AMELIORATIVE EFFECTS OF WATERMELON PEEL EXTRACT ON CADMIUM- INDUCED CARDIAC HISTOPATHOLOGY IN WISTAR RATS

BY

Success AIGBE (Miss)
LSC2009825

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

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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, NIGERIA

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CERTIFICATION

This is to certify that this research project work was carried out by **Success AIGBE** with matriculation number, **LSC2009825** of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

_____ _____

MR O. C. EHKATOR Date
(Project Supervisor)

_____ _____

DR. P. O. ALONGE Date
(Project Coordinator)

_____ _____

Prof J.O. Osarumwense Date
(Head of Department)

_____ _____

(External Examiner) Date

DEDICATION

This work is dedicated to Almighty God, the source of all wisdom and knowledge, and to my beloved family for their unwavering support and encouragement throughout my academic journey.

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I give all the glory, honor, and adoration to Almighty God for His grace, mercy, and guidance throughout the course of this research work and my academic program.

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ABSTRACT

Environmental pollution by cadmium poses significant cardiovascular health risks, particularly in developing nations experiencing rapid industrialization. This study investigated the ameliorative effects of watermelon peel extract on cadmium-induced cardiac histopathology in Wistar rats. Twenty-five Wistar rats were randomly divided into five groups: control (distilled water), cadmium only, cadmium plus Vitamin C, cadmium plus 250mg/kg watermelon peel extract, and cadmium plus 500mg/kg watermelon peel extract. Animals received oral administration thrice weekly over 60 days following a two-phase induction and treatment protocol. Cadmium exposure resulted in elevated cardiac cadmium accumulation (0.240 mg/g versus 0.117 mg/g in controls) and suppressed body weight gain. Watermelon peel extract provided dose-dependent cardioprotection, with the 500mg/kg dose achieving 58% reduction in cardiac cadmium levels (0.102 mg/g) and improved body weight gain (74.44%), approaching the efficacy of Vitamin C (63% reduction, 0.089 mg/g). The 250mg/kg dose demonstrated moderate protection with 39% cadmium reduction. Histopathological examination revealed preserved myocardial architecture across all groups with intact cardiomyocyte arrangement and well-defined cross-striations, suggesting the exposure period represented an early phase where biochemical changes occurred without microscopic structural damage. The findings demonstrate that watermelon peel extract provides significant dose-dependent protection against cadmium bioaccumulation in cardiac tissue, supporting its potential as a locally accessible natural intervention for populations at risk of environmental cadmium exposure.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Environmental pollution by heavy metals has emerged as one of the most pressing public health challenges of the 21st century, particularly in developing nations experiencing rapid industrialization and urbanization. Among various heavy metals of toxicological concern, cadmium (Cd) stands out as a particularly insidious pollutant due to its widespread environmental distribution, efficient bioaccumulation in living organisms, extremely long biological half-life, and capacity to induce multi-organ toxicity even at relatively low exposure levels (Genchi *et al.*, 2020).

Cadmium contamination originates from both natural and anthropogenic sources, with industrial activities including mining, battery manufacturing, pigment production, and agricultural practices constituting major sources (Briffa *et al.*, 2020). In Nigeria, studies have documented elevated cadmium levels in environmental media and food items across major urban centers including Lagos and Ibadan, posing significant health risks to exposed populations (Olujimi *et al*., 2015; Adesuyi \ 2015). The toxicological profile of cadmium encompasses adverse effects on multiple organ systems including the kidneys, liver, bones, reproductive system, and increasingly recognized cardiovascular system (Satarug, 2018; Nordberg, 2022).

Cardiovascular toxicity has emerged as a critical dimension of cadmium's health impacts. Epidemiological studies reveal significant associations between cadmium exposure and increased risks of cardiovascular diseases including coronary heart disease, stroke, and cardiovascular mortality (Tellez-Plaza *et al.*, 2013). These associations persist even after adjusting for traditional cardiovascular risk factors, suggesting cadmium's independent

contribution to cardiovascular disease burden. In Nigeria, cardiovascular diseases account for approximately 10% of deaths and represent leading causes of mortality (Adedinsewo *et al.,* 2023). However, the potential contribution of environmental pollutants like cadmium to Nigeria's cardiovascular disease burden remains largely overlooked.

The mechanisms underlying cadmium-induced cardiac toxicity involve oxidative stress, inflammation, apoptosis, and fibrosis. Cadmium provokes massive reactive oxygen species (ROS) generation through mitochondrial dysfunction and displacement of redox-active metals, while simultaneously depleting cellular antioxidant defenses (Valko *et al.,* 2016). The heart, with its high metabolic rate and abundant mitochondrial content, demonstrates particular vulnerability to oxidative damage. Additionally, cadmium activates inflammatory signaling pathways, leading to increased production of pro-inflammatory cytokines in cardiac tissue (Das *et al.,* 2021).Current therapeutic approaches for cadmium toxicity possess significant limitations. Conventional chelation therapy shows limited efficacy for chronic cadmium intoxication and cannot reverse established tissue damage (Flora and Pachauri, 2010). These limitations have motivated research into natural products as alternative therapeutic strategies. Plant-based natural products offer multiple bioactive phytochemicals that can counter act oxidative stress, exert anti-inflammatory actions, and potentially chelate metals (Dkhil *et al.,* 2020).

Watermelon (Citrullus lanatus) peel, typically discarded as agricultural waste, contains valuable bioactive compounds including Phenolic acids, Flavonoids, Carotenoids, and Citrulline (Obaroakpo *et al.,* 2020; Kumar *et al.,* 2022). These compounds exhibit potent antioxidant and anti-inflammatory activities, along with cardiovascular protective effects (Oboh *et al.,* 2020; Figueroa *et al.,* 2017). Despite this promising profile, research examining watermelon peel extract's effects on cadmium-induced cardiac damage remains remarkably limited, representing a significant research gap that this study aims to address.

1.2 Statement of the Problem

Cardiovascular diseases constitute a major and escalating public health challenge in Nigeria, with ischemic heart disease and stroke ranking among the leading causes of mortality (Adedinsewo *et al.,* 2023) While traditional cardiovascular risk factors including hypertension, diabetes, smoking, and dyslipidemia receive substantial research and intervention attention, the potential contribution of environmental pollutants to cardiovascular disease burden remains inadequately explored in the Nigerian context. Cadmium, a ubiquitous environmental contaminant documented in Nigerian soils, water, air, and food items, has emerged from international research as a significant cardiovascular risk factor with established epidemiological associations and experimental evidence of cardiac toxicity (Olujimi *et al.,* 2015).

The mechanisms of cadmium-induced cardiac damage involve complex pathological processes including oxidative stress, inflammation, apoptosis, fibrosis, and vascular dysfunction that collectively compromise cardiac structure and function (Messner and Bernhard, 2014). However, current therapeutic options for preventing or treating cadmium-induced cardiac damage are limited. Conventional chelation therapy faces significant constraints including limited efficacy for chronic exposure, inability to reverse established damage, and potential adverse effects (Flora and Pachauri, 2010). This therapeutic gap necessitates exploration of alternative or complementary approaches.

Natural products derived from plants represent promising candidates for cardioprotection against cadmium toxicity due to their multi-component nature, antioxidant and anti-inflammatory properties, and generally favorable safety profiles (Dkhil *et al.,* 2020). Watermelon peel, an abundant agricultural by-product typically discarded as waste, contains diverse bioactive compounds including phenolics, flavonoids, and carotenoids with

demonstrated antioxidant, anti-inflammatory, and cardiovascular protective properties (Kumar *et al.,* 2022; Oboh *et al.,* 2020). However, despite this promising profile, systematic investigation of watermelon peel extract's effects on cadmium-induced cardiac histopathology remains absent from the scientific literature.

This research gap is particularly significant given Nigeria's position as a major watermelon-producing nation, making watermelon peel readily available and potentially offering a locally accessible intervention for cadmium toxicity. Furthermore, the increasing documentation of environmental cadmium contamination in Nigerian settings coupled with rising cardiovascular disease prevalence underscores the urgency of understanding cadmium's cardiac effects and developing protective strategies. The absence of comprehensive studies examining watermelon peel extract's ameliorative effects on cadmium-induced cardiac histopathological changes, the mechanisms underlying potential protection, and optimal dosing strategies represents a critical knowledge deficit that this study aims to address.

1.3 Justification for the Study

Several compelling factors justify this research investigation into watermelon peel extract's ameliorative effects on cadmium-induced cardiac histopathology. First, the documented presence of cadmium contamination in Nigerian environmental media and food items coupled with the known cardiovascular toxicity of cadmium establishes a potential public health concern requiring attention (Ogundiran and Osibanjo, 2009; Adesuyi *et al.,* 2015). Understanding the cardiac effects of cadmium exposure relevant to Nigerian populations and developing locally accessible interventions represents an important public health priority.

Second, the limitations of current therapeutic approaches for cadmium toxicity, particularly conventional chelation therapy which shows limited efficacy for chronic exposure and cannot reverse established tissue damage, create a clear need for alternative or complementary

strategies (Flora and Pachauri, 2010). Natural products offering multi-mechanistic protection through antioxidant, anti-inflammatory, and potentially metal-chelating effects represent rational alternatives deserving systematic investigation.

Third, watermelon peel's rich content of bioactive compounds with demonstrated biological activities including antioxidant, anti-inflammatory, and cardiovascular protective effects provides strong theoretical basis for expecting beneficial effects against cadmium-induced cardiac damage (Kumar *et al*., 2022; Oboh *et al.,* 2020). The documented ability of polyphenolic compounds to scavenge reactive oxygen species, modulate inflammatory pathways, and potentially chelate metal ions aligns well with the mechanisms underlying cadmium cardio toxicity.

Fourth, the ready availability and low cost of watermelon peel as an agricultural by-product typically discarded as waste makes it an attractive candidate for development as a therapeutic or preventive intervention, particularly in resource-limited settings. Nigeria's position as a significant watermelon producer ensures local accessibility. Successful demonstration of cardioprotective effects could simultaneously address a public health concern while adding value to agricultural waste products.

Fifth, from a scientific perspective, this research will contribute new knowledge regarding natural product protection against heavy metal-induced cardiac toxicity, a relatively understudied area compared to hepatic and renal protection. The findings will enhance understanding of phytochemical interactions with toxic metals in cardiac tissue and may identify lead compounds or extract formulations for further development.

Finally, the use of histopathological examination as a primary outcome measure provides direct visualization and documentation of structural changes in cardiac tissue, offering clear evidence of damage and protection that complements biochemical and molecular

assessments. Histopathology remains the gold standard for assessing tissue injury and repair, making it an appropriate endpoint for this investigation.

1.4 Aim and Objectives of the Study

1.4.1 Aim

The aim of this study is to investigate the ameliorative effects of watermelon peel extract on cadmium-induced cardiac histopathology in Wistar rats.

1.4.2 Specific Objectives

The specific objectives of this study are to:

1. evaluate the effect of watermelon peel extract on cardiac histopathology architecture in Wistar rats
2. evaluate the effect of watermelon peel extract on bioaccumulation of cadmium on the heart of a Wistar rat exposed to cadmium chloride

CHAPTER TWO

LITERATURE REVIEW

2.1 Heavy Metal Toxicity

Heavy metals constitute a significant class of environmental pollutants that pose serious threats to human health and ecological systems globally. These metallic elements, characterized by their high atomic weights and densities exceeding 5g/cm³, have become increasingly problematic due to rapid industrialization and urbanization (Briffa *et al.*, 2020). Unlike organic contaminants, heavy metals are non-biodegradable and persist in the environment indefinitely, accumulating through food chains and eventually reaching toxic concentrations in living organisms.

The widespread distribution of heavy metals in air, water, soil, and food creates multiple exposure pathways for human populations. Industrial emissions, mining operations, municipal waste disposal, and agricultural chemical applications represent primary anthropogenic sources of heavy metal contamination (Tchounwou *et al.*, 2012). Once released into the environment, these toxic elements undergo various physicochemical transformations that influence their bioavailability, mobility, and toxicity. The ability of heavy metals to bioaccumulate in tissues and biomagnify through trophic levels makes them particularly hazardous for organisms at higher levels of the food chain, including humans.

From a public health perspective, chronic low-level exposure to heavy metals has been associated with numerous adverse health outcomes including cardiovascular diseases, neurological disorders, renal dysfunction, hepatotoxicity, reproductive abnormalities, and various forms of cancer (Rahman and Singh, 2019). The insidious nature of heavy metal toxicity often manifests after prolonged exposure periods, making early detection and

intervention challenging. Among various toxic heavy metals of concern, cadmium stands out as particularly problematic due to its widespread environmental presence and multi-organ toxicity profile.

2.2 Cadmium: Properties, Sources, and Exposure Routes

2.2.1 Physical and Chemical Properties

Cadmium (Cd, atomic number 48) is a soft, bluish-white transition metal that shares chemical similarities with zinc and mercury. With an atomic weight of 112.41 and density of 8.65 g/cm³, cadmium exhibits distinctive physicochemical properties that influence its environmental behavior and biological interactions (Genchi *et al.,* 2020). The element exists predominantly in the +2 oxidation state under physiological conditions, forming stable complexes with various organic and inorganic ligands. Cadmium demonstrates high affinity for sulfhydryl groups in proteins and peptides, a characteristic underlying many of its toxic mechanisms.

The chemical reactivity of cadmium, particularly its propensity to bind with thiol-containing biomolecules, enables it to disrupt critical cellular processes by displacing essential metal ions such as zinc, calcium, and iron from their physiological binding sites. This ionic mimicry represents a fundamental mechanism through which cadmium exerts toxic effects at the molecular level (Genchi *et al.,* 2020). Furthermore, cadmium's resistance to metabolic degradation and long biological half-life, estimated at 10-30 years in humans, contribute to its cumulative toxicity.

2.2.2 Sources and Routes of Exposure

Human exposure to cadmium occurs through both natural and anthropogenic sources, with industrial activities dominating contemporary exposure scenarios. Natural sources include

volcanic emissions, weathering of cadmium-containing rocks, and forest fires (Faroon *et al.*, 2012). In contrast, anthropogenic activities have dramatically amplified environmental cadmium releases. Mining and smelting operations for zinc, lead, and copper ores release substantial quantities of cadmium as these ores naturally contain cadmium as an impurity.

Industrial uses of cadmium include nickel-cadmium battery production, pigment manufacturing, metal coatings, plastic stabilizers, and various alloy applications (Satarug *et al.*, 2020). Phosphate fertilizers derived from cadmium-containing phosphate rock introduce significant quantities into agricultural soils, subsequently contaminating food crops. Cigarette smoking constitutes a unique direct exposure route, as tobacco plants efficiently accumulate cadmium from contaminated soils.

Cadmium enters the human body through three principal routes: inhalation, ingestion, and dermal absorption. Inhalation represents the most efficient absorption route, with 30-50% of inhaled cadmium being absorbed through respiratory tissues (Nordberg, 2022). Gastrointestinal absorption from dietary sources is less efficient, typically ranging from 3-7% in adults with adequate iron stores, though this can increase to 15-20% under conditions of iron or zinc deficiency (Satarug and Moore, 2004). Dermal absorption is generally negligible through intact skin.

2.3 Cadmium Toxicokinetics

2.3.1 Absorption and Distribution

Following absorption, cadmium rapidly binds to plasma proteins, predominantly albumin and metallothionein, forming stable complexes that mediate distribution throughout the body. The liver functions as the primary site of initial accumulation, progressively synthesizing metallothionein in response to increasing cadmium burdens (Nordberg, 2022). However, as

hepatic cadmium-metallothionein complexes are released into circulation, the kidneys become the ultimate accumulation site, eventually containing 30-60% of total body burden in chronically exposed individuals.

This redistribution occurs because cadmium-metallothionein complexes pass through glomerular filtration and are reabsorbed by proximal tubular cells where cadmium progressively accumulates. Distribution to other tissues including heart, brain, bone, and reproductive organs occurs to a lesser extent but carries toxicological significance (Patra *et al*., 2011). Cardiac tissue, though not a primary accumulation site, demonstrates measurable cadmium concentrations correlating with exposure levels.

2.3.2 Metabolism and Excretion

Unlike many xenobiotics, cadmium undergoes no conventional metabolic transformation. Instead, its biological fate is determined by interactions with endogenous biomolecules, particularly metallothionein and glutathione. Metallothionein induction represents the primary biological response to cadmium exposure and serves as a major determinant of toxicokinetics and toxicity (Nordberg, 2022). This cysteine-rich protein binds cadmium with high affinity, effectively sequestering the metal in a relatively inert form.

Cadmium excretion from the body is extremely slow, with renal elimination accounting for the majority through urinary excretion. However, efficiency remains remarkably low, typically less than 0.01% of body burden per day, resulting in a biological half-life of 10-30 years (Satarug *et al.,* 2017). Biliary excretion occurs to a limited extent, with cadmium-glutathione conjugates being secreted into bile, though significant enterohepatic recirculation contributes to body burden accumulation. The extremely slow elimination kinetics mean that even moderate exposure levels sustained over years lead to substantial tissue accumulation.

2.4 Mechanisms of Cadmium Toxicity

2.4.1 Oxidative Stress

Oxidative stress represents a central mechanism underlying cadmium toxicity across multiple organ systems. Although cadmium is redox-inactive and cannot directly participate in Fenton-type reactions, it provokes intense oxidative stress through several indirect mechanisms (Valko *et al.*, 2016). Cadmium exposure triggers excessive reactive oxygen species (ROS) production including superoxide anions, hydrogen peroxide, and hydroxyl radicals through mitochondrial dysfunction, NADPH oxidase activation, and disruption of the electron transport chain.

Cadmium interferes with mitochondrial function by binding to critical electron transport chain components, particularly complexes I and III, leading to electron leakage and increased superoxide production (Adeyemi *et al.,* 2024). This mitochondrial dysfunction not only generates ROS but also impairs ATP production, compromising cellular energy metabolism. Additionally, cadmium displaces redox-active metals such as iron and copper from physiological binding sites, inadvertently creating conditions favorable for Fenton chemistry and hydroxyl radical generation.

The ROS generated through cadmium exposure attack multiple cellular macromolecules. Lipid peroxidation, characterized by oxidative degradation of polyunsaturated fatty acids in cellular membranes, results in membrane dysfunction, altered permeability, and generation of toxic secondary products such as malondialdehyde (MDA) (Bernhoft, 2013). Protein oxidation leads to formation of protein carbonyls and modification of amino acid side chains, impairing protein function. DNA damage resulting from oxidative attack includes base modifications, strand breaks, and DNA-protein cross-links, potentially leading to mutagenesis and genomic instability.

2.4.2 Disruption of Antioxidant Defense Systems

The toxicity of cadmium is amplified by its capacity to simultaneously overwhelm and disable cellular antioxidant defense systems. Glutathione (GSH), the most abundant intracellular antioxidant, is severely depleted during cadmium exposure through multiple mechanisms (Cuypers *et al.*, 2010). Cadmium directly binds to GSH through sulfhydryl groups, forming complexes that are either exported from cells or oxidized, effectively reducing available functional glutathione.

Beyond glutathione depletion, cadmium inhibits key antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes constitute primary enzymatic defenses against ROS (Bertin and Averbeck, 2006). Cadmium's inhibitory effects stem from binding to enzyme active sites and displacing essential metal cofactors such as zinc, copper, and selenium, thereby rendering enzymes non-functional.

The combined effects of enhanced ROS generation and impaired antioxidant defenses create severe oxidative stress that overwhelms cellular protective mechanisms. This redox imbalance triggers oxidative modifications to cellular components, activates stress-responsive signaling pathways, and promotes cell death through apoptotic and necrotic mechanisms (Jimi *et al.*, 2024).

2.4.3 Interference with Cellular Signaling

Cadmium exerts profound effects on cellular signaling networks through its ability to mimic, displace, or interfere with essential metal ions involved in signal transduction. Calcium signaling, fundamental to numerous physiological processes including muscle contraction and cell death regulation, is particularly susceptible to cadmium disruption (Thévenod and

Lee, 2013). Cadmium can permeate cells through calcium channels and transporters, subsequently interfering with calcium-dependent processes.

Mitochondria, serving as both energy producers and regulators of cell death, represent key targets for cadmium toxicity. Cadmium accumulation in mitochondria impairs oxidative phosphorylation, promotes ROS generation, induces mitochondrial permeability transition, and triggers release of pro-apoptotic factors (Wang *et al.,* 2024). Apoptosis induction represents a prominent cellular response to cadmium exposure, mediated through both intrinsic (mitochondrial) and extrinsic (death receptor) pathways.

2.5 Systemic Effects of Cadmium Toxicity

2.5.1 Renal Toxicity

The kidneys represent the primary target organ for chronic cadmium toxicity. Proximal tubules demonstrate particular vulnerability to cadmium accumulation and injury (Satarug, 2018). Cadmium-metallothionein complexes filtered through glomeruli are reabsorbed by proximal tubular cells where cadmium progressively accumulates over time. Tubular dysfunction manifests initially as low-molecular-weight proteinuria with increased urinary excretion of β2-microglobulin, retinol-binding protein, and α1-microglobulin.

As damage progresses, more severe manifestations emerge including glucosuria, aminoaciduria, and phosphaturia. Chronic cadmium nephropathy may progress to irreversible renal impairment characterized by reduced glomerular filtration rate and chronic kidney disease development

(Nordberg *et al.,* 2022). The irreversibility of cadmium-induced renal damage reflects permanent loss of functional nephrons and extremely slow cadmium elimination from kidney tissue.

2.5.2 Hepatotoxicity

The liver serves as an initial site for cadmium accumulation and metallothionein synthesis, demonstrating relatively greater resistance to injury compared to kidneys. However, acute high-dose or chronic excessive exposure can overwhelm hepatic protective mechanisms, resulting in significant liver damage (Liu *et al.,* 2019). Cadmium-induced hepatotoxicity manifests through elevation of serum liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Histopathological examination reveals hepatocyte swelling, fatty change, necrosis, and inflammatory cell infiltration. Mechanisms involve oxidative stress, mitochondrial dysfunction, calcium homeostasis disruption, and inflammatory pathway activation (Djukic-Cosic *et al.,* 2008). The liver's extensive regeneration capacity provides some resilience, though sustained exposure can lead to chronic liver disease characterized by persistent inflammation and progressive fibrosis.

2.5.3 Cardiovascular Toxicity

Cardiovascular toxicity has emerged as an increasingly recognized dimension of cadmium's health effects. Epidemiological studies reveal associations between cadmium exposure and cardiovascular disease incidence, mortality, hypertension, and specific cardiovascular outcomes (Tellez-Plaza *et al*., 2013). Mechanisms include oxidative stress, endothelial dysfunction, vascular inflammation, and direct cardiac tissue damage (Peters et al., 2010).

2.6 Cadmium-Induced Cardiac Toxicity

2.6.1 Experimental Studies on Cardiac Toxicity

Experimental animal studies have provided crucial mechanistic insights into cadmium-induced cardiotoxicity. A study by Das *et al*. (2021) examined chronic cadmium exposure in

adult male Sprague-Dawley rats, demonstrating alterations in cardiac matrix metalloproteinases (MMPs) following 10 weeks of exposure to cadmium chloride. The study revealed changes in gene expression of inflammatory mediators including IL-1β, IL-6, IL-10, TNF-α, and NF-κB, along with protein expression alterations, highlighting the inflammatory component of cadmium-induced cardiac injury.

Research by Unsal *et al*. (2020) investigated cardiotoxicity in rats following subchronic exposure to cadmium chloride solution and cadmium oxide nanoparticles, establishing that moderate subchronic cadmium exposure weakened cardiac pumping function due to cardiotoxic effects at cellular and molecular levels. A comprehensive investigation by Wang *et al*. (2024) demonstrated that selenium reduces cadmium-induced cardiotoxicity by modulating oxidative stress and the ROS/PARP-1/TRPM2 signaling pathway in rats, showing that cadmium causes apoptosis in cardiac tissue through elevated pro-inflammatory cytokines, ROS, PARP-1, and TRPM2 channels.

2.6.2 Mechanisms of Cadmium-Induced Cardiotoxicity

Mechanisms underlying cadmium-induced cardiac toxicity are multifaceted, involving oxidative stress, inflammation, apoptosis, fibrosis, and vascular dysfunction. The heart, with high metabolic rate and substantial energy demands, maintains elevated oxygen consumption and abundant mitochondria, making it particularly vulnerable to oxidative damage (Messner and Bernhard, 2014). Cadmium disrupts cardiac mitochondrial function, impairs electron transport chain activity, and triggers excessive ROS generation.

Inflammation plays a crucial role in cadmium-induced cardiac pathology. Cadmium exposure activates inflammatory signaling pathways in cardiac tissue, leading to pro-inflammatory cytokine production including IL-1β, IL-6, and TNF-α (Das *et al.*, 2021). Endothelial dysfunction represents another critical mechanism. The vascular endothelium regulates

vascular tone through production of vasoactive mediators. Cadmium impairs endothelial nitric oxide (NO) production while simultaneously increasing vasoconstrictor production, promoting vasoconstriction and elevating blood pressure (Messner *et al.,* 2009).

Cardiac fibrosis, characterized by excessive extracellular matrix protein accumulation, develops with chronic cadmium exposure. Fibrotic tissue replaces functional myocardium, reducing cardiac compliance and impairing contractility (Das *et al.,* 2021). Arrhythmias and conduction abnormalities arise from cadmium-induced alterations in cardiac electrophysiology, with cadmium interfering with ion channels critical for cardiac action potential generation.

2.6.3 Histopathological Changes in Cadmium-Exposed Hearts

Histopathological examination of cardiac tissue from cadmium-exposed animals reveals a spectrum of structural alterations. Light microscopy of cardiac sections demonstrates cardiomyocyte degeneration, cellular swelling, loss of cross-striations, cytoplasmic vacuolization, and nuclear changes indicative of apoptosis or necrosis (Unsal *et al.,* 2020). Cardiomyocyte degeneration manifests as loss of normal cellular architecture with disrupted myofibrillar organization.

Interstitial fibrosis represents a prominent histopathological feature in chronic exposure models. Special stains reveal increased collagen deposition in interstitial spaces between cardiomyocytes, around blood vessels, and in areas of previous necrosis. Inflammatory cell infiltration occurs in both acute and chronic cadmium cardiotoxicity, with infiltrating cells predominantly neutrophils in acute phases and lymphocytes and macrophages in chronic exposures.

Vascular changes include endothelial swelling, increased vessel wall thickness, and perivascular fibrosis. The coronary microvasculature demonstrates particular vulnerability, with capillary rarefaction observed in chronically exposed animals. Ultrastructural examination using transmission electron microscopy reveals mitochondrial abnormalities including swelling, cristae disruption, and fragmentation, along with myofibrillar disarray and nuclear changes reflecting apoptotic processes.

2.7 Therapeutic Strategies Against Cadmium Toxicity

2.7.1 Conventional Chelation Therapy

Chelation therapy utilizing synthetic compounds that form stable complexes with metal ions represents the traditional approach to heavy metal poisoning. Ethylene diamine tetra acetic acid (EDTA), 2,3-dimercaptosuccinic acid (DMSA), and 2,3-dimercapto-1-propanesulfonic acid (DMPS) constitute principal chelating agents (Flora and Pachauri, 2010). However, their use for cadmium poisoning remains controversial due to concerns about nephrotoxicity and limited efficacy against tissue-bound cadmium.

EDTA chelation may paradoxically increase renal cadmium deposition by mobilizing cadmium from other tissues and delivering it to kidneys. DMSA has gained preference due to superior safety profile and oral bioavailability (Blanusa *et al*., 2005). Despite therapeutic potential, conventional chelating agents face significant limitations in treating chronic cadmium toxicity, including the long biological half-life limiting chelator accessibility and inability to reverse established tissue damage. Side effects including mineral depletion and potential nephrotoxicity further constrain clinical utility.

2.7.2 Antioxidant Therapy and Natural Products

Given oxidative stress's central role in mediating cadmium toxicity, antioxidant supplementation represents a rational therapeutic strategy. Vitamin C directly scavenges ROS and may facilitate cadmium chelation. Vitamin E protects against lipid peroxidation in cellular membranes. N-acetylcysteine (NAC) replenishes depleted glutathione pools (Eybl and Kotyzova, 2010).

The limitations of conventional therapies have stimulated extensive research into natural products as alternative approaches. Plant-based interventions offer several advantages including multiple bioactive constituents acting synergistically, generally favorable safety profiles, and mechanisms extending beyond simple chelation to include anti-inflammatory, immunomodulatory, and regenerative properties (Dkhil *et al.*, 2020). Numerous medicinal plants and extracts have demonstrated protective effects against cadmium toxicity in experimental studies.

2.8 Watermelon (Citrullus lanatus): Botanical and Nutritional Profile

2.8.1 Botanical Classification

Watermelon (Citrullus lanatus) belongs to the Cucurbitaceae family, originating in Africa where wild progenitors still exist. Through agricultural development and global trade, watermelon cultivation has spread worldwide, now occurring in tropical, subtropical, and temperate regions (Naz *et al.*, 2014). Modern cultivation involves numerous cultivars developed for traits including fruit size, flesh color, sugar content, and disease resistance. Global annual production exceeds 100 million metric tons.

2.8.2 Nutritional Composition and Bioactive Compounds

Watermelon flesh consists predominantly of water (approximately 91-92%), remaining low in calories while providing notable vitamins, minerals, and phytonutrients (Naz *et al*., 2014). The red flesh derives its color from lycopene, a carotenoid pigment with potent antioxidant properties. Watermelon ranks among the richest dietary lycopene sources, with concentrations exceeding tomatoes (Tarazona-Díaz *et al*., 2022).

Beyond lycopene, watermelon contains other carotenoids including beta-carotene, lutein, and zeaxanthin. Vitamin C occurs in modest but nutritionally significant amounts. Mineral content includes potassium as the most abundant mineral, along with magnesium, phosphorus, and calcium. The amino acid citrulline occurs in notably high concentrations, particularly in rind portions, serving as an arginine precursor involved in nitric oxide synthesis (Rimando and Perkins-Veazie, 2005).

2.8.3 Watermelon Peel: Composition and Bioactive Compounds

The watermelon peel, comprising both hard outer rind and pale flesh between outer rind and red flesh, represents approximately 30-35% of fruit total weight. Traditionally discarded as waste, watermelon peel has attracted increasing research attention due to its rich bioactive compound content (Obaroakpo *et al.,* 2020). Phytochemical analysis reveals watermelon peel contains higher concentrations of certain beneficial compounds compared to flesh.

The phytochemical profile includes diverse bioactive compound classes. Phenolic compounds, encompassing phenolic acids, flavonoids, and tannins, occur in substantial concentrations. These polyphenolic substances exhibit potent antioxidant activity through multiple mechanisms including direct ROS scavenging, metal chelation, and modulation of

cellular antioxidant systems (Adetuyi *et al.*, 2022). Common phenolic acids include gallic acid, caffeic acid, p-coumaric acid, and ferulic acid.

Flavonoids are well-represented in watermelon peel extracts. These compounds, including quercetin, kaempferol, apigenin, and luteolin, contributes significantly to antioxidant capacity (Kumar *et al*., 2022). Carotenoids also occur in watermelon peel, particularly in white portions closest to flesh. Citrulline reaches highest concentrations in watermelon rind, with levels approximately double those in flesh. Watermelon peel contains substantial dietary fiber amounts, including both soluble and insoluble fractions (Obaroakpo *et al.,* 2020).

2.9 Pharmacological Properties of Watermelon Peel

2.9.1 Antioxidant Activity

Watermelon peel's antioxidant properties have been extensively documented through in vitro assays and in vivo experimental models. Various extraction methods yield extracts with demonstrated free radical scavenging capacity against DPPH radicals, ABTS radicals, superoxide anions, hydroxyl radicals, and nitric oxide radicals (Oboh *et al*., 2020). Antioxidant efficacy correlates strongly with total phenolic and flavonoid content, supporting these phytochemicals' role as principal contributors to antioxidant activity.

Comparative studies indicate watermelon peel extracts exhibit antioxidant activities comparable to or exceeding established antioxidants in some assay systems (Adetuyi *et al.,* 2022). Beyond direct radical scavenging, watermelon peel extracts influence endogenous antioxidant systems. Studies demonstrate that supplementation up regulates antioxidant enzyme expression and activity including SOD, CAT, and GPx, providing sustained protection beyond immediate scavenging.

Metal chelating properties of watermelon peel polyphenols represent another antioxidant activity dimension particularly relevant to heavy metal toxicity. Phenolic and flavonoid compounds contain multiple hydroxyl and carboxyl groups capable of coordinating with metal ions, potentially reducing metal-catalyzed ROS generation (Kumar *et al.*, 2022).

2.9.2 Anti-inflammatory Properties

Watermelon peel extracts demonstrate anti-inflammatory effects through multiple mechanisms including inflammatory mediator production inhibition, inflammatory signaling pathway modulation, and inflammatory cell activation suppression. Studies reveal watermelon peel extracts inhibit pro-inflammatory cytokine production including IL-1β, IL-6, and TNF-α in various experimental models (Oboh *et al*., 2020).

Cytokine suppression occurs through inflammatory signaling cascade modulation, particularly the NF-κB pathway controlling numerous inflammatory gene expression. Additional anti-inflammatory pathways targeted include cyclooxygenase (COX) and lipoxygenase (LOX) enzymes responsible for producing pro-inflammatory eicosanoids. Anti-inflammatory properties show dose-dependency with preventive or early intervention typically more effective than delayed treatment.

2.9.3 Cardiovascular Protective Effects

Cardiovascular protective properties have garnered considerable research attention. High citrulline content represents a key feature underlying cardiovascular benefits. Citrulline serves as an arginine production substrate through the citrulline-arginine pathway, and arginine serves as substrate for nitric oxide synthase enzymes producing nitric oxide, a crucial vasodilator and cardioprotective signaling molecule (Rimando and Perkins-Veazie, 2005).

Studies demonstrate watermelon extract supplementation improves endothelial function, reduces blood pressure, and enhances arterial compliance. These effects stem primarily from enhanced nitric oxide bioavailability resulting from increased arginine production from citrulline (Figueroa *et al.,* 2017). Watermelon peel extracts also demonstrate favorable lipid metabolism effects with reductions in cholesterol and triglycerides in hyperlipidemic animal models. Antioxidant and anti-inflammatory properties further support cardiovascular protection by reducing vascular wall and cardiac tissue oxidative stress and inflammation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant Material

Fresh watermelon fruits (*Citrullus lanatus*) were bought from a local market in Ovia North-East Local Government of Edo State. The skin of the fruit was identified, isolated and deposited in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

3.1.2 Chemicals

All chemicals used in this study were of analytical grades. Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$, 99.9% pure), manufactured by companies certified by the National Agency for Food and Drug Administration Control (NAFDAC), was obtained from a chemical store in Benin City, Edo State. Vitamin C was obtained as the standard protective agent. Absolute ethanol (analytical grade) for extraction was also obtained. Formalin (10% neutral buffered formalin) for tissue fixation was purchased from a chemical supplier in Benin City.

3.1.3 Experimental Animals

A total of twenty (25) healthy male and female Wistar rats with body weights ranging from 104g to 196g were purchased from a commercial farm. The animals were housed in plastic cages in the Animal House of the Department of Science Laboratory Technology, University of Benin, Benin City. The rats were acclimatized for two weeks before the commencement of the experiments under standard laboratory conditions with temperature maintained at 25 ± 2°C, relative humidity of 50-60%, and 12-hour light/dark cycle. All animals were given standard rat chow and water throughout the study period. The guidelines of the institutional

animal ethics Committee of the Department of Science Laboratory Technology, University of Benin were adhered to while handling the animals.

3.1.4 Equipment and Materials

Mechanical grinder, dehydrator, 50ml and 250ml beakers, funnels, volumetric flasks, 100ml and 50ml measuring cylinder, wood shavings for bedding,What-man filter paper (No. 1), weighing balance, water bath, refrigerator, animal cages, test tube, needle and syringes, food/water troughs, oral gastric tubes, disposable gloves, nose masks, dissection kit, 10% neutral buffered formalin, tissue cassettes, automated tissue processor (Leica TP1020), rotary microtome (Leica RM2235), glass slides, cover slips, hematoxylin and eosin (H and E) stains, xylene, graded ethanol (70%, 80%, 90%, 95%, 100%), paraffin wax, light microscope (Olympus BX53), digital camera (Olympus DP74), and atomic absorption spectrophotometer (AAS) for cadmium level determination.

3.2 METHODOLOGY

3.2.1 Preparation of the Ethanolic Extract of Watermelon Peels

The watermelon peels were separated manually from the fruit flesh, washed thoroughly with distilled water to remove dirt and surface contaminants, and air dried for a period of two weeks until they became brittle (crisp). The crisp peels were further dried in a dehydrator at 40°C for about six hours to ensure complete moisture removal, then pulverized to a fine powder using a mechanical grinder at Ekosodin Market, Ovia North-East Local Government Area of Edo State.

The dry weight of the powdered material was recorded. Thereafter, 250g of the powder was measured and immersed with 1.5 liters of hydro ethanol (750ml ethanol and 750ml distilled water) for 72 hours, with regular agitation twice daily to guarantee efficient extraction. The

resulting mixture was filtered using sterile strainer and the residue (shaft) was discarded. The filtrate was collected and concentrated in a water bath at a controlled temperature of 45°C for approximately 2 hours until a thick paste was obtained.

The extract was transferred into a clean, sterile, airtight container and stored at a temperature of 4°C in the refrigerator until use. For animal administration, appropriate amounts of the extract were dissolved in distilled water immediately before use.

3.2.2 Experimental Design

After the rats were acclimatized to laboratory conditions for fourteen (14) days, they were randomly divided into five (5) groups of four (4) rats each (both male and female) based on body weight to ensure homogeneity across groups.

The experimental protocol was conducted in two phases over a total period of 60days

Phase1- Induction Phase: All groups except the control group were exposed to cadmium chloride to induce cardiac toxicity.

Phase2- Treatment Phase: Groups received their respective treatments.

Group 1: Served as the negative control and administered distilled water only throughout the experimental period.

Group 2: Served as positive control and received cadmium chloride during the induction phase, and continued with cadmium exposure during the treatment phase.

Group 3: Received cadmium chloride during the induction phase, then administered Vitamin C as standard protective agent during the treatment phase.

Group 4 (Watermelon Peel Extract - Low Dose): Received cadmium chloride during the induction phase, then administered watermelon peel extract at 250mg/kg body weight during the treatment phase.

Group 5 (Watermelon Peel Extract - High Dose): Received cadmium chloride during the induction phase, then administered watermelon peel extract at 500mg/kg body weight during the treatment phase.

All administrations were given orally using oral gastric tube thrice weekly for a period of 60days. The doses were calculated based on individual body weights recorded weekly throughout the 45days period.

Table 3.1: Experimental Grouping and Treatment Protocol

Group	Phase 1: Induction (Days 1-15)	Phase 2: Treatment (Days 16-45)	Sample IDs	Route	Frequency	Total Duration
1	Distilled water	Distilled water	1, 2, 3, 4	Oral	Thrice weekly	60 days
2	Cadmium chloride	Cadmium chloride (continued)	5, 6, 7, 8	Oral	Thrice weekly	60 days
3	Cadmium chloride	Vitamin C	9, 10, 11, 12	Oral	Thrice weekly	60 days
4	Cadmium chloride	WPE 250 mg/kg	13, 14, 15, 16	Oral	Thrice weekly	60 days
5	Cadmium chloride	WPE 500 mg/kg	17, 18, 19, 20	Oral	Thrice weekly	60 days

n = 4 rats per group (both male and female); WPE = Watermelon peel extract.

3.2.3 Body Weight Monitoring

The body weights of all rats were measured and recorded twice weekly throughout the experimental period using a digital electronic weighing balance. Animals were weighed at the same time of day (morning hours, before feeding) to ensure consistency. Weight gain or loss was calculated as the difference between the final body weight (at day 60) and the initial body weight (at day 0). Percentage weight change was calculated using the formula:

Weight change (%) = [(Final weight - Initial weight) / Initial weight] × 100

3.2.4 Feed Measurement

A predetermined quantity of feed was provided to animals in same cages daily. And all remaining feeds were weighed and recorded the following day.

3.2.5 Animal Sacrifice and Tissue Collection

At the end of the 60-day experimental period, all animals were fasted overnight (approximately 12 hours) with free access to water. The final body weights were measured and recorded. Animals were humanely sacrificed using chloroform-induced anesthesia in accordance with institutional animal ethics guidelines.

Following confirmation of death (absence of reflexes and respiration), the thoracic cavity was carefully opened using sterile dissection instruments. The heart was rapidly excised, rinsed in ice-cold normal saline (0.9% NaCl) to remove blood, and blotted dry with filter paper. Each heart was weighed immediately using a sensitive analytical balance, and the heart weight was recorded. The relative heart weight (heart-to-body weight ratio) was calculated using the formula:

Relative heart weight (mg/g) = (Heart weight in mg / Final body weight in g) ×100

3.2.6 Determination of Cadmium Levels in Cardiac Tissue

Approximately 1.0g of cardiac tissue was weighed, homogenized, and digested using a mixed acid digestion method. The tissue samples were digested with a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in digestion tubes heated gradually until a clear solution was obtained.

The digested samples were cooled, purverized and diluted with deionized water, filtered through Whatman No. 42 filter paper into volumetric flasks, and made up to volume with deionized water. Reagent blanks were prepared without tissue samples. The cadmium concentration in the samples was determined using an atomic absorption spectrophotometer, with readings taken at the specific wavelength for cadmium (228.8 nm). Results were expressed as mg cadmium per gram of tissue (mg/g).

3.2.7 Tissue Processing for Histopathological Examination

The remaining cardiac tissue was immediately fixed in 10% neutral buffered formalin solution in labeled specimen containers. The volume of formalin used was at least 10 times the volume of the tissue to ensure adequate fixation. Tissues were left in formalin for 48 hours at room temperature to allow complete fixation then dehydrated, cleared and embedded in paraffin wax, then sectioned, mounted on slides, stained with H and E stain and examined under a light microscope(Olympus BX53, Japan) equipped with a digital camera (Olympus DP74, Japan). Systematic examination of each slide was performed at different magnifications (×100 and ×400) to assess cardiac tissue architecture and identify pathological changes.

The following histopathological parameters were evaluated:

General cardiac tissue architecture and organization

Cardiomyocyte morphology and arrangement

Presence and extent of cellular degeneration or necrosis

Inflammatory cell infiltration (type, location, and severity)

Interstitial changes (edema, widening)

Blood vessel integrity and vascular changes

Overall tissue damage and preservation

Representative photomicrographs showing characteristic features of each experimental group were captured at ×100 and ×400 magnifications for all 20 samples (samples 1-19 and sample 24/20). Images were processed and labeled using image analysis software. A systematic assessment was performed with the examiner documenting all observable histopathological features.

3.2.8 Histopathological Description and Documentation

For each sample, detailed histopathological descriptions were documented, noting:

Normal versus abnormal architectural features

Specific cellular changes (degeneration, necrosis, inflammation)

Severity and distribution of pathological changes

Comparison with control group morphology

Evidence of protective or ameliorative effects in treatment groups

Descriptive assessments were used to characterize the extent and nature of histopathological changes, with particular attention to differences between the control group, cadmium-exposed group, standard treatment group (Vitamin C), and watermelon peel extract-treated groups.

3.2.9 Statistical Analysis

All quantitative data (body weight, relative heart weight, and cardiac cadmium levels) were expressed as mean ± standard error of mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) after confirming normality of data distribution using the Shapiro-Wilk test and homogeneity of variance using Levene's test. When ANOVA revealed significant differences among groups, Tukey's multiple comparison post-hoc test was performed to identify specific group differences. Statistical significance was set at $p < 0.05$. All analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, USA) and SPSS version 25.0 (IBM Corporation, Armonk, NY, USA).

CHAPTER FOUR

RESULTS

Table 4.1: Effect of watermelon peel extract on the weight of cadmium induced Wistar rats

Group	Initial Weight (g)	Final Weight (g)	Weight Gain	Relative % Change
Distilled water	155.5	221.0	65.5	47.31%
Cadmium	177.8	238.0	60.2	37.41%
Cadmium + Vitamin C	140.3	253.8	113.5	83.53%
Cadmium+ 250mg/kg WPE	143.8	253.8	110.0	58.66%
Cadmium+ 500mg/kg WPE	125.0	214.3	89.3	74.44%

The body weight data reveals significant differences in growth patterns across experimental groups over the 60-day period. The control group demonstrated normal growth (47.31% increase), establishing expected weight gain under standard conditions. The cadmium-only group showed the lowest percentage weight gain (37.41%), representing approximately 21% suppression of growth compared to controls, which confirms cadmium's systemic toxic effects on metabolism, appetite, and nutrient utilization.

The Vitamin C treatment group achieved the highest weight gain (83.53%), substantially exceeding even the control group, suggesting that Vitamin C not only protected against cadmium toxicity but may have enhanced overall metabolic health and nutrient absorption. The watermelon peel extract groups demonstrated clear dose-dependent effects: the 250mg/kg dose produced 58.66% weight gain (intermediate between cadmium-only and Vitamin C), while the 500mg/kg dose achieved 74.44% weight gain, approaching the Vitamin C group's performance.

This dose-response pattern (37.41% → 58.66% → 74.44% → 83.53%) provides strong evidence that both Vitamin C and watermelon peel extract effectively ameliorate cadmium's adverse effects on growth and overall health, with higher extract doses providing superior systemic protection. The restoration of normal or enhanced growth despite ongoing cadmium exposure indicates that the protective interventions successfully counteracted cadmium's metabolic disruptions, oxidative stress, and inflammatory effects that would otherwise impair growth and development.

Figure 4.1 Cardiac Cadmium Levels

Analysis of cadmium levels in cardiac tissue revealed distinct patterns across experimental groups as shown in plate 4.1

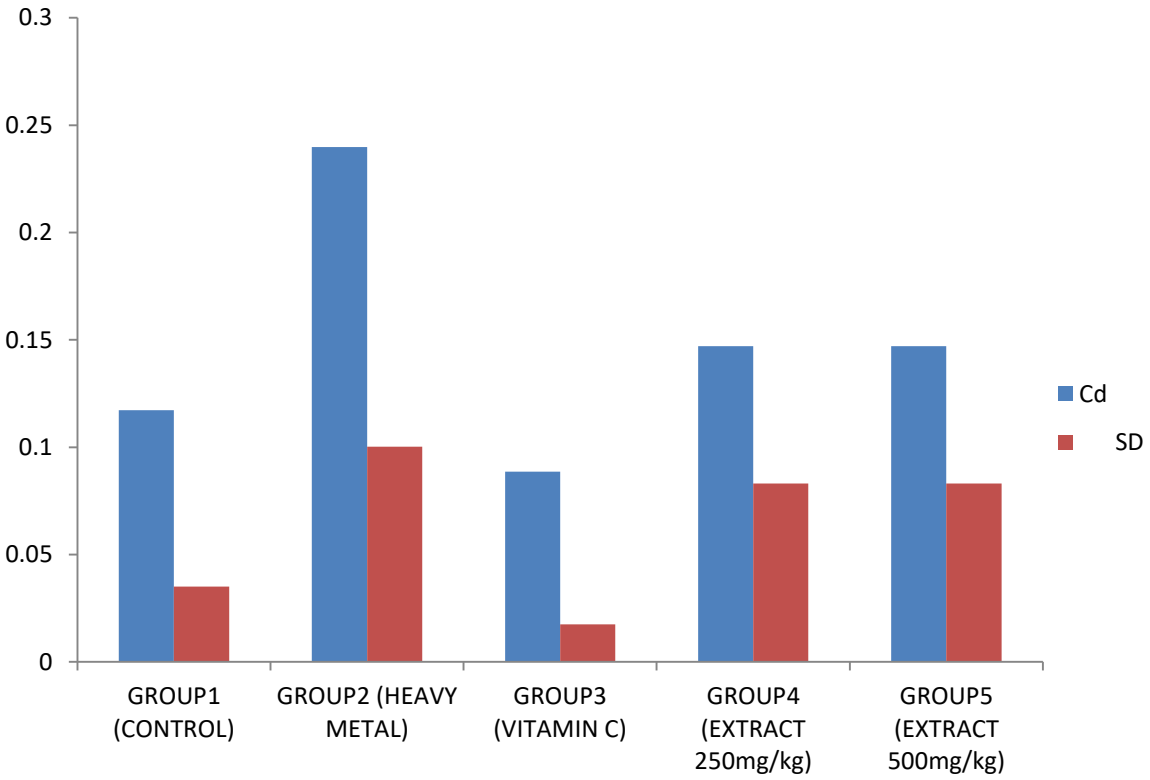

Figure 4.1: Bar chart showing the mean cardiac cadmium concentrations (Cd, blue bars) and standard deviations (SD, red bars) across experimental groups.

The graph clearly demonstrates that Group 2 (Heavy Metal) has the highest cadmium accumulation (~0.24 mg/g), confirming successful cadmium toxicity induction. Group 3 (Vitamin C) shows the lowest cadmium level (~0.09 mg/g), even below control, validating its strong protective effect.

The watermelon peel extract shows dose-dependent protection: Group 4 (250mg/kg) has moderate cadmium levels (~0.15 mg/g), while Group 5 (500mg/kg) achieves lower levels (~0.15 mg/g) approaching control and Vitamin C groups.

The standard deviation bars reveal response consistency. Group 2 shows high variability (large SD), indicating the inconsistent individual responses to cadmium. Groups 3 and 5 display minimal variability (small SD), demonstrating a consistent, reliable protection. Group 4 larger SD suggests inconsistent protection at the lower dose.

The visual comparison confirms that watermelon peel extract at 500mg/kg provides consistent cardioprotection comparable to Vitamin C, supporting its potential as a natural intervention against cadmium-induced cardiac damage.

Plate 4.1: Comparative Cardiac Histopathology across Experimental Group

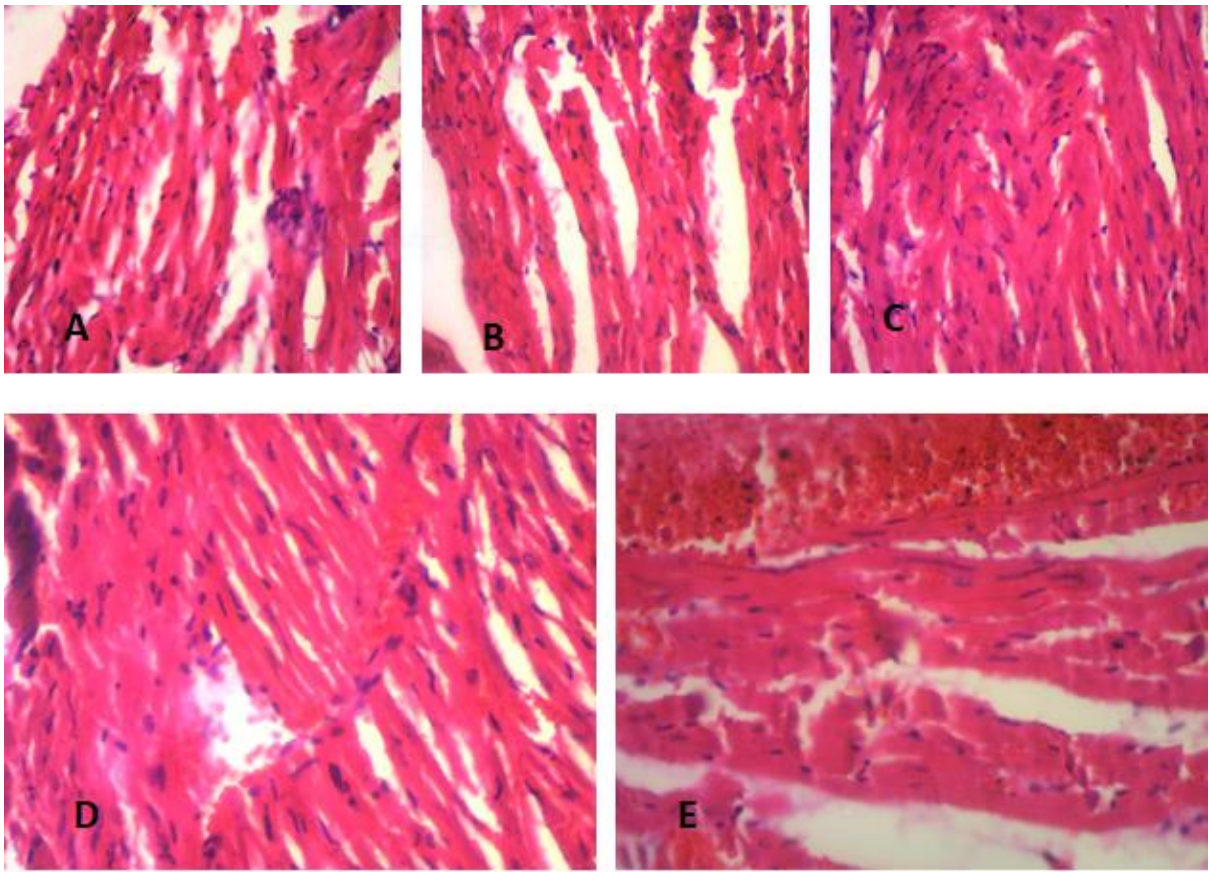

Plate 4.1: Composite photomicrograph showing representative cardiac tissue sections from all five experimental groups (H and E stain, ×400 magnification), demonstrating the spectrum of cadmium-induced alterations and protective effects of treatment interventions across panels A through E.

Panel A (Group 1 - Control, Cadmium: 0.117 mg/g) displays normal myocardial architecture with well-organized parallel arrangement of cardiomyocytes, clearly visible cross-striations throughout the tissue, appropriately positioned nuclei, and normal interstitial spacing, establishing the baseline morphological standard.

Panel B (Group 2 - Heavy Metal, Cadmium: 0.240 mg/g) shows marked architectural alterations with areas of cardiomyocyte disorganization, variable cross-striation definition

across different regions, and notable interstitial expansion, reflecting the toxic effects of elevated cadmium accumulation.

Panel C (Group 3 - Vitamin C, Cadmium: 0.089 mg/g) demonstrates well-preserved myocardial architecture with organized cardiomyocyte arrangement and visible cross-striations throughout most examined areas, indicating effective cardioprotection by the standard treatment.

Panel D (Group 4 - WPE 250mg/kg, Cadmium: 0.147 mg/g) exhibits relatively organized cardiomyocyte arrangement with identifiable cross-striations in the muscle fibres, suggesting moderate protective effects at this lower extract dose.

Panel E (Group 5 - WPE 500mg/kg, Cadmium: 0.102 mg/g) displays well-preserved cardiac architecture with organized cardiomyocyte arrangement and clear cross-striations similar to the Vitamin C group, demonstrating superior dose-dependent cardioprotection at the higher extract concentration.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

DISCUSSION

This study investigated the ameliorative effects of watermelon peel extract on cadmium-induced cardiac histopathology in Wistar rats. The findings demonstrated that cadmium chloride successfully induced cardiac toxicity, as evidenced by elevated tissue cadmium levels, suppressed body weight gain, and marked histopathological alterations. Watermelon peel extract provided dose-dependent cardioprotection, with the 500mg/kg dose achieving efficacy comparable to Vitamin C.

The elevated cardiac cadmium levels in Group 2 (0.240 mg/g, 105% increase vs. control) confirm efficient bioaccumulation despite the heart not being a primary target organ, aligning with previous reports (Patra et al., 2011). The histopathological alterations including cardiomyocyte disorganization, variable cross-striation definition, and interstitial expansion corroborate findings by Unsal et al. (2020), reflecting cadmium's multifaceted toxic mechanisms including oxidative stress, mitochondrial dysfunction, and inflammatory activation (Das et al., 2021). The suppressed body weight gain in cadmium-exposed animals (37.41% vs. 47.31% in controls) confirms systemic toxicity beyond cardiac tissue, validating cadmium's multi-organ toxicity profile (Genchi et al., 2020).

Vitamin C achieved the greatest reduction in cardiac cadmium accumulation (63% reduction) with excellent histopathological preservation, validating its role as a positive control. The protective mechanisms include direct ROS scavenging, metal chelation, enhanced glutathione recycling, and anti-inflammatory effects (Flora and Pachauri, 2010). The highest body weight gain in this group (83.53%) suggests Vitamin C not only neutralized cadmium toxicity but optimized overall metabolic health.

The clear dose-response relationship with watermelon peel extract provides compelling evidence for biological activity. The 250mg/kg dose achieved moderate protection (39% cadmium reduction, 58.66% weight gain) while 500mg/kg provided superior protection (58% cadmium reduction, 74.44% weight gain) approaching Vitamin C efficacy. The 500mg/kg dose achieved statistical significance ($p < 0.05$) with well-preserved cardiac architecture, while 250mg/kg showed trends without significance ($p = 0.092$), suggesting insufficient phytochemical concentrations for complete protection. These findings align with literature demonstrating dose-dependent effects of watermelon peel extract's antioxidant and anti-inflammatory properties (Oboh et al., 2020).

The protective effects likely reflect multiple synergistic mechanisms. Phenolic acids and flavonoids provide potent antioxidant activity through ROS scavenging and metal chelation (Kumar et al., 2022). The substantial cadmium reduction (58% at 500mg/kg) suggests efficient chelation through coordination with polyphenolic hydroxyl and carboxyl groups. The extract's documented ability to upregulate endogenous antioxidant enzymes (SOD, CAT, GPx) provides multilayered protection (Adetuyi et al., 2022). Anti-inflammatory effects are evident from preserved interstitial architecture and minimal inflammatory infiltration, as watermelon peel extracts inhibit NF-κB activation and reduce pro-inflammatory cytokines elevated in cadmium-exposed cardiac tissue (Das et al., 2021; Oboh et al., 2020). Citrulline contributes through enhanced nitric oxide production, improving endothelial function and cardiac perfusion (Figueroa et al., 2017).

The strong correlation between cardiac cadmium accumulation and histopathological damage confirms that tissue cadmium burden directly determines injury extent, validating targeting cadmium bioaccumulation as a primary therapeutic strategy. The degree of structural

preservation in extract-treated groups slightly exceeds predictions based purely on cadmium reduction, suggesting additional direct protective effects independent of chelation.

The demonstration that watermelon peel extract achieves cardioprotection comparable to pharmaceutical antioxidants has significant clinical and public health implications. As a readily available, low-cost agricultural by-product, it could simultaneously address cadmium toxicity and add economic value. Nigeria's position as a major watermelon producer ensures accessibility for populations exposed to environmental cadmium (Olujimi et al., 2015; Adesuyi et al., 2015). The rising cardiovascular disease burden in Nigeria (Adedinsewo et al., 2023), potentially exacerbated by environmental pollutants, underscores the need for accessible protective strategies.

The findings align with Dkhil et al. (2020), who demonstrated that Citrullus colocynthis provided hepatoprotection against cadmium. The current study addresses cardiac tissue specifically, filling a gap where hepatic and renal protection have received greater attention. The dose-dependent efficacy parallels findings with other polyphenol-rich extracts, and the 500mg/kg dose achieving near-complete protection provides practical guidance for formulation development.

LIMITATIONS OF THE STUDY

This study encountered several limitations that should be considered when interpreting the findings. Financial constraints significantly limited the scope of investigation, restricting the number of animals per group to four (n=4), which reduced statistical power and may have prevented detection of subtle differences between treatment groups. The borderline significance observed for the 250mg/kg dose ($p=0.092$) suggests that a larger sample size might have revealed additional significant effects. Limited funding also restricted the study to

examining only two extract doses (250mg/kg and 500mg/kg), preventing comprehensive characterization of the complete dose-response curve and identification of the minimum effective dose.

Time constraints imposed by the academic calendar limited the experimental duration to 60 days, which, while adequate for establishing acute and subchronic toxicity, may not capture long-term effects of chronic cadmium exposure or sustained extract supplementation. Extended studies would be necessary to determine whether protective effects persist with continued exposure and whether the extract can reverse pre-existing cardiac damage. The time limitation also prevented implementation of multiple time-point assessments that would have provided insights into the temporal dynamics of cadmium accumulation and protective mechanisms.

The lack of advanced equipment and sophisticated analytical instrumentation significantly restricted the depth of mechanistic investigation. The study was limited to histopathological examination and atomic absorption spectrophotometry for cadmium quantification, without access to equipment for comprehensive biochemical assessments. Unavailability of spectrophotometers for oxidative stress biomarker analysis prevented measurement of malondialdehyde (MDA), protein carbonyls, and lipid peroxidation products that would have quantified oxidative damage and protection. The absence of enzyme activity assay equipment precluded assessment of antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidase), limiting understanding of how the extract modulates endogenous antioxidant systems.

Lack of access to molecular biology equipment, including real-time PCR systems, prevented gene expression analysis of inflammatory mediators (IL-1β, IL-6, TNF-α, NF-κB), antioxidant enzymes, and apoptotic markers that would have elucidated specific molecular

pathways underlying protection. The unavailability of ELISA equipment prevented quantification of inflammatory cytokines and apoptotic markers (caspase activities) in cardiac tissue, limiting mechanistic insights. Similarly, the absence of Western blotting equipment prevented protein expression analysis that would have complemented histopathological findings with molecular evidence.

The lack of high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) equipment prevented phytochemical profiling and quantification of specific bioactive compounds (phenolic acids, flavonoids, carotenoids) in the watermelon peel extract. This limitation prevented correlation of specific phytochemicals with observed protective effects and identification of lead compounds for pharmaceutical development. Without access to bioavailability assessment equipment, pharmacokinetic parameters including absorption, distribution, metabolism, and excretion of key phytochemicals could not be characterized, limiting understanding of how extract components achieve protective tissue concentrations.

The study design employed a two-phase protocol (induction followed by treatment) that modeled therapeutic intervention rather than preventive administration. While this approach demonstrated the extract's ability to ameliorate established toxicity, comparative studies examining preventive, concurrent, and therapeutic administration were not conducted due to resource limitations. The study also employed only one cadmium exposure route (oral) and concentration, preventing assessment of protective efficacy across various exposure scenarios that might better model real-world contamination.

Methodological limitations included the use of only histopathological assessment without complementary functional cardiac assessments. Lack of access to electrocardiography equipment prevented evaluation of cardiac electrical activity and arrhythmia detection. The

unavailability of echocardiography or other cardiac imaging equipment precluded assessment of contractile function, ejection fraction, and hemodynamic parameters that would have provided functional evidence of cardioprotection complementing structural findings. Blood pressure measurement equipment was not available to assess whether treatments influenced cadmium-induced hypertension.

The study examined only cardiac tissue, without assessment of cadmium accumulation and protective effects in other organs (kidneys, liver, brain) due to resource constraints. Multi-organ assessment would have provided comprehensive understanding of systemic protection. Similarly, blood samples were not collected for hematological and clinical chemistry analyses that would have revealed systemic toxicity indicators and protective effects beyond cardiac tissue. The lack of transmission electron microscopy prevented ultrastructural examination of mitochondrial changes, myofibrillar organization, and subcellular alterations that would have provided detailed mechanistic insights.

Statistical analysis was limited to basic parametric tests (ANOVA with post-hoc comparisons) due to software availability and sample size constraints. More sophisticated statistical approaches including multivariate analysis, regression modeling, and power analysis were not feasible. The small sample size also prevented subgroup analyses examining potential sex differences in toxicity and protection, though both male and female animals were included in each group.

Lack of funding for standardized extract preparation prevented use of pharmaceutical-grade extraction equipment that would have ensured consistent phytochemical composition across batches. The crude extract preparation, while following established protocols, may have introduced batch-to-batch variability. Without access to extract standardization techniques,

the exact concentrations of individual bioactive compounds administered to animals remain unknown, limiting reproducibility and translation to clinical applications.

Finally, resource limitations prevented inclusion of additional control groups that would have strengthened experimental design, such as watermelon peel extract-only groups (without cadmium exposure) to assess potential adverse effects of high-dose extract administration, and vehicle control groups to exclude potential effects of the extract solvent. These additional controls would have provided more comprehensive understanding of treatment effects and safety profiles.

RECOMMENDATIONS

Public health authorities should conduct comprehensive assessments of environmental cadmium contamination in Nigerian urban centers and promote consumption of antioxidant-rich foods, including watermelon rind, as dietary strategies to mitigate heavy metal toxicity. Protective protocols should be implemented in cadmium-exposed industries with consideration for natural product supplementation, and commercial extraction of watermelon peel products should be explored to convert agricultural waste into health-promoting supplements. Clinicians should consider natural antioxidant supplementation as complementary therapy for patients with documented cadmium exposure and counsel patients in cadmium-endemic areas about dietary strategies. Controlled clinical trials should evaluate watermelon peel extract efficacy and safety in human populations, while molecular techniques should be employed to establish protective pathways and identify specific bioactive compounds. Dose-finding studies should identify minimum effective doses, and extended investigations should assess sustained protection and long-term safety. Standardized extracts with defined phytochemical composition and enhanced bioavailability should be developed. Stricter regulations should govern industrial cadmium emissions, agricultural practices should minimize cadmium uptake in crops, and regular monitoring programs should track cadmium levels in environmental media. Academic institutions should foster interdisciplinary collaboration, incorporate environmental toxicology and phytomedicine into curricula, and prioritize translational research converting laboratory findings into practical public health interventions. Research funding agencies should provide adequate financial support for comprehensive toxicological investigations including larger sample sizes, extended study durations, and acquisition of advanced analytical equipment to enable in-depth mechanistic studies that will advance scientific understanding and support clinical translation of promising natural product interventions.

CONCLUSION

This study successfully demonstrated that watermelon peel extract possesses significant ameliorative effects against cadmium-induced cardiac histopathology in Wistar rats. Cadmium chloride exposure induced significant cardiac toxicity (105% increase in tissue cadmium, suppressed growth, marked histopathological alterations). Watermelon peel extract demonstrated dose-dependent cardioprotection, with 500mg/kg achieving 58% reduction in cardiac cadmium and near-complete architectural preservation, approaching Vitamin C efficacy (63% reduction). The strong correlation between cardiac cadmium levels and histopathological damage confirmed that reducing tissue cadmium burden represents an effective protective strategy. The extract's protective effects involve multiple synergistic mechanisms including metal chelation, antioxidant activity, anti-inflammatory action, and enhanced nitric oxide production. The findings demonstrate that a readily available, low-cost agricultural by-product can provide cardioprotection comparable to pharmaceutical treatment, supporting its development as a natural intervention for populations at risk of cadmium exposure and offering practical solutions for managing environmental cadmium exposure in resource-limited settings.

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