specific Objectives

1. To evaluate the effect of cadmium chloride on the livers ofWistar rats.

2 To examine and compare the histological architecture of liver tissues in Wistar rats across control, cadmium, standard, and extract-treated groups.

CHAPTER TWO

LITERATURE REVIEW

2.1. Heavy Metals

Heavy metals are a group of metallic chemical elements characterized by their high density (above 5g/m) and Toxicity. On the periodic table, heavy metals are found in periods 4-6 and groups 11-15, and they include transition metals, metalloids, basic metals, lanthanides, and actinides.

Heavy metals are classified into biological essential and non-biological essential heavy metals (Tchounwou *et al*., 2012; Dagdag *et al*., 2023). Biologically essential heavy metals are less toxic at low concentrations. They are required for biological processes in living organisms, for example, zinc (Zn), copper (Cu), and iron (Fe) (Jomova *et al*., 2025). Non-biological essential heavy metals are non-biodegradable metallic elements that are not required for biological processes in living organisms and can be toxic even at low concentrations. Non-biological essential heavy metals include arsenic, cadmium, lead, and mercury (Jomova *et al*., 2025).

2.2. Cadmium

Cadmium, with the chemical symbol Cd and an atomic number of 48, is a soft, silvery-white metal that closely resembles zinc and mercury, the other stable elements in group 12 of the periodic table. It typically exhibits a +2 oxidation state, similar to zinc. Like mercury, cadmium has a relatively low melting point, distinguishing it from the transition metals in groups 3 to 11. Due to the lack of partially filled d or f electron orbitals in its elemental or common ionic forms, cadmium and its group 12 counterparts are often excluded from traditional transition metal classification. Found in the Earth's crust at concentrations ranging from 0.1 to 0.5 ppm, cadmium

was independently discovered in 1817 by Stromeyer and Hermann in Germany while analyzing zinc carbonate impurities.

Cadmium, often referred to as a metal of the 20th century, is primarily obtained as a byproduct during the extraction of zinc. It naturally exists in the environment, being present in soil, rocks, coal, and certain mineral fertilizers. According to the Agency for Toxic Substances and Disease Registry (ATSDR), cadmium is the 7th-most-toxic heavy metal (Jaishankar *et al*., 2014). Due to its harmful health effects, cadmium and its compounds have been classified as Group 1 carcinogens (known human carcinogens) by the International Agency for Research on Cancer (Jomova *et al*., 2025).

Cadmium is released into the environment through both natural occurrences and human activities, exposing animals and humans in different ways. Water pollution by cadmium often results from industrial discharge, surface runoff, and its accumulation in soil and sediments. People can be exposed to cadmium by inhaling contaminated air, drinking polluted water, or eating cadmium-laced food (Almeer *et al.,* 2019; Mitra*et al*., 2022). Unlike some essential metals (such as iron or zinc), the human body has no physiological need for cadmium and no efficient mechanism for excreting it. Once absorbed, it accumulates over a lifetime, with a biological half-life estimated at 10-30 years (Sinicropi*et al.,* 2010;Bernhoft *et al*., 2013).

Cadmium enters the human body primarily through inhalation and ingestion, with minimal absorption through the skin. Breathing in cadmium-contaminated air (from cigarette smoke, industrial emissions, or welding fumes) allows cadmium particles to be absorbed quickly through the lungs into the blood stream.The percentage of inhaled cadmium dust absorbed ranges from 10% to 50%, depending on particle size (Jomova *et al*., 2025). Cadmium from contaminatedfood and water (e.g., rice, leafy vegetables, shellfish) is absorbed through the

gastrointestinal tract (Qu and Zheng, 2024;Jomova *et al*., 2025). Absorption through the digestive tract ranges from about 10–44% (Charkiewicz*et al*.,2023), depending on factors like nutritional status and the form of cadmium present.

Continuous exposure to low doses is linked to chronic respiratory illnesses such as asthma, bronchitis, and emphysema, as well as to high blood pressure (Genchi *et al.,* 2020;Charkiewicz *et al*., 2023). Prolonged contact with cadmium is also associated with severe health issues such as cancer, leukemia, kidney tubular disorder and genetic mutations (Genchi *et al.,*2020). Research has revealed that even small amounts of heavy metal exposure can damage human organs. In cases of acute ingestion, symptoms such as stomach pain, vomiting, nausea, dizziness, muscle spasms, shock, and seizures may occur shortly after exposure.

2.2.1. Industrial Uses of Cadmium

Despite cadmium's toxicity, it is widely used in various industrial applications due to its unique physical and chemical properties. One of its primary uses is in the production of nickel-cadmium (Ni-Cd) rechargeable batteries (Nordberg and Nordberg, 2022), which are commonly found in electronic devices, emergency systems, and power tools. It is also used as a pigment in the manufacture of plastics, ceramics, and glass, producing vibrant red, yellow, and orange colors that are stable under heat and light (Genchi *et al.,*2020).

Additionally, cadmium is employed in electroplating processes to provide corrosion resistance to metals, especially in aerospace and marine industries. It serves as a stabilizer in polyvinyl chloride (PVC) plastics (Godt *et* al., 2006), improving durability and resistance to degradation. Cadmium is also used in certain metal alloys, nuclear reactor control rods due to its neutron-absorbing ability, and in the production of thin-film solar panels using cadmium telluride. It also

finds applications in semiconductors and infrared devices.

2.2.2. Distribution and Accumulation of Cadmium

After cadmium enters the bloodstream, it binds primarily to proteins, such as those found in red blood cells or plasma (Bernhoft *et al*., 2013; Vacchi-Suzzi *et al.,* 2016). It is then transported throughout the body, where it follows a distinct distribution pattern. The liver is the first organ it reaches after being absorbed through the gastrointestinal tract (Godt *et* al., 2006), where it stimulates the synthesis of metallothionein, a protein that binds to heavy metals. This hepatic metallothionein cadmium complex is subsequently released into circulation and filtered by the kidneys, where it accumulates and causes nephrotoxicity. The majority of cadmium in the body, between 50% and 70% accumulates primarily in the kidneys and liver. The renal cortex, especially the proximal tubular cells, is particularly susceptible to cadmium build-up, making the kidneys a major target organ for toxicity. In these organs, zinc serves as a protective mechanism by stimulating metallothionein that binds to cadmium, helping to regulate its presence and partially reduce its toxicity (Yang and Shu, 2015). This accumulation can lead to long-term health effects due to cadmium's slow excretion and prolonged biological half-life.

2.2.3. Mechanism of Cadmium Toxicity

Cadmium harms biological systems through several molecular pathways, with oxidative stress being the most prominent. Unlike many other metals, cadmium cannot directly generate reactive oxygen species (ROS) due to its fixed +2 oxidation state but displaces other metallic ions such as zinc, iron, and copper from their protein complexes, thereby inducing oxidative stress (Jomova*et al*., 2025;Yang andShu, 2015). Cadmium triggers oxidative damage by weakening antioxidant defenses and disrupting mitochondrial electron transport (Thévenod and Lee, 2013).

Cadmium-induced oxidative stress also arises from the inhibition of antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase, leading to oxidative damage of lipids,

proteins, and DNA (Cannino *et al.,* 2009). It reduces intracellular glutathione (GSH) by binding to its -SH group and promoting its expulsion from cells, thereby compromising antioxidant defenses (Genchi *et al.,* 2020). It also interferes with mitochondrial function by inhibiting mitochondrial complex III, leading to the build-up of unstable semiquinone molecules. These accumulated semi-ubiquinones donate electrons to oxygen molecules, generating superoxide radicals and increasing the production of reactive oxygen species (ROS) (Wang *et al*., 2004; Zhao *et al*., 2022).

Beyond oxidative stress, cadmium also induces epigenetic alterations, including DNA methylation, histone modifications, and changes in microRNA expression. These shifts can silence tumor-suppressor genes and activate oncogenes, increasing cancer risk (Arita and Costa, 2009).Cadmium also acts as an endocrine disruptor, mimicking estrogen by binding to its receptors and altering hormones such as insulin, thyroid hormones, and steroids, thereby influencing multiple endocrine pathways (Genchi *et al*., 2020).

2.3 Histopathological Manifestations on Cadmium Hepatotoxicity

2.3.1 Acute Hepatotoxic Changes

Acute cadmium exposure produces characteristic histopathological changes in the liver. Light microscopy reveals hepatocellular necrosis, particularly in centrilobular regions where cadmium preferentially accumulates. The necrotic hepatocytes show loss of nuclear detail, cytoplasmic eosinophilia, and cellular swelling. Inflammatory cell infiltration, predominantly neutrophils and macrophages, was observed in necrotic and adjacent areas (Dudley *et al*., 1985).

Sinusoidal congestion and dilation are common findings, reflecting vascular dysfunction and disturbances in hepatic microcirculation. Kupffer cell hyperplasia and hypertrophy indicate activation of the innate immune response. Cytoplasmic vacuolation may be observed, representing hydropic degeneration or lipid accumulation (El-Refaiy *et al*., 2013).

2.3.2 Chronic Exposure Changes

Chronic cadmium exposure leads to progressive histopathological alterations. Chronic exposure promotes hepatic fibrosis characterized by excessive accumulation of extracellular matrix proteins, particularly collagen. This fibrotic response involves the activation of hepatic stellate cells, which transform into myofibroblast-like cells that produce large amounts of collagen (Liu *et al*., 2009).

Portal and periportal fibrosis develops initially, potentially progressing to bridging fibrosis that connects portal tracts and central veins. In severe cases, cirrhotic changes, including nodular regeneration and disruption of normal lobular architecture, may occur. Chronic inflammation with lymphocytic infiltration persists, and bile duct proliferation may be observed (Järup and Akesson, 2009).

2.3.3 Ultrastructural Changes

Electron microscopic studies reveal ultrastructural alterations in cadmium-exposed hepatocytes, including mitochondrial swelling with disrupted cristae, dilatation of the endoplasmic reticulum, disaggregation of ribosomes, and nuclear chromatin condensation.

2.4. Treatment of Cadmium-Induced Toxicity

The treatment of cadmium toxicity is primarily supportive, as there is no specific antidote for poisoning. The condition seriously affects the kidneys and respiratory system, making the

immediate and complete removal of the patient from the source of exposure the most critical first step. Management begins with a thorough initial assessment to determine the route of exposure, followed by prompt decontamination, such as gastric lavage for recent ingestions, and baseline laboratory testing.

Supportive care forms the cornerstone of treatment. For respiratory exposure, this involves monitoring for and managing complications such as pneumonitis or acute respiratory distress syndrome (ARDS), with oxygen support and possibly mechanical ventilation. The use of corticosteroids for lung inflammation remains controversial. In cases of ingestion, care focuses on gastrointestinal irrigation and symptomatic relief.

Chelating agents such as calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$), 2,3-dimercaptosuccinic Acid (DMSA), and 2,3-dimercapto-1-propanesulfonic acid (DMPS) form stable complexes with cadmium that can be excreted through urine (Flora, 2010). These agents are most effective when administered shortly after acute exposure but show limited efficacy in cases of chronic exposure, where cadmium has already accumulated intracellularly (Bernhoft, 2013). Chelation can mobilize cadmium from relatively inert storage sites, potentially increasing its toxicity to target organs; hence, it is not routinely recommended by major health bodies such as the CDC. Additionally, chelators can deplete essential metals and cause adverse effects, including renal damage, gastrointestinal disturbances, and allergic reactions. It may only be considered after a rigorous risk-benefit analysis for cases of severe acute poisoning with extremely high blood cadmium levels.

Antioxidant supplementation has been investigated as a protective strategy. Vitamin C and E, selenium, N-acetylcysteine, and other plant-derived antioxidants have shown protective effects in

experimental studies (Poli *et al*.,2022). These agents reduce oxidative damage by scavenging free radicals, enhancing endogenous antioxidant defenses, and preserving cellular glutathione levels. Novel approaches, such as nanoparticle-based antidotes for targeted metal removal, are still in experimental phases. Dietary interventions, including ensuring adequate intake of calcium, iron, and zinc, can help reduce cadmium absorption and serve as a supportive measure.

Long-term management is essential, particularly for chronic exposure, which often causes irreversible kidney damage. Ongoing care includes eliminating all exposure sources, annual monitoring of kidney function, and screening for bone density loss. The prognosis for acute toxicity is good with prompt care, but chronic toxicity can lead to progressive kidney disease requiring lifelong nephrology follow-up. Ultimately, prevention through occupational safety measures, smoking cessation, and environmental controls is the most effective strategy against cadmium-induced illness.

2.5. Watermelon (*Citrullus lanatus*)

Citrullus lanatus, commonly known as watermelon, gets its name due to its high water content of about 9% and its large, round, sweet flesh, which resembles that of a melon. The scientific name has roots in both Greek and Latin. "*Citrullus"* originates from the Greek word for citrus, referring to the fruit itself, while *"lanatus*" is a Latin term meaning "woolly," referencing the fine hairs found on the plant's stems and leaves.

2.5.1. Taxonomic Classification

Watermelon *(Citrullus lanatus*) belongs to the family Cucurbitaceae, which includes other important crops such as cucumbers, melons, and squashes. Watermelon is made up of the pulp, seed, rind, and peel. The genus *Citrullu s*comprises several species, with *C. lanatus* being the most economically important. The complete taxonomic classification is as follows:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Cucurbitales

Family: Cucurbitaceae

Genus: Citrullus

Species: C. lanatus (Erhirhie and Ekene, 2013).

PLATE 2.1:*Citrullus lanatus.* (Erhirhie and Ekene, 2013)

2.5.2 Origin and Distribution

Watermelon originated in Africa, with wild varieties still found in the Kalahari Desert. Historical records indicate that its cultivation began more than 4,000 years ago in areas such as Egypt and Sudan (Erhirhie and Ekene, 2013).

Currently, watermelon is grown across tropical and temperate regions worldwide. Leading producers include China, Turkey, Iran, Brazil, and the United States. With global production exceeding 100 million tons annually, watermelon ranks among the most significant horticultural crops worldwide (Paris, 2015).

2.5.3 Nutritional Composition

Watermelon flesh is composed of 92% water, making it a highly hydrating food. Despite its high water content, it contains valuable nutrients including vitamin A,B6, and C, as well as minerals such as potassium, magnesium, and trace amounts of zinc and iron. The flesh is low in calories (approximately 30 kcal per 100g) but rich in bioactive compounds (USDA, 2019).

2.5.4. Ethnomedicinal Value of *Citrullus lanatus* (Watermelon)

Citrullus lanatus (Watermelon) holds significant ethnomedicinal value across diverse traditional healing systems worldwide, serving as both food and medicine for thousands of years. Watermelon is traditionally used in folk medicine due to its abundance of bioactive compounds and, as a member of the Cucurbitaceae family, is a source of multiple minerals, vitamins, and proteins in the pulp, seed, and rind. The peel, rind, and seeds are usually discarded or used as animal supplements. The peels are known to have analgesic properties (Kumari *et al*.,2013), the rind possesses a vasorelaxant effect (Rimando and Perkins-Veazie, 2005), and the seed triggers the release of insulin from the islet of Langerhans, thereby regulating blood sugar level (Omigie and Agroreyo, 2014). Preliminary research suggests that eating watermelon may help lower

blood pressure, attributed to its high water content (Sharma *et al*., 2020). Watermelon is used in Northern Sudan to treat burns, swellings, rheumatism, and gout, and as a laxative (Gupta *et al.,* 2023).

The contemporary relevance of watermelon's ethnomedicinal applications is supported by modern scientific research, which confirms many traditional uses while identifying specific bioactive compounds responsible for therapeutic effects. Modern analysis shows that watermelon provides various health benefits, including hydration and vitamin C, serving as a source of antioxidants that fight free radicals and may play protective roles against many health conditions. The integration of traditional knowledge with contemporary research methodologies supports the continued use of watermelon in complementary healthcare approaches. At the same time, its accessibility and safety profile make it particularly valuable for communities seeking affordable, culturally appropriate therapeutic options that bridge traditional wisdom with evidence-based healthcare practices

2.6. Nutraceutical Properties of Watermelon

Phytochemicals in watermelon have been reported for their pharmacological activities and therapeutic properties, such as analgesic, laxative, gastroprotective, hepatoprotective, antimicrobial, anti-inflammatory, antioxidant, and antiulcer activities.

2.6.1. Antioxidant Activity

The pulp and rind of watermelon contain high amounts of antioxidants as a result of the pigments lycopene and β-carotene found in it. Lycopene is a carotenoid that provides multiple health benefits, neutralizing harmful radicals that can damage cell membranes and DNA. It also regulates lipid biosynthesis by influencing the enzymes in the cholesterol biosynthesis pathway (Abu-Reidah *et al*., 2013).

Polyphenolic compounds are primary antioxidants present in watermelon; they are classified into phenolic acid, flavonoids, lignans, and stilbenes. The intake of watermelon induces the antioxidant potential in the body and helps improve cellular processes like signaling and adhesion (Manivannan *et al.,* 2020). The antioxidant capacity of watermelon rind extract has been demonstrated through various in vitro assays, including DPPH radical scavenging, ABTS radical scavenging, and ferric reducing antioxidant power (FRAP) assays. The antioxidant activity is attributed to the synergistic effects of phenolic compounds, flavonoids, carotenoids, and vitamins present in the rind (Tarazona-Diaz *et al*., 2013)

2.6.2 Antimicrobial Properties

The antimicrobial activity of watermelon is attributed to its bioactive compounds. Compounds like glycosides, steroids, saponins, and tannins work synergistically to provide antimicrobial effects. Studies has shown that watermelon rind extracts have a strong inhibitory effect against Gram-positive bacteria such as *Staphylococcus aureus* by the disruption of the bacteria's cell wall and interference with metabolic processes, and Bacillus cereus, when extracted with methanol, inhibited the growth of B. cereus with a minimum inhibitory concentration (MIC) of 8.0mg/ml (Neglo *et al*., 2021).

The antimicrobial activity of watermelon rind extends to Gram-negative bacteria, which are more resistant to antimicrobial agent due to their complex cell wall structure. Studies have shown the effectiveness of watermelon rind extracts against Escherichia coli, a common cause of foodborne illness. The methanolic extract of watermelon rind showed antimicrobial activity against E. coli with an MIC of 128mg/ml (Neglo *et al*., 2021). These antimicrobial activities operate through multiple mechanisms, such as cell wall disruption, protein and enzyme inhibition, DNA damage in bacterial cells, and membrane permeabilization (Athanasiadis *et al.,* 2023).

Research has shown that the peel of watermelon has the highest antimicrobial effect, which was followed by the seed and the rind, as it contains phytochemicals like saponins, flavonoids, alkaloids, free reducing sugar, and tannin, which are absent in other parts of the fruit (Neglo *et al*., 2021). The initial study to quantify the antibacterial activity of the peel was carried out by Harith *et al*. with the aim of investigating the activity of the peel on a Gram-positive bacterium, *Staphylococcus epidermis*, using a disc diffusion method (Harith *et al.,* 2018). The watermelon peel extract at a concentration of 20mg/mL showed the same antibacterial activity as the positive control against S. epidermidis.

2.6.3. Cardiovascular Health

Watermelon has been shown to lower blood pressure due to its high L-citrulline content (Bailey *et al.,* 2016). L-citrulline is a nonessential amino acid that has been shown to improve cardiovascular health by enhancing the availability of nitric oxide in the body (Volino-Souza *et* al., 2020). The rind has a high level of potassium, which acts as a vasodilator, helping reduce stress on blood vessels and arteries and potentially reducing the risk of atherosclerosis, heart attacks, strokes, and coronary heart disease. Watermelon has a high level of lycopene, which may help reduce the risk of heart disease and protect the cells from cell damage (Nadeem *et al*., 2022).

2.6.4. Anti-Inflammatory Action

The anti-inflammatory properties of watermelon are attributed to phytochemicals such as lycopene, citrulline, and other polyphenolic compounds. Lycopene is the primary bioactive compound in watermelon, which is approximately 6,888 micrograms per 152g, and it possesses anti-inflammatory and anticancer activities (Nadeem *et al*., 2022).

Madhavi *et al.*conducted an in vivo and in vitro study of the anti-inflammatory activity of watermelon seed oil in a rat model of carrageenan-induced paw edema. The result showed a significant reduction in carrageenan-induced rat paw edema, with percentage reductions in paw volume of 44.44% and 55.56% at 50mg/kg and 100mg/kg, respectively (Madhavi *et al.,* 2012).

2.6.5. Hepatoprotective Activity

Juice gotten from watermelon pulp has been reported to have tissue-protective effects (Ko *et al*., 2005; Oyenihi *et al*., 2016). The protective effect of watermelon juice against hepatotoxins, such as carbon tetrachloride (CCl4), has also been demonstrated (Altas *et al*., 2011; Chaturvedi *et al*., 2014). Watermelon juice contains bioactive compounds, which are known for their free radical scavenging activities and antioxidant effects. Hence, Oyenihi*et al*. conducted a study evaluating the antioxidant effects of watermelon juice pretreatment on acute ethanol-induced oxidative stress in the brains and livers of rats. The results show that treatment with watermelon juice prior to ethanol exposure significantly increased liver glutathione (GSH) concentration and moderately reduced (32%) malondialdehyde (MDA) concentration in ethanol-treated rats (Oyenihi *et al*., 2016).

Michael et al. conducted a study to investigate the effect of the methanol extract of watermelon rind on lead-induced hepato-renal dysfunction in male Wistar rats. The study found that treating lead-exposed rats with watermelon rind decreased serum urea and creatinine levels compared to lead-exposed rats alone. Watermelon treatment also increased serum glutathione peroxidase activity and reduced glutathione concentration in lead-exposed rats (Micheal *et al*., 2021).

2.6.6. Anti-Ulcer Activity

Watermelon is rich in vitamin A, which may help prevent and shrink stomach ulcers. Watermelon seeds have been reported to have several medicinal uses, including treating ulcers.

Abdu *et al.* conducted a study to evaluate the anti-ulcer activity of the methanolic seed extract of watermelon on indomethacin-induced ulcers in Swiss Albino mice. The study shows that the methanolic watermelon seed extract at 200-800mg/kg decreases gastric volume and total acidity, while increasing gastric pH, in the indomethacin-induced ulcer model. The extract produced a significant ($p < 0.01$) and dose-dependent reduction in ulcer index (Abdu *et al.,* 2023).

2.7. Phytochemical Properties of Watermelon Rind

Watermelon peel is a rich source of diverse bioactive compounds, making it nutritionally and therapeutically valuable. Its phytochemical profile is characterized by high levels of citrulline, lycopene, and polyphenols, along with other constituents such as flavonoids, coumarins, and stilbenes.

2.7.1. Phenolic Compounds

Watermelon peel contains a large amount of polyphenol, which are known for their high antioxidant capabilities that helps neutralize harmful free radicals. A study by Feizy et al. showed that watermelon peel contains 2473.45mg gallic acid equivalent (GAE)/100g (Feizy *et al*., 2020). Phenolic compounds found in watermelon peels include flavonoids (luteolin, Kaemferol, and quercetin), tannins, and lignans (Priastomo *et al.,* 2025).

2.7.2. Carotenoid Content

The major carotenoids in watermelon include lycopene, phytofluene, lutein, and β-carotene, with lycopene being the major component, approximately 6888ug/152g (Nadeem *et al*., 2022). Lycopene is known to have several beneficial health effects, including antioxidant activity, anticancer properties, and protection of multiple organs.

2.7.3. Amino Acids and *Citrulline*

Watermelon rind consists of a high amount of *citrulline*. This non-essential amino acid is converted to arginine, which plays a role in the urea cycle to help remove ammonia from the body (Collins *et al*., 2007). These amino acids aid in the production of nitric acid, which helps to relax and dilate blood vessels, thereby improving blood circulation (Oseni and Okoye, 2013; Bailey *et al.,* 2016). Watermelon contains the highest concentration of *citrulline*, with levels ranging between 0.7 and 3.6mg per gram of fresh weight (Hong *et al.,* 2015).

2.7.4. Synergistic Health Benefits

The combination of phytochemicals in watermelon rind, including lycopene, phenolic compounds, vitamins, and *citrulline*, creates a synergistic effect that enhances its anti-inflammatory, anticancer, and antioxidant properties. This makes the rind a potent, sustainable source of bioactive compounds.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Equipment and Materials

Animal cages, Beakers (50ml and 250ml), Measuring cylinder (100ml and 500ml), Distilled water, Food and water troughs, Wood shavings for bedding, Nose masks, Disposable gloves, Test tubes, Needles and syringes (2ml and 5ml), Blender (Kenwood model KCB239K), Analytical weighing balance, Cotton wool, Chloroform, Oral gastric tubes, Dissecting set, Universal bottles, Plain bottles, Ethylenediaminetetraacetic acid (EDTA) bottles, Micropipettes (0-500microliters),Water bath, Oven and Strainer were utilized.

3.1.2 Plant Material

Fresh watermelons (*Citrullus lanatus*) were purchased from a local market in Oredo Local Government Area of Edo State. The fruit was identified, isolated, and deposited at the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

3.1.3 Chemicals

All chemicals utilized in this research were of analytical grade. Specifically, 100g of Cadmium (99.9% pure) and 100ml of absolute ethanol (99.7%), manufactured by National Agency for Food and Drug Administration Control (NAFDAC) certified companies, were procured from a chemical store in Benin City.

3.1.4 Experimental Animals

Twenty-five (25) healthy Wistar rats weighing between 104g and 196g of both sexes were purchased from a commercial farm in Benin City, Edo State. The animals were housed in plastic

cages at the Department of Science Laboratory Technology's animal facility at the University of Benin, Benin City. The animals were acclimatized for 2 weeks under standard laboratory conditions, with a 12-hour light/dark cycle, before the commencement of the experiments. All animals had unrestricted access to water and were fed regular animal pellets. The animals were handled in accordance with the rules and guidelines set by 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 Plant Preparations

Watermelon fruits were thoroughly washed to remove dirt. Subsequently, the rind was separated from the other parts of the fruit and air-dried for two weeks (14 days) until it reached a brittle consistency. The dried rind was finely pulverized using a Kenwood model KCB239K blender and stored in a properly labeled jar.

3.2.2 Plant Extraction

250g of pulverized watermelon rind was weighed into an extraction jar and dissolved with 1.5 liters of hydro-ethanol (750ml of ethanol and 750ml of distilled water) for 72 hours, with regular agitation every 6 hours to ensure proper extraction. The mixture was filtered through a strainer. The filtrate was concentrated in a water bath at a controlled temperature for several hours, and the yield was weighed to calculate the percentage yield. The resulting extract was stored in a clean, sterile airtight container, labeled and refrigerated at 4 °C.

3.2.3 Experimental Design

After a fourteen-day acclimatization period to the laboratory conditions, the rats were divided into five groups, each containing four (4) rats:

Group I served as the negative control and received 1ml of distilled water

Group II served as the positive control and received cadmium

Group III received cadmium and vitamin C

Group IV and V received cadmium and hydro-ethanolic extracts of watermelon rind at doses of 250mg/kg and 500mg/kg, respectively.

For all administrations, an oral gastric tube was used via the oral route.

3.2.4 Dose Preparation

3.2.4.1 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 15mg/kg.

3.2.4.2 Dose preparation of *citrullus lanatus* (watermelon) rind

2.5g of watermelon rind 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 rind to be administered (preparation was repeated after 3days and left over were discarded)

3.2.4.3 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 given the solution with the use of oral gastric tube.

3.2.5 Measurement of Animal Body Weight (BW)

Body weight (BW) was initially measured at the start of the experiment and then twice weekly until day 60. Mean weekly BW gains were computed for each group. The percentage weight gain/loss was determined using the formula:

Final body weight on day 60(g) – initial body weight on day 0(g)/initial body weight(g) x 100

3.2.6 Measurement of Food Intake (FI)

Each group was provided with a feed container containing a predetermined quantity of feed. The animals had unrestricted access to food and water. The amount of feed consumed by each group was measured daily and recorded.

3.2.7 Measurement of Relative Liver Weight (RLW)

The liver weights were weighed as described. The relative liver weights were determined as follows:

Relative Liver Weight (RLW) =Liver weight/Animal weight at day 60 x 100

3.2.8 Collection of Tissues For Testing

At the end of the duration, the final body weight of all rats was measured and documented. Selected animals were humanely sacrificed under chloroform-induced anesthesia. Thereafter, their livers were harvested, weighed, and the organ–body ratio was recorded.

3.2.9 Histological Examination

Tissues were fixed in 10% formalin, processed, and embedded in paraffin. Sections of 5 µm thickness were stained with hematoxylin and eosin (H&E) for microscopic examination under light microscopy. Histopathological changes, including cellular degeneration, inflammation, necrosis, and architectural distortion, were assessed. The remaining portion of the liver wascollected and placed in a universal bottle containing 2ml of normal saline for heavy metal analysis.

3.3 Heavy Metal Analysis

The collected specimens were decomposed chemically to quantify various metal concentrations. In the laboratory, liver samples were dried in an oven until a constant weight was achieved, with weight recorded at regular intervals to confirm stability. The dried organs were then pulverized into a fine powder using a mortar and pestle. The pulverized sample was transferred to a digestion bottle, and concentrated nitric acid was added. The mixture was covered and allowed to digest at room temperature for 24 hours to ensure complete breakdown of the organic matrix and release the heavy metals into the solutions. After the digestion period, the solutions were filtered using Whatman filter paper No. 42 into a 100 mL volumetric flask, filled to volume with distilled water, and stored in plastic reagent bottles. These prepared samples were primed for analysis via Atomic Absorption Spectroscopy (AAS) to unveil their metallic composition.

CHAPTER FOUR

RESULT

Table 4.1: Effect of watermelon rind extract on weight of cadmium induced rats

GROUP	Initial weight	final weight	Relative %
Control	155.5	228.5	47.3125
Cadmium	177.75	238	37.405
Vitamin C	140.25	253.75	83.525
WMRE 250mg/kg	131.25	241.25	83.53
WMRE 500mg/kg	125	214.25	74.4425

The lowest mean body weight was observed in the Cd-only (positive control) group, while the highest body weight was recorded in the vitamin C group and watermelon rind extract 250mg/kg co-treatment group. The Cd + 500 mg/kg extract groups showed body weight profiles close to the negative control, suggesting a dose-dependent mitigation of Cd-induced growth suppression.

Table 4.2: Weight of liver after Cadmium exposure

GROUP	**Weight of liver (g)**
Control	6.4625
Cadmium	8.9
Vitamin C	8.315
WMRE 250mg/kg	8.6775
WMRE 500mg/kg	6.43

The highest liver weight (both absolute and relative) was observed in the Cd-only group, indicative of mild hepatomegaly possibly due to early inflammatory or adaptive responses. The lowest liver weights were seen in the negative control and Cd + 500 mg/kg extract groups, with the latter closely approximating normal values.

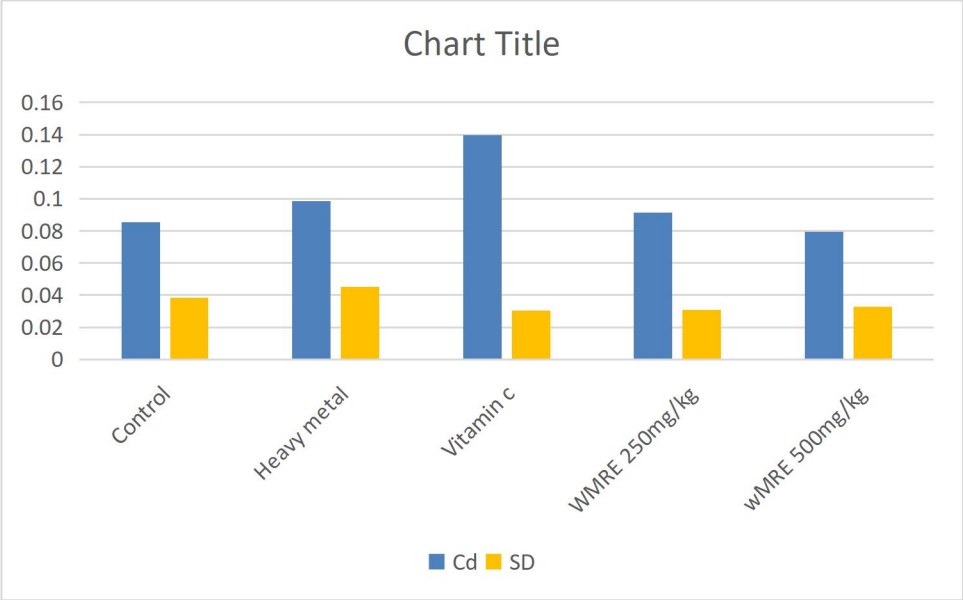

FIGURE 4.1: Bioaccumulation of Cadmium in liver of Wistar rats

The lowest Cd concentrations were observed in the Cd + watermelon rind extract (500 mg/kg) group and notably in the control group, which showed a marked reduction compared to the Cd-only group. The Cd + 250 mg/kg group exhibited intermediate levels, suggesting partial chelation or excretion enhancement. The standard group showed a significant increase in the level of cadmium compared to other groups.

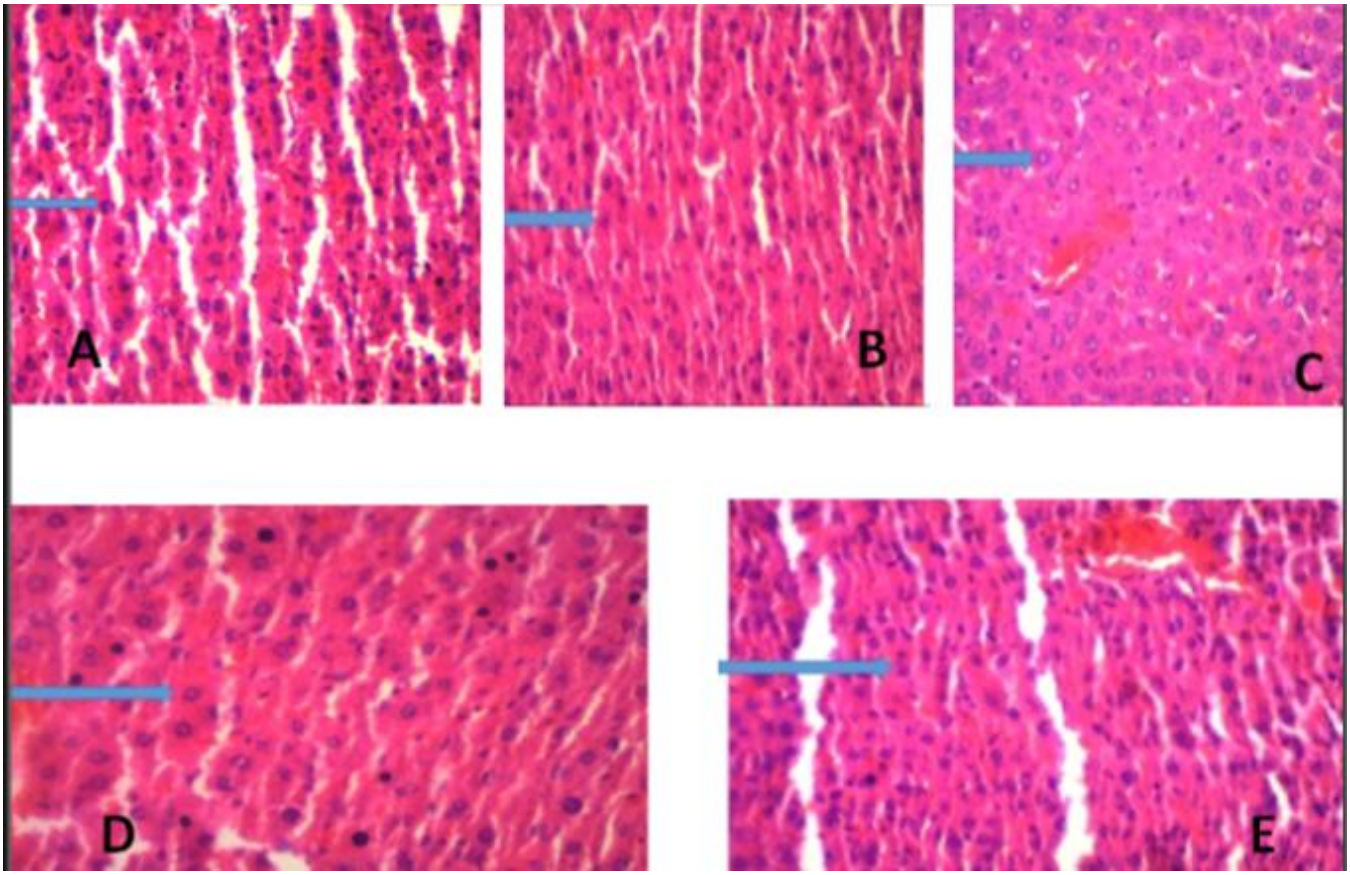

Plate 4.1: EFFECT OF WATERMELON RIND EXTRACT ON THE HISTOLOGY OF THE LIVER

A shows a representative image of control group liver tissue from a Wistar rat. B shows a representative image of cadmium group liver tissue from a Wistar rat. C shows a representative image of vitamin C and cadmium group liver tissue from a Wistar rat. D shows a representative image of watermelon rind extract (250mg/kg) and cadmium group liver tissue from a Wistar rat. E shows a representative image of watermelon rind extract (500mg/kg) and cadmium group liver tissue from a Wistar rat. A section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nucleus with indistinct nucleoli across all groups-features in keeping with normal hepatocytes.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Cadmium (Cd), a pervasive environmental heavy metal, poses significant risks to human and animal health through bioaccumuation in vital organs, particularly the liver, where it induces oxidative stress, lipid peroxidation, and histopathological alterations (Rana and Sharma, 1994; Anwar *et al.,* 2020). The increasing prevalence of Cd contamination in food, water, and soil underscores the urgency for effective, natural hepatoprotective agents, of which watermelon rind (*Citrullus lanatus*), traditionally regarded as agricultural waste, has emerged as a promising source of bioactive compounds, including flavonoids, phenolic acids, and citrulline with potent antioxidant and metal-chelating properties (Tarazona-Díaz *et al.,* 2014).

Histopathological assessment of liver tissues from Wistar rats in the present study revealed uniform preservation of normal hepatic architecture across all experimental groups. Hepatocytes in the negative control (distilled water), cadmium (Cd)-exposed, Cd plus vitamin C, and Cd co-administered with hydro-ethanolic extracts of watermelon rind (*Citrullus lanatus*) at 250 mg/kg and 500 mg/kg doses consistently displayed eosinophilic cytoplasm surrounding centrally placed normochromic nuclei with indistinct nucleoli, indicative of intact parenchyma without evidence of vacuolation, necrosis, fibrosis, or inflammatory infiltration.

This outcome is noteworthy, particularly in the Cd-exposed group, where the lack of discernible pathological alterations contrasts sharply with established models of Cd hepatotoxicity in Wistar rats. Cadmium, a potent environmental toxicant, typically elicits oxidative stress-mediated liver injury through reactive oxygen species (ROS) overproduction, mitochondrial dysfunction,

anddisruption of cellular homeostasis, resulting in dose- and time-dependent histopathological changes such as hepatocellular degeneration, centrilobular necrosis, sinusoidal congestion, and Kupffer cell hyperplasia (Anwar *et al.,* 2020; Rana *et al.,* 1994). The absence of such features here may reflect experimental nuances, including a potentially subtoxic Cd dosage, oral administration route minimizing bioavailability compared to intraperitoneal injections used in many studies, or the short exposure duration insufficient to manifest overt morphology despite biochemical perturbations (Dudley *et al.,* 1985). Strain-specific resilience in Wistar rats has also been documented, where genetic variability influences Cd accumulation and detoxification capacity (Sentürker *et al.,* 2004).

Despite the unanticipated normalcy in the Cd-alone group, the sustained hepatic integrity in rind extract-co-treated cohorts strongly implies a hepatoprotective prophylactic effect. Watermelon rind, often discarded as agro-waste, harbors bioactive phytochemicals including flavonoids, phenolic acids, lycopene, and citrulline, which confer robust antioxidant properties capable of neutralizing Cd-induced ROS and bolstering endogenous defenses like superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Tarazona-Díaz *et al.,* 2014; Adedapo *et al.,* 2021). Hydro-ethanolic extraction likely optimized the yield of these lipophilic and hydrophilic antioxidants, enabling membrane stabilization and inhibition of lipid peroxidation pathways central to Cd pathogenesis. This interpretation is bolstered by biochemical-histological correlations in analogous rind extract studies; for example, ethanol extracts of watermelon rind mitigated lead acetate-induced hepato-renal oxidative damage in male Wistar rats, evidenced by normalized serum transaminases (AST/ALT), reduced malondialdehyde (MDA) levels, and preserved tissue architecture (Adedapo *et al.,* 2021). Similarly, aqueous extracts of watermelonseeds demonstrated hepatoprotection against ethanol toxicity by attenuating oxidative markers and restoring hepatocyte morphology in Wistar rats (Obidike *et al.,* 2018).

Broader comparative literature on plant-based interventions against Cd liver toxicity reinforces these findings. Moringaoleifera leaf extracts, rich in isothiocyanates and polyphenols, have alleviated Cd-induced hepatotoxicity in rats by enhancing antioxidant enzyme activities, decreasing histopathological scores of necrosis and inflammation, and modulating metal-binding proteins (Aslam *et al.,* 2016). Grape seed proanthocyanidins similarly exerted ameliorative effects on Cd-exposed rat livers, reducing ROS accumulation, inflammatory cytokine release, and degenerative changes through Nrf2 pathway activation (Wang *et al.,* 2016). Even watermelon juice, encompassing rind-derived components, has shown hepato- and neuroprotective benefits against ethanol oxidation via free radical scavenging (Oseni *et al.,* 2017). These parallels suggest that the rind extract's efficacy may stem from comparable mechanisms, including chelation of Cd ions and upregulation of phase II detoxifying enzymes, positioning *Citrullus lanatus* byproducts as viable, sustainable alternatives to synthetic antioxidants like vitamin C observed in this study.

In essence, while the subclinical Cd insult limits direct demonstration of reversal, the histopathological normalcy in rind-treated groups highlights its latent hepatoprotective potential, warranting validation through integrated endpoints such as serum enzymology, oxidative stress profiling, and gene expression analyses. Dose-response escalations and chronic exposure models would further delineate therapeutic windows, potentially translating rind extracts into nutraceutical strategies for heavy metal detoxification in vulnerable populations.

Body weight is a sensitive, non-invasive marker of overall health and systemic toxicity. Chronic or subchronic Cd exposure typically induces weight loss due to reduced feed intake, gastrointestinal disturbances, and metabolic disruption (Thévenod and Lee, 2013). In this study, the lowest mean body weight was observed in the Cd-only (positive control) group, while the

highest body weight was recorded in the negative control (distilled water) and watermelon rind extract 500 mg/kg co-treatment group. The Cd + 250 mg/kg and Cd + 500 mg/kg extract groups showed body weight profiles closer to the negative control than the Cd-only group, suggesting a dose-dependent mitigation of Cd-induced growth suppression.

This finding aligns with (Adedapo *et al,* 2021), who reported that ethanol extracts of watermelon rind significantly reversed lead acetate-induced body weight reduction in male Wistar rats, attributing recovery to improved antioxidant status and reduced oxidative burden on metabolic pathways. Similarly, (Oseni *et al,* 2017) observed that watermelon juice prevented ethanol-induced weight loss in rats, supporting the role of *Citrullus lanatus* bioactives in maintaining anabolic balance under toxic stress. The higher dose (500 mg/kg) appearing more effective than 250 mg/kg indicates a possible threshold effect in phytochemical-mediated metabolic protection.

Hepatic Cd concentration is a direct measure of metal bioaccumulation and target organ exposure. The lowest Cd concentrations were observed in the Cd + watermelon rind extract (500 mg/kg) group and notably in the control group, which showed a marked reduction compared to the Cd-only group. The Cd + 250 mg/kg group exhibited intermediate levels, suggesting partial chelation or excretion enhancement. The standard group showed a significant increase in the level of cadmium compared to other groups, which was an unexpected result suggesting that Vitamin C may provide antioxidant protection against cadmium toxicity without necessarily reducing tissue accumulation. This result corresponds with the findings of Tarasub *et al.* (2012) and Mumtaz *et al.* (2019) which demonstrated that vitamin C alone does not reverse cadmium-induced pathological changes and does not completely prevent metals from accumulating in the liver, though it does help reduce oxidative damage to cellular structures. This dissociation between metal chelation and antioxidant protection indicates that vitamin C's therapeutic benefit

in cadmium toxicity operates primarily through neutralization of reactive oxygen species rather than enhancement of metal elimination. The present study extends these observations by quantitatively demonstrating that vitamin C co-administration may paradoxically increase tissue cadmium levels possibly through enhancement of intestinal absorption via formation of more readily absorbable ascorbate metal complexes, as proposed by Suzuki *et al*. (1984) while potentially still providing subclinical protection against oxidative cellular damage not captured by the histopathological assessment employed in this investigation.

This dose-dependent reduction in hepatic Cd accumulation is a significant finding and compares favorably with (Al-Otaibi *et al*, 2018), who demonstrated that pomegranate peel extract lowered tissue Cd levels in exposed rats through enhanced urinary excretion and possible metal-binding by polyphenols mechanisms likely shared by watermelon rind's phenolic and flavonoid constituents. Additionally, (Messaoudi *et al,* 2023) reported that *Citrullus lanatus* seed extracts reduced heavy metal retention in liver and kidney, reinforcing the metal-chelating potential of watermelon-derived phytocompounds. The superior effect at 500 mg/kg suggests saturation of binding sites or enhanced induction of metallothionein or efflux transporters at higher doses.

Relative liver weight (liver-to-body weight ratio) often increases in response to Cd due to edema, inflammatory infiltration, or compensatory hyperplasia (Rana and Sharma, 1994). In this study, the highest liver weight (both absolute and relative) was observed in the Cd-only group, indicative of mild hepatomegaly possibly due to early inflammatory or adaptive responses. The lowest liver weights were seen in the negative control and Cd + 500 mg/kg extract groups, with the latter closely approximating normal values.

This normalization of liver weight in the high-dose extract group is consistent with (Obidike *et al.* 2018), who found that aqueous watermelon seed extract prevented ethanol-induced liver

enlargement in rats by reducing oxidative inflammation. Similarly, (Wang *et al.* 2016) reported that grape seed proanthocyanidins attenuated Cd-induced liver swelling through Nrf2-mediated antioxidant and anti-inflammatory pathways a mechanism potentially operational here given the polyphenol richness of watermelon rind.

5.2 Conclusion

The histopathological findings from this study demonstrate that hydro-ethanolic extract of watermelon rind, at doses of 250 mg/kg and 500 mg/kg, effectively preserved normal liver architecture in Wistar rats co-exposed to cadmium (Cd), mirroring the protective outcomes observed with vitamin C and the negative control which position watermelon rind as a viable, cost-effective, and sustainable natural antidote against environmental hepatotoxins. Thus, this investigation not only validates the therapeutic repurposing of agro-waste but also reinforces the value of plant-based interventions in combating heavy metal toxicity.

This study faced several limitations, including financial constraints and lack of specialized equipment prevented comprehensive assessment of certain biomarkers relevant to the study. Intermittent power supply disrupted laboratory procedures and affected experimental timelines. Time constraints also limited the scope and sample size of the research.

5.3 Recommendations

Based on the outcome of this research work, I further recommend that;

1. Future studies should complement histopathological assessments with serum liver function tests (ALT, AST, ALP), oxidative stress markers (MDA, SOD, GPx).
2. Higher Cd doses or chronic exposure protocols to induce measurable liver damage should be employed, enabling clearer evaluation of the extract's curative rather than solely preventive efficacy.
3. Standardized hydro-ethanolic extraction protocols with phytochemical profiling (e.g., HPLC for flavonoids and citrulline) to ensure reproducibility and bioactive consistency across studies should be developed.
4. Synergistic Formulations to investigate combinations of watermelon rind extract with established hepatoprotectants (e.g., silymarin, N-acetylcysteine) to enhance therapeutic outcomes in heavy metal detoxification should be carried out.

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